PODIUM ABSTRACTS

S1.1 A COMPARATIVE STUDY BETWEEN UNCEMENTED AND HYBRID TOTAL HIP ARTHROPLASTY IN OCTOGENARIANS

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Uncemented total hip arthroplasty (THA) implants have become the standard for younger patients on account of increased implant survivorship and multiple other advantages. Nevertheless, uncemented THA remains controversial in elderly patients. The evidence base for this is limited, as previous studies have compared octogenarians to a younger control group. The aim of this prospective cohort study is to evaluate the outcome of octogenarian patients undergoing uncemented THA with a control group of similarly aged patients undergoing hybrid THA with a minimum 5 years follow up. Clinical outcomes including intra and postoperative complications, blood transfusion, revision rate and mortality were recorded. Radiological analysis of pre and postoperative radiograph assessed bone quality, implant fixation and any subsequent loosening. 143 patients, (mean age 86.2 yrs.) were enrolled in the study. 76 patients underwent uncemented THA and 67 underwent hybrid THA. The uncemented cohort had fewer intraoperative and postoperative complications. The uncemented cohort also had a lower transfusion rate (p=0.002). Mean hospital stay (p=0.27) was comparable between the 2 groups. Two patients underwent revision surgery in either cohort. Our study demonstrates uncemented THA is safe for the octogenarian patient and we recommend that age should be not be a barrier of choice of implant. However intraoperative assessment of bone quality should guide surgeon to the optimum decision regarding uncemented and hybrid implant.

S1.2 THE ASSOCIATION BETWEEN RADIOGRAPHIC AND FUNCTIONAL OUTCOMES AFTER THA


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Restoring native hip anatomy and biomechanics is important to create a well-functioning total hip arthroplasty (THA). Hip offset and leg length are regarded as the most important biomechanical characteristics. This study investigated their association with clinical outcomes including patient reported outcome measures (PROMs) and functional tests. This prospective cohort study was conducted in 77 patients undergoing primary THA (age=65±11 years). Hip offset and leg length were measured on anteroposterior radiographs of the hip pre- and postoperatively. Participants completed the Western Ontario & McMaster Universities Osteoarthritis Index (WOMAC) and performed functional tests (i.e. gait, single leg stance, sit-to-stand, block step-up) preoperatively, and 3 and 12 months postoperatively. A wearable motion sensor was used to derive biomechanical parameters. Associations between radiographic and functional outcomes were investigated with the Spearman's rho correlation coefficient. Subgroup comparisons were conducted for patients with more than 15% decreased or increased femoral offset after THA. Differences in postoperative offset and leg length had little impact on clinical outcomes. Femoral offset subgroups demonstrated no significantly different WOMAC function scores. In functional tests, patients with >15% decreased femoral offset after THA demonstrated more sagittal plane motion during block step-up (14.43° versus 10.66°; p=0.04) while patients with >15% increased femoral after THA demonstrated more asymmetry of frontal plane motion during block step-up (34.05% versus...
14.18%; p=0.03). To create a well-functioning THA, there seems to be a reasonable safe zone regarding the reconstruction of offset and leg length.

**S1.3 EFFECTS OF DESIGN MODIFICATION IN ZWEYMULLER CEMENTLESS TOTAL HIP PROSTHESIS**


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SL-PLUS MIA stem (Smith & Nephew Orthopaedics AG) is a modified implant of Zweymuller type SL-PLUS standard stem (Smith & Nephew Orthopaedics AG). We constructed finite element (FE) models and analysed equivalent stresses in the femur. In addition, we measured bone mineral density (BMD) in the femur by dual-energy X-ray absorptiometry (DEXA) after THA. The purpose of this study was to investigate the equivalent stress and to compare the results of the FE analyses with changes in BMD after THA. Twenty-one patients (18 women and 3 men) who underwent primary cementless THA with SL-PLUS MIA or SL-PLUS formed the basis of this study. Eleven patients received SL-PLUS MIA and ten patients received SL-PLUS. Zones were defined according to Gruen's system (zones 1~7). Computed-tomography (CT) images of the femur were taken before and at 1 week after THA. FE models of the femur and prosthesis were obtained from CT data by Mechanical Finder (Research Center of Computational Mechanics Inc., Tokyo, Japan), software that creates FE models showing individual bone shape and density distribution. Equivalent stresses were analysed in zones 1 to 7 and compared to the DEXA data. FE studies revealed that there was no significant difference in equivalent stress between SL-PLUS MIA and SL-PLUS. BMD was maintained after THA in zones 3, 4, and 5, whereas BMD decreased in zones 2, 6, and 7. In zone 1, BMD decreased in SL-PLUS MIA stem group by 14%, while BMD was maintained in SL-PLUS standard stem.

**S1.4 THE INFLUENCE OF A TOTAL KNEE ARTHROPLASTY ON HINDFOOT ALIGNMENT AND VICE VERSA: A SYSTEMATIC REVIEW**

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Patients with a hindfoot deformity impose a particular challenge when performing a total knee arthroplasty (TKA). This could be attributed to the lack of insights concerning the outcome towards the hindfoot alignment. Our objective was to perform a systematic review of the literature to investigate the influence of TKA on hindfoot alignment and vice-versa. In accordance with the Methodological Index For Non-Randomized Studies (MINORS) statement standards, we performed a systematic review. Electronic databases Pubmed, EMBASE, Web of Science, Google Scholar and Cochrane Library were searched to identify capable studies studying the influence between TKA and hindfoot malalignment. We indentified four prospective cohort studies, seven retrospective cohort studies and one case-control study. All twelve articles addressed the influence of TKA on hindfoot alignment. Seven out of nine studies which noticed an improvement of hindfoot alignment after TKA, found a significant improvement (p<0.05). Additionally three of these studies reported a significant improvement only in valgus hindfeet (p<0.05). On the topic of hindfoot alignment influencing TKA, we identified two studies. These studies reported an impact of hindfoot alignment on the weightbearing and described that 87% of hindfeet remained in valgus alignment after TKA. Available data suggests that alignment in valgus hindfeet can improve after TKA, though long term results are not present. Contrary to last, improvement of hindfoot alignment is not expected in varus hindfeet. Furthermore hindfoot alignment
deformity may cause a reduction of the long term survival of the knee prosthesis and therefore should be taken into account.

S1.5 PAIN AND TOURNIQUET USE IN TKA. WHAT IS THE EVIDENCE? A SYSTEMATIC REVIEW AND META-ANALYSIS

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Tourniquets have been used for many years during total knee arthroplasty (TKA). With a growing demand for TKA in recent years, tourniquet use has been surrounded by ongoing controversy due to many conflicting advantages and disadvantages of tourniquet use. Quantifying the case for or against tourniquet use in TKA, in terms of patient focused outcomes, is a priority. This meta-analysis analysed, the never before assessed, impact of tourniquet use during TKA on post-operative pain. We completed a systematic review and meta-analysis using PRISMA reporting guidelines to assess the impact of tourniquet use on patients post-TKA. Post-operative pain was the primary outcome. Secondary outcomes were post-operative range of motion (ROM) and length of stay (LOS). The initial search yielded 230 studies, of which 14 met the inclusion criteria. A post-operative increase in pain and reduction in ROM when using a tourniquet appeared significantly more likely when compared to no tourniquet use during TKA, yet with no overall difference in post-operative LOS. Subgroup meta-analysis demonstrated a trend that favoured the half-course tourniquet for reduced post-operative pain in patients when compared to full tourniquet use during TKA. This systematic review and meta-analysis concluded that the after-effects of tourniquet use in TKA patients and its impact on post-operative pain and ROM are indeed significant. We recommend further randomized controlled trials (RCTs) focusing on TKA patient outcomes of post-operative pain and ROM. Conflict of interest: The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

S1.6 POSTOPERATIVE MOBILITY OF TRAUMATISED GERIATRIC PATIENTS: A PILOT STUDY USING ACTIBELT TECHNOLOGY


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Capturing objective data of the postoperative changes in the mobility of patients is expected to generate a better understanding of the effect of postoperative treatment. Until recently, the collection of gait-related data was limited to controlled clinical environments. The emergence of accurate wearable accelerometers with sufficient runtime, however, enables the long-term measurement and extraction of mobility parameters, such as “real-world walking speed”. An interim analysis of 1967 hours of actibelt data (3D accelerometer, 100 Hz) from 5 patients (planned total 20) with a femur fracture and 5 patients (planned total 20) with a humerus fracture from a geriatric population at two different sites of the university hospital of the Ludwigs-Maximilian-University in Munich was performed. Mobility data was captured during several days of stationary treatment starting directly after surgery and during a short follow-up visit six weeks after the surgery. Preliminary results show an increase of the mean walking speed between the two visits independent of the type of fracture. Patients with a humerus fracture tended to walk faster than patients with a femur fracture during both visits. The data also
reveals an unexpected low level of mobility during the stationary stay. Mobile accelerometry can be used to evaluate different postoperative mobilisation strategies and even provide near-time feedback in geriatric trauma patients.

S2.1 EARLY RADIOLOGIC ASSESSMENT OF REVISION TOTAL HIP ARTHROPLASTY WITH THE RECLAIM MODULAR REVISION HIP SYSTEM

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Revision total hip arthroplasty (THA) presents with increasing challenges, potentially compromising the integrity of a revision. The objective of this study was to assess radiologic outcomes of patients who underwent revision THA with a modular tapered stem (Reclaim, DePuy Synthes). This study retrospectively examined all revision Reclaim THAs between 2012 and 2016. Radiologic assessment compared x-rays at two time points: immediately after surgery and the most recent x-ray available. Leg length discrepancy, subsidence and line-to-line fit was assessed. Significant subsidence was considered ≥10mm. Adequate line-to-line fit was considered ≥30mm of bicortical contact. Descriptive statistics included clinical factors (i.e. age, Paprosky classification). P values <0.05 were considered significant. A total of 81 femoral revisions were completed. There were 42 females and 38 males with a mean age of 71 years (range, 46-89). Of these, 6 were revised (dislocation, fracture or infection), and 7 were lost to follow up. Average follow up time was 18 months (range, 1-46 months). Femoral revisions were classified as Paprosky 3a or 3b. Mean stem subsidence was 4.15mm (range, 0-25.6mm). Subsidence of the femoral stem was <10mm in 88% of patients. A total of 62% of patients had both subsidence <10mm and ≥30mm of bicortical contact. In patients with <10mm subsidence, 70% had ≥30mm of bicortical contact. There was a positive trend between cortical contact and stem stability (OR 2.3). The Reclaim modular femoral system has demonstrated radiographic stability. Inadequate initial fit is a potential determinant of subsidence.

S2.2 CONED HEMI-PELVIS AND TOTAL HIP ARTHROPLASTY IN ACUTE COMPLEX ACETABULAR FRACTURES OF THE ELDERLY

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Acetabular fractures in the elderly are associated with high levels of morbidity and mortality and are becoming more common. Treatment is complicated by osteoporosis and multiple comorbidities. We present the early results of the use of a coned hemi-pelvis component and total hip arthroplasty in the primary treatment of these injuries. We have prospectively monitored a series of seventeen patients (18 cases) with a mean follow-up of sixteen (4-36) months. They have been reviewed clinically and radiographically. The mean patient age was 78 (64-87), and they had a mean ASA score of 3.3 (3-5). There were (Letournel classification) three elementary fractures, and 15 associated fractures. Mean operative time was 94 (61-134) minutes. There were seven minor post-operative complications. One patient suffered a pre-operative bilateral sciatic nerve injury, partially resolved. Sixteen of 17 patients were allowed to mobilise full weight bearing day one post-operatively. Mean length of hospital stay was 12 (5-27) days. Mortality at 30 days was 0%, and at one year 8%. There have been no thromboembolic events, dislocations or deep infections and no cases of prosthesis migration. Early weight bearing is essential for a successful outcome in this cohort. The coned hemi-pelvis bypasses the fracture, creating an immediately stable construct that allows immediate weight bearing. This is the first description of
an innovative use of this prosthesis in the treatment of a complex fracture that is traditionally associated with poor outcomes. Early results suggest this to be a safe technique with an acceptable early complication rate.

S2.3 DO PATIENTS ARRIVING WITH A MULTILIGAMENT KNEE INJURY AT THE EMERGENCY DEPARTMENT OF A MAJOR TRAUMA CENTRE UNDERGO ADEQUATE NEUROVASCULAR ASSESSMENT?

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Acute multiligament knee injuries (MLKI) are rare, high energy traumatic injuries associated with an increased risk of lower limb complications. The objectives of this study were to investigate the adequacy of clinical assessment for neurovascular status, compartment syndrome, and deep vein thrombosis in the emergency department (ED) following acute MLKI. The authors conducted a retrospective case note review of 19 patients with MLKI presenting at the ED of a Major Trauma Centre during a 7.5-year period between June 2009 and December 2016. MLKIs were diagnosed by MRI or examination under anaesthesia and confirmed intraoperatively. Arterial assessment consisted of documented capillary refill time, dorsalis pedis and posterior tibial pulse assessment (through palpation or Doppler ultrasound), and ankle-brachial pressure index (ABPI) calculation. Neural assessment was adequate if there was documented assessment of both sensory and motor function of the superficial peroneal, deep peroneal and tibial nerves individually. Data was collected for 19 patients (17 male, 2 female). The mean age was 34 (range: 14-61). The most common injury mechanism was road traffic accident. Neurovascular assessment was suboptimal in all categories: only one patient received a satisfactory lower limb neurological assessment and no patients received complete vascular assessments. Neurovascular assessment of multiligament knee injuries was suboptimal. Reasons for this included poor documentation and lack of certain specific clinical assessments, such as ABPI calculation. We propose the introduction of an acute knee injury pro forma highlighting the components of a full lower limb neurovascular examination to rectify this problem.

S2.4 AN EXPERIMENTAL INVESTIGATION OF CEMENT APPLICATION METHODS IN BALLOON KYPHOPLASTY

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Balloon kyphoplasty (BKP) is a minimally invasive surgical technique used to correct kyphosis and vertebral compression fractures. BKP uses cement to fill a void created by the inflation of a balloon in a vertebra, it can be used as an alternative to vertebroplasty to reduce cement extravasation. Issues such as poor interdigitation of the cement and the trabecular bone can arise with the BKP method. This can be due to a compacted layer created during the procedure which can cause complications post-surgery. The primary aim of this study was to investigate alternative cement application methods which could improve the mechanical strength of the bone-cement interface. Three alternative methods were investigated, and cylindrical bone-cement specimens were created for all methods (BKP and three alternatives). An important part of this study was to replicate the compacted layer created by the inflation of the balloon tamp in BKP. Synthetic trabecular bone specimens (Sawbones®, Pacific Research Laboratories, Vashon Island, Washington, USA) were pre-loaded in compression and the resultant compacted layers were found to replicate the compacted layers found in surgery. Mechanical testing was carried out with an MTS Model 858 Bionix® Servohydraulic load frame using static tensile and torsion loads. Static tests revealed that two of the three alternative methods were an improvement.
on BKP, with a high statistical significance in relation to the mechanical performance of the bone-cement interface (P < 0.001). This data illustrates the potential to improve the standard BKP technique, in terms of bone-cement interface performance.

**S2.5 ASSESSMENT OF THE CURRENT PRACTICE OF THE USE OF INTRAOPERATIVE NEUROPHYSIOLOGICAL MONITORING IN SPINAL SURGERY**

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There is an inherent risk of iatrogenic new neurological deficit (NND) arising at the spinal cord, cauda equina and nerve root during spinal surgery. Intraoperative neurophysiological monitoring (IONM) can be employed to preserve spinal cord function during spinal surgery. IONM techniques include somatosensory and motor evoked potentials, amongst others. A Canadian survey of 95 spinal surgeons showed that 62.1% used IONM and a similar survey in France of 117 spinal surgeons showed that only 36% used IONM. Unavailability was a common reason for its disuse. Current literature by the British Society of Clinical Neurophysiology has outlined the importance of IONM in preventing NND and the need for the implementation of guidelines for IONM. The lack of an established guideline has resulted in a varied approach in the use of IONM in England. There has been no previous attempt to ascertain the current use of IONM in England. Our study is aimed at assessing the variability of the use of IONM in England as well as identifying the rationale amongst surgeons that dictate their use of IONM. We are in the process of investigating the indications of use of IONM for cervical and lumbar spine procedures in 252 spinal surgeons from 33 hospitals with spinal services. Our survey will illustrate the current use of IONM in spinal surgery in England. It will highlight some of the reasons for the variability of use of IONM and identify factors that can contribute to a more standardised use of IONM in spinal surgery.

**S2.6 LESSONS LEARNT FROM IMMOBILIZATION STUDY FOR IMPROVING THE USAGE OF MOBILE ACCELEROMETRY IN CLINICAL TRIALS**

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Lower limb fractures are commonly treated with cast immobilization, and as a main consequence of strict immobilization this typically leads to loss in muscle mass, decrease of bone density and decline in functional abilities. Body-worn sensors are increasingly used to assess outcome in clinical trials by providing objective mobility parameters in a real-world environment. The aim of this study is to investigate the usability aspects and potential changes in mobility parameters in partial-immobilization patients in real-world conditions. Six healthy young males (age 22.2 ± 1.2 years; weight 76.5 ± 6.7 kg, height 185.8 ± 6.1 cm. Mean ± standard deviation) wore a leg cylinder cast with walker boot to immobilize their dominant leg for two consecutive weeks. Subjects were asked to continuously wear a tri-axial accelerometer on the waist (actibelt) during waking hours for 6 weeks including 2 weeks before, during and after cast immobilisation. The total amount of days of continuous recording was 339 days with a total wearing time of 120 days. Software packages which allow to detect steps and to estimate real-world walking speed were used to analyse the accelerometry data. It was suspected that knee immobilization would affect strongly the waveform of the signal with an impact on the accuracy of the speed algorithm, whereas the step detection should be more robust. This effect was confirmed in a preliminary study performed to quantify the accuracy under immobilization conditions. On the other hand, step numbers are known to be sensitive to fluctuations in wearing time which was not uniform throughout the
entire study. We concluded that in this setting step frequency is the most reliable parameter. Step frequency showed a systematic decrease in the values during the immobilization period which recovered to pre-immobilisation values after cast removal. This confirms the usability of accelerometry and sensitivity of its mobility parameters for clinical outcome assessment.

**S3.1 OUTCOMES OF CONSERVATIVELY MANAGED COMPLEX ACETABULAR FRACTURES IN THE ELDERLY**

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Acetabular fractures in the elderly are associated with high levels of morbidity and mortality. Conservative management is reserved for those unfit for extensive reconstruction, or those who achieve ‘secondary congruence’ of a complex fracture. We present demographic data and the results of conservative management in patients over 65 years of age. The Fracture Outcome Research Database (FORD) at our unit was interrogated for all patients over 65 years, who had sustained an acetabular fracture between June 2008 and June 2016. 410 patients were identified. Following exclusions, thirty-two patients were included for analysis. They had a mean age of 80 (66-91), and a mean ASA equivalent score of 3.1 (2-4). Mean follow up was five (1-9) years. Twenty-five patients lived in their own home and seven in a nursing home. Thirty had low energy injuries, two high energy. Twenty-four (75%) had anterior column posterior hemitransverse fractures, seven (22%) had associated both column and one (3%) had a T-type fracture. The mean length of inpatient stay was 43 days (4-140). Maximum post-operative mobility was limited to a hoist in eight (25%), a frame with or without assistance in 15 (47%), a stick in five (16%) and independence in four (13%). Thirty-day mortality was 6% and one-year mortality 22%. The data demonstrates that conservative treatment in this cohort leads to long inpatient stays, poor mobility and significant levels of mortality. Complex reconstruction remains demanding on both surgeon and patient. Innovative ways of managing these patients are needed to improve outcomes.

**S3.2 THE EFFECT OF A HINDFOOT DEFORMITY ON THE FULL LEG ALIGNMENT ASSESSED BY WEIGHT-BEARING CT**

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Hindfoot disorders are complex 3D deformities. Current literature has assessed their influence on the full leg alignment, but the superposition of the hindfoot on plain radiographs resulted in different measurement errors. Therefore, the aim of this study is to assess the hindfoot alignment on Weight-Bearing CT (WBCT) and its influence on the radiographic Hip-Knee-Ankle (HKA) angle. A retrospective analysis was performed on a study population of 109 patients (mean age of 53 years ± 14,49) with a varus or valgus hindfoot deformity. The hindfoot angle (HA) was measured on the WBCT while the HKA angle, and the anatomical tibia axis angle towards the vertical (TAx) were analysed on the Full Leg radiographs. The mean HA in the varus hindfoot group was 9,19°±7,94, in the valgus hindfoot group -7,29°±6.09. The mean TAx was 3,32°±2.17 in the group with a valgus hindfoot and 1,89°±2.63 in the group with a varus hindfoot, which showed to be statistically different (p<0.05). The mean HKA Angle was -1,35°±2.73 in the valgus hindfoot group and 0,4°±2.89 in the varus hindfoot group, which showed to be statistically different (p<0.05). This study demonstrates a higher varus in both the HKA and TAx in valgus hindfoot and a higher tibia valgus in varus hindfoot. This contradicts the previous assumption that a varus hindfoot is associated with a varus knee or vice
versa. In clinical practice, these findings contribute to a better understanding of deformity corrections of both the hindfoot and the knee.

**S3.3 IMPACT OF A MEDIAL CALCANEAL OSTEOTOMY ON THE LONGITUDINAL FOOT ARCH DETERMINED BY WEIGHTBEARING CT IN 2D AND 3D**

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An adult acquired flatfoot deformity (AAFD) is a complex 3D deformity. Surgical correction consists of a medial calcaneal osteotomy (MCO) but shows limitations due to the current 2D assessment. Therefore, the aim is to determine the influence of an MCO on the longitudinal foot arch assessed by 2D and 3D weightbearing CT (WBCT). Seventeen patients with a mean age of 44.5 years (range 18–66 yrs) were retrospectively included. MCO was indicated in a stage II AAFD (N=15) and a post-traumatic valgus deformity (N=2). Pre- and post-operative imaging was obtained from a WBCT. The height of the longitudinal foot arch was measured as the distance from the navicular tuberositas to the floor (Navicular Height, NH) on 2D CT images (NH2D) and computed on 3D CT data (NH3D). Additionally, 3D assessment could compute the degree of exorotation (α) of the navicular bone towards the vertical axis. The mean pre-operative NH2D and NH3D were respectively 29.57mm ± 7.59 and 28.34mm ± 6.51. These showed to be statistically different from the mean post-operative NH2D and NH3D, respectively 31.62mm ± 6.69 and 31.67mm ± 6.47 (p < 0.001). A statistical difference was also found when comparing the mean degree of exorotation in pre- and post-operative, respectively: αpre=14.08° ± 4.92 and the αpost=19.88° ± 3.50 (p < 0.001). This study demonstrates a significant correction of the longitudinal foot arch after a MCO. The novelty is attributed to the accurate degree of rotation assessment using WBCT. This information could be assistive to optimise a pre-operative planning.

**S3.4 DIAGNOSIS FOR LABRUM TEAR USING RADIAL SEQUENCE 3D MULTIPLE ECHO RECOMBINED GRADIENT ECHO MRI: COMPARISON WITH ARTHROSCOPIC FINDINGS**


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In this study, we evaluated the labrum tear using radial sequence 3D Multiple Echo Recombined Gradient Echo (MERGE) MRI without arthrography based on modified Czerny’s classification, comparing with actual arthroscopic findings. A total of 61 hips including 27 hips of femoroacetabular impingement (FAI), 19 hips of borderline development dysplasia of the hip (BDDH) and 15 hips of early stage osteoarthritis (OA) were enrolled this retrospective study. MRI findings evaluated in each three regions of interest; anterior region, anterolateral region, and lateral region. The cases with severe degeneration that is not concordant with any original Czerny’s classification is defined as stage4. We compared MRI findings with arthroscopic findings and calculated the sensitivity, specificity, and likelihood ratio in terms of the existence of labrum tear. MRI findings revealed labrum tear more frequently in anterolateral than lateral (p<0.001). Especially in FAI group, labrum tear was more frequently observed by MRI in anterolateral than lateral (p=0.006). In comparison with MRI findings and arthroscopic findings, the sensitivity was 97%, specificity was 79% and likelihood ratio was 4.59 as average of all regions in terms of the existence of labrum tear. In each region, sensitivity and specificity was 97% and 50% in anterior, 97% and 100% specificity in anterolateral, 94% and 81% in lateral, respectively. Thus, MERGE MRI revealed excellent sensitivity and specificity for diagnosis of labrum tear, especially in anterolateral region. The cases with severely degenerated labrum were classified as newly defined stage 4, which was recognized frequently in OA cases.
S3.5 INTRINSIC ANATOMICAL RISK FACTORS IN HIGH ANKLE SPRAINS DETERMINED BY 3D CT ANALYSIS

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High ankle sprains (HAS) cause subtle lesions in the syndesmotic ligaments of the distal tibiofibular joint (DTFJ). Current intrinsic anatomical parameters of the DTFJ are determined based on 2D imaging and uncertainty remains whether they differ in a HAS patients. The aim of this study is therefore two-fold: radiographic parameters will be determined in 3D and compared in a healthy vs sprained group. Ten patients with a mean age of 42.56 (SD = 15.38) that sustained a HAS and twenty-five control subjects with a mean age of 47.44 (SD = 6.55) were retrospectively included. The slices obtained from CT analysis were segmented to have a 3D reconstruction. The following DTFJ anatomical parameters were computed using CAD software: incisura width, incisura depth, incisura length, incisura angle, and incisura-tibia ratio. The mean incisura depth in the sprained group was 3.93mm (SD = 0.80) compared to 4.76 mm (SD = 1.09) in the control group, which showed a significant difference ($P < 0.05$). The mean incisura length in the group of patients with HAS was 30.81 mm (SD = 3.17) compared to 36.10mm (SD = 5.27) in the control group which showed a significant difference ($P < 0.05$). The other DTFJ anatomical parameters showed no significant difference. This study shows a significant difference in both incisura depth and incisura length between HAS patients and control subjects. These parameters could be used to identify potential anatomical intrinsic risk factors in sustaining a HAS.

S3.6 A SIMPLE AND LOW-COST DRILLING SIMULATOR FOR TRAINING PLUNGING DISTANCE AMONG ORTHOPEDIC SURGERY RESIDENTS


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Drilling through bone is a complex action that requires precise motor skills of an orthopedic surgeon. In order to minimize plunging and soft tissue damage, the surgeon must halt drill progression precisely following penetration of the far cortex. The purpose of this study was to create a low-cost and easy-to-use drilling simulator to train orthopedic residents in reducing the drill plunging depth. This prospective observational study was performed in the division of orthopedic surgery of a single tertiary medical center. The participants included 13 residents and 7 orthopedic specialists. The simulator consisted of a synthetic femur bone model and ordinary modeling clay, and the training unit consisted of a disposable plastic tube (~US$14), clamps (~US$58) and a power drill + drill bit (standard hospital equipment). Plunging depths were measured by the simulator and compared between orthopedic specialists, the 6 “senior residents” (3+ years) and the 7 “junior residents” during a training session. Measurements were taken again 2 weeks following the training session. Initially, the plunging depths of the junior residents were significantly greater compared to those of the orthopedic specialists (7.00 mm vs 5.28 mm, respectively, $p < 0.038$). There was no similarly significant difference between the senior residents and the orthopedic experts (6.33 mm vs. 5.28 mm, respectively; $p = 0.18$). The senior residents achieved plunging depths of 5.17 mm at the end of the training session and 4.7 mm 2 weeks later compared to 7.14 mm at the end of the training session and 6 mm 2 weeks later for the junior residents. This study demonstrated the capability of a low-cost drilling simulator as a training model for reducing the plunging depth during the drilling of bone and soft tissue among junior and senior residents.
S4.1 COMMERCIALISING UNIVERSITY TECHNOLOGY

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Universities have an obligation to ensure that Intellectual Property (IP) outputs are properly captured and exploited according to various National and European guidelines. There are two main ways which University technology development can take on the road to commercialisation: 1. Licensing the technology to an existing company: A license is permission to do something the granting party (the licensor) has the right to otherwise prohibit. In the context of IP licensing, it is a grant, by the owner of the property, to another (the licensee) of the right to use the IP in question for commercial purposes; 2. Starting a new company: An important university objective is to explore and pursue opportunities for the exploitation of its intellectual property rights. For universities and its inventors, spin out companies often provide an effective means to achieve this objective. A spin out is created when the University creates a new company out of one of its existing departments, institutions or by an inventor. The decision of which path to take is critical and various elements can effect this decision such as the inventors own objectives, the market niche for the technology, the stage of technical development, the potential reward for each option and the types of support structures available. This talk will summarise the main points to consider when deciding on the most appropriate way to commercialise technologies developed in Universities.

S4.2 FINDING THE ‘SWEET SPOT’: TIMING PATENT APPLICATIONS TO YOUR ADVANTAGE

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Patents are among the most important assets that a technology company can have. Building a patent portfolio involves balancing competing demands. Technical development, cashflow management and attracting investment must all happen against a backdrop of unforgiving patent application deadlines. Getting it right can pave the way to commercial success; but getting it wrong can kill a business before it even starts. Of course, filing patent applications too late can be fatal. But there’s also a risk of filing too soon; an applicant can be locked into a spiral of patent fees before it can afford the cost. Drawing on more than thirty years of experience in the IP field, Sean Cummings will help to navigate the patent maze. He will explain how the international patent system works and how to exploit the system to maximise options while minimising outlay. He will give tips for accelerating the process when granted patents are needed quickly and for slowing down the clock when cashflow is tight. And he will identify the ‘sweet spot’ for assessing inventions and attracting investment in the early stages when patent costs are still modest.

S5.1 OPERATIVE TIMES OF ORTHOPAEDIC TRAINEES AND CONSULTANT- IS THERE A DIFFERENCE?

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Surgical training in the UK is under increasing pressure with a high demand for service provision. This raises concerns about the resultant negative impact this is having on training opportunities for surgical trainees in theatre due to a high demand for surgical procedures to be performed expediently by consultants. This is due to the assumption that trainee take significantly longer time to operate in theatre and thus result in a slow progress of theatre lists. Our study evaluated the differences in operative time between orthopaedic trainees and orthopaedic consultants, as well as provide realistic timings for each stage encompassed within the entire duration a patient is in theatre. From our trauma unit electronic theatre database, we retrospectively collected data for six Joint Committee of Surgical Training (JCST) mandatory procedures. Information collected included patients' ASA grading, total surgical time and grade of surgeons. A total of 956 procedures were reviewed: 71.8% hip procedures, 14.2% intramedullary nail fixations and 14.2% ankle fixations. 46.2% and 53.8% of the procedures were performed by consultants and trainees as first surgeon, respectively. On average, consultants were found to be 13 minutes quicker in performing the hip procedures and this difference was found to be statically significant (p < 0.05). However, trainees were found to be quicker in performing intramedullary femoral nailings and simple ankle fixations, but consultant were faster at performing intramedullary tibial nailings and complex ankle fixations. However, the differences were not found to be statistically significant (p > 0.05).

S5.2 DOES RHIZOMELIA DWARFISM TRULY EXIST IN THE PATIENTS WITH ACHONDROPLASIA?

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Although achondroplasia has been cited as the most common form of rhizomelic dwarfism, no report in the literature has given the data on the ratio of their upper and lower limb segments. We performed a paired study of 91 achondroplasia patients with age and gender matched normal control group. Their upper and lower extremity radiographs were evaluated, and their radio-humeral and tibia-femoral ratios were compared. The ratios were compared using the Wilcoxon rank sum test. A p value of <0.05 was considered significant. The mean age of the patients was 15.8 years (95% confidence interval (CI), 13.1-18.6), and there were 45 males and 46 females. The radio-humeral ratio for the upper extremity of achondroplasia patients was 0.76 (95% CI, 0.75-0.76) which was significantly different from the normal control group of 0.79 (95% CI, 0.77-0.80) (p=0.001). The tibio-femoral ratio of the achondroplasia patients was 0.79 (95% CI, 0.78-0.79), which was not significantly different from the normal control group of 0.78 (95% CI, 0.77-0.79) (p=0.346). Rhizomelia is the predominant form of dwarfism in upper extremity, but not in lower extremity in achondroplasia. The functional deficits from these patients seem to come from the generalized limb shortness, and not specifically from rhizomelia.

S5.3 SURGICAL TRAINEES’ BELIEFS REGARDING THEIR INTRA-OPERATIVE PARTICIPATION AND ITS EXPLANATION DURING THE INFORMED CONSENT PROCESS IN A PAEDIATRIC SETTING: A QUALITATIVE STUDY

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The process of gaining informed consent can be a complex and much debated pursuit, especially within a paediatric setting. The role of the trainee surgeon and its explanation to children and their families prior to an operation has not been explored from the resident surgeons’ point of view. Ten face-to-face interviews were
conducted with orthopaedic surgery trainees at a tertiary level paediatric hospital in Toronto, Canada. These were transcribed and subsequently thematically coded by 3 reviewers. Three main themes were identified from the interviews. 1) Surgical trainees feel their level of participation and autonomy gradually increases dependent on their observed skills and level of training. 2) Trainees feel the consent process is adequate but acknowledge it is often purposely vague with regards to their intra-operative involvement as this is often unpredictable and it avoids patient/family anxiety. 3) Trainees believe families are aware of their participation however most likely underestimate their role during operations. Trainees in surgical specialties believe their level of autonomy is variable dependent on a number of factors and that this impacts on the ability to be more specific when gaining informed consent. This must be balanced with a family’s right to an appropriate understanding of their child’s operation and who is performing it. It may be that further patient education regarding trainees and their role in operations would help develop a more thorough and patient centred informed consent process.

S5.4 CAN ERRORS DUE TO SOFT TISSUE ARTIFACT BE REDUCED WITH USE OF PROBABLISTIC POSE ESTIMATION?
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Conventional marker based optical motion capture (mocap) methods for estimating the position and orientation (pose) of anatomical segments use assumptions that anatomical segments are rigid bodies and the position of tracking markers is invariant relative to bones. Soft tissue artefact (STA) is the error in pose estimation due to markers secured to soft tissue that moves relative to bones. STA is a major source of pose estimation error and is most prevalent when markers are placed over joints. Mocap and bi-plane videoradiography data were recorded synchronously while three individuals walked on a treadmill. For all three, pose of the thigh and shank, and movement of markers relative to the bones, were determined from the videoradiography data (DSX, C-Motion). Independently, pose of thighs and shanks was estimated using mocap data (Visual3D, C-Motion). Our measures of error in the mocap pose estimation were the relative thigh and shank translations. X-ray data from two subjects were used to generate a regression model for the antero/posterior movement of the lateral knee marker against internal/external hip rotation. The mocap translation errors of the third subject, attributed to STA of the knee marker, were 15.6mm and 32.0mm respectively. The pose of the third subject was then estimated using a probabilistic algorithm incorporating our regression model. Mocap translation errors were reduced to 10.6mm (thigh) and 4.4mm (shank). The results from these data suggest that errors in pose estimation due to STA may possibly be reduced via the application of algorithms based on probabilistic inference to mocap data.

S5.5 EVALUATION OF A SURGICAL WOUND CLOSURE SYSTEM
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A suitable wound closure is an indispensable requirement for an uncomplicated and expedient recovery after an abdominal surgery. The closure technique will have a great impact on the healing process of the wound. Surgical complications, such as wound dehiscence (sometimes associated with evisceration), infection, hernia, nerve injury and incisional pain are very common in the postoperative period of an abdominal surgery. Besides, although their development can be promoted by other risk factors like age, sex, lifestyle, diet, health condition,
the closure method can also influence the emergence of these undesirable complications. For this reason, and having the wellbeing and quality of life of the patients in mind, particularly high-risk patients, a closure system consisting of anchors applied on either side of the wound that aims to reduce the tension caused on the surrounding tissues of a wound and, consequently, decrease the risk of herniation was evaluated in a pilot animal study and compared with the traditional suture approach.

**S5.6 INTRA-ARTICULAR INJECTIONS OF EXPANDED MESENCHYMAL STEM CELLS WITH AND WITHOUT ADDITION OF PLATELET-RICH PLASMA**

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Intra-articular injections of human mesenchymal stromal cells (MSCs) and platelet-rich plasma (PRP) have been intensively investigated as therapies for knee osteoarthritis (OA) with positive outcomes. In this work we evaluated whether a combination of the treatments (MSCs + PRP) would be beneficial compared to MSCs alone (MSCs) and standard corticosteroid injection (Control group). Forty seven patients (24 males and 23 females; 53.3 ± 10.7 years old) with radiographic symptomatic knee OA (Dejour grades II–IV) were randomized to receive intra-articular injections of MSCs (n = 16), MSCs + PRP (n = 14) or corticosteroid (n=17). MSCs were obtained after mononuclear cells separation from bone marrow aspiration collected from both posterior iliac crests using Sepax automated closed system and expanded in culture until reaching the number of $4 \times 10^7$. PRP was obtained by double-centrifugation of whole blood according to a protocol developed in house. After 12 months follow-up, the MSCs and MSCs+PRP groups achieved higher percentages of expected improvement when comparing to the corticosteroid group for the KOOS-symptoms, pain, function and daily living, domains and global score. For the population older or equal to 60 years old the MSCs+PRP group showed significant superiority for the KOOS-ADL domain at 12 months. Cytokines quantification evidenced anti-inflammatory aspects of the treatments. This work evidences the safety and efficacy of intra-articular injection of MSCs for the treatment of early knee OA, with greater improvement with PRP addition particularly to the older population.

**S6.1 ADDITIVE MANUFACTURING FOR CRANIO-MAXILLOFACIAL SURGERIES**

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Originally the use of Additive Manufacturing (AM) in the medical field begun with simple 3D printed tissue and organ models. The technology has now evolved giving the ability to produce polymers, metal, ceramic personalized implants. What is the status and use of AM technology in cranio-maxillofacial surgeries? What are the recent advances in personalized implants? What are the technical, clinical and legal challenges in the use of AM this entails? These and other interesting questions, will be discussed in this presentation.

**S6.2 3D PRINTING OF BONE-LIKE SCAFFOLDS CONTAINING MESOPOROUS GLASSES TO TREAT OSTEOPOROTIC FRACTURES**

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Osteoporosis is a worldwide spread, silent disease steadily increasing due to demographic shift; it results in bone loss and increased porosity that lead to an increase in bone fragility and to low-energy fractures. In such a contest, we worked on the development of 3D scaffolds engineered to mimic the features of human healthy bone. Healthy and osteoporotic bone microCT scans were obtained from tissues discarded during surgical interventions (Istituto Ortopedico Rizzoli-Italy). The obtained .STL file was used to 3D print a type I collagen solution to mimic bone matrix whereas mesoporous bioactive glass/nano-hydroxyapatite were embedded within the collagen fibers to mimic the inorganic phase of human bone. The rheological properties of the Type I collagen/mesoporous glass suspensions were investigated at different collagen concentration and temperatures. The possibility of incorporating growth factors (IGF and β-TGF) in the scaffold struts was investigated proposing several approaches and their retained activity was assessed. Different co-culture of osteoblasts and osteoclasts set-ups were explored in order to define the influence of both chemical and topographical stimuli on the osteoblast-osteoclast coupling.

S6.3 BIOFABRICATION APPROACHES TO ENGINEER COMPLEX BONE AND CARTILAGINOUS STRUCTURES

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Current treatments of cartilage defects, including chondrocyte implantation and several tissue engineering strategies, often result in a repair tissue that does not replicate the architecture and depth-dependent properties of the native tissue. As a result, these therapies often only delay the occurrence degenerative diseases, such as osteoarthritis. Additionally, when the damage is extended to the subchondral bone, the regeneration of both bone and cartilage is major challenge, due to the dissimilar composition of the two tissues and the inherent challenge in recreating their strong interface, thus favouring the integration in vivo of the neo-tissue. The recent progresses in the field of biofabrication are opening new avenues for the treatment of damaged articulating joints. In particular, bioprinting technologies allow coordinating the deposition of multiple cell types and materials, thus permitting to mimic the complex architecture of osteochondral structures. In this lecture, the latest development in the field of (stem) cell-laden hydrogels, also termed bioinks, to create zonal-biomimetic cartilage constructs will be discussed, together with the integration of multiple (bio)printing strategies (i.e. co-fabrication of hydrogels, reinforcing polymers and bioceramics), and the impact of these technologies towards the generation of fully biofabricated, high-performance engineered osteochondral grafts, with potential application as tissue engineering constructs for regenerative medicine in orthopaedics.

S6.4 EVALUATION OF A NOVEL IN-SITU MONITORING TECHNIQUE DURING THE ADDITIVE MANUFACTURING OF TITANIUM ALLOYS

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Additive Manufacturing techniques such as Selective laser melting (SLM) are increasingly used in the fabrication of hip, knee and other orthopaedic implants. This is due to the ability of these techniques to print geometrically complex parts with osteoconductive features, resulting in a decreased chance of aseptic loosening. To facilitate wider adoption of SLM, in-situ process monitoring is required. This paper examines the robustness of a novel monitoring systems ability to detect voids within the bulk of a component with varying part density. This work reports the results of a printing study carried out with Ti6Al4V parts using a production scale Renishaw system. This system is equipped with the recently developed in-situ monitoring system, called InfiniAM Spectral. InfiniAM measures the level of optical emissions emitted during the build process. The Spectral software creates a 3D representation of the part, in near real time, based on the level of emissions detected. In this work, Spectral 3D images are compared with those generated after printing using a micro CT scanner. The latter creates a virtual 3D representation of the part and has the ability to detect part defects and voids, as well as quantify part density, within the body of a component. In this work, parts were designed with voids of diameters in the range 200 to 600 μm. The sensitivity of the in-situ monitoring system was correlated with post process analysis of the void dimensions. Additionally, the detection of part density variation due to a variation of input energy, was also evaluated.

S7.1 SEX AND GENDER: WHY DOES IT MATTER?
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There is a growing requirement by governmental and other funders of research, that investigators pay attention to and integrate considerations of sex and gender in their health research studies. Doing so, the argument goes, will reduce data waste, lead to the generation of more complete and accurate evidence to apply to the delivery of health care, and hopefully improve outcomes for both male and female patients. Yet, it is not always clear what sex and gender mean and how best to apply these to the study of diverse health conditions and health service delivery. In this presentation sex, gender and other related factors will be considered in the context of fractures, fracture repair, and post-operative management. Examples of sex and gender bias, sex and gender differences, and the integration of sex and gender in research on fracture and fracture repair will be presented.

S7.2 METHODS & DESIGN: INTEGRATING SEX AND GENDER INTO RESEARCH
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We need to shift our focus to integrating sex and gender into research proposals, so we can answer some of the most basic unanswered questions in the field of fracture management. Current evidence in guidelines indicate a near-to-linear increase from the 1990s for inclusion of sex and gender. However, these recommendations remain expressed in absolute terms, with little explanatory power, affecting uptake and implementation in clinical practice. This co-branded session, with members of the Orthopaedic Research Society – International section of fracture repair (ORS-ISFR), will provide participants with guiding principles and tools to assist researchers and grant reviewers understand what it means to include sex and gender in meaningful ways: from formulating research questions, recruitment strategies, to conducting sex-stratified analyses. In this presentation, we will consider diverse approaches, methods and, analyses to elevate sex and gender within trauma. A strong emphasis on the ways and means of including marginalized and vulnerable populations in research will be addressed.
S7.3 GENDERED INNOVATIONS

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The presentation of musculoskeletal disease differs in men and women, and recognition of the differences between men and women’s burden of disease and response to treatment is critical to optimizing care. In this presentation, I will discuss the expanding evidence in the literature that examine the role of sex and gender in musculoskeletal disease, including how its examination increases the innovations and contributions, as well as expands the knowledge about musculoskeletal disease, conditions, and injury in a broad sense. We will discuss the role that structural anatomy differences, hormones, and genetics play in differential disease expression, to the historical biases in the subject populations of clinical and basic research projects. Participants will be provided with examples and opportunities to evaluate orthopaedic science through a sex and gender lens, and what impact this may play in setting the stage for both clinical practice and scientific investigation.

S7.4 FOOT AND ANKLE ASSOCIATION WITH AGE – MRI STUDY


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The aim of this study was to evaluate the relationship between the location of the insertion point of the AT into the posterior aspect of the calcaneus and the PF. Two hundred and two feet were evaluated from MRI scans. Ninety-seven women and one hundred and five men with a mean age of 40.15±18.58 were included in this study. Two independent investigators measured the horizontal distance from the most anterior point of the calcaneus to the most posterior part of the PF (A), including the horizontal length of the calcaneus (B). Moreover, distance between the most inferior point of the calcaneus and the most inferior part of the AT insertion into the calcaneus (C) and height of the posterior aspect of the calcaneus (D) were measured. Patients were divided into three groups based on age (I - patients younger than 18, II - 18-65, III - older than 65) The all obtained mean values showed high sexual dimorphism between genders. However, when standardized ratios were compared, no statistically significant sexual differences were noted (p>0.05). Although previous studies have reported a correlation between the PF, age and gender, this correlation was not found in our study. Based on the obtained results, this study concludes that age and sex do not influence the morphology of the PF. However, aging strongly affects the location of the AT insertion point. Therefore, we believe this is the key factor which influence the relationship between the AT and PF.

S8.1 PATIENT-SPECIFIC PLANNING OF PROXIMAL FEMORAL AUGMENTATION: IN VITRO AND IN SILICO APPROACHES

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Femoroplasty is the process of injecting cement (cement augmentation) into the proximal femur to prevent osteoporotic hip fractures. Femoroplasty increases the strength and energy to failure of the femur and can be
performed in a minimally-invasively manner with lower hospitalization costs and reduced recovery. Our hypothesis was that efficient cement augmentation strategies can be identified via computational optimization. Therefore, using patient-specific planning we can minimize cement volume while increasing bone strength and reducing the risk of fracture. We proposed an in-silico methodology that was validated with in vitro experiments. A discrete particle model for cement infiltration was used to determine the optimum volume and filling pattern of the cement such that the best outcome was achieved. Several artificial bones were scanned before and after cement augmentation to applied previous in silico methodology. Then those femurs were mechanically tested (non-augmented and augmented). Therefore, in silico methodology was validated. Cement augmentation significantly increased the yield load. Predicted yield loads correlated well with the experiments. Results suggest that patient-specific planning of femoroplasty reduces the risk of hip fracture while minimizing the amount of cement required.

S8.2 BIOMECHANICS OF ORTHOPAEDIC SURGICAL CUTTING PROCESSES – EXPERIMENTAL AND COMPUTATIONAL MODELLING

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The complex structural arrangement of bone gives rise to anisotropic, rate-dependent failure behaviour, which varies significantly depending on tissue composition and architecture. This presents significant challenges in the development of orthopaedic surgical cutting instruments, which are required to generate sufficient forces to penetrate bone tissue, while minimizing the risk of thermal and mechanical damage to the surrounding environment. Currently, instrument designers rely heavily on empirical-based strategies to understand tool-bone interactions, with significant amounts of prototyping and validation experiments required throughout the design process. The aim of this study is to develop an experimentally-validated predictive computational model of orthopaedic cutting processes in three dimensions to understand the role of various cutting parameters on cutting forces and chip formation. An experimental model of orthogonal cutting was developed, whereby an adaptable cutting tool fixture driven by a servo-hydraulic uniaxial test machine was used to carry out high-rate cutting tests on Sawbone® trabecular bone analogues. A three-dimensional computational model was also developed using Abaqus/Explicit. The constitutive model describing material behaviour considers strain-rate and pressure-dependant yield behaviour using a Drucker-Prager elastic-plastic damage model, with Strain-hardening and rate-dependent model constants determined through dynamic uniaxial high-strain rate compression tests of material cubes. An excellent correlation between experimental and computational models was found, with the computational model accurately predicting tool cutting forces and chip development ahead of the tool during the cutting process. It was identifying that lower tool rake-angles resulted in the formation of larger discontinuous chips and higher cutting forces, while higher rake angles tended to lead to more continuous chip formation and lower cutting forces.

S8.3 MODELLING OF SURGICAL TECHNIQUE FOR SCAPHOULNATE INSTABILITY

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The treatment of scapholunate (SL) ligament injuries is addressed by surgical procedures to stabilize the carpal joint. Open techniques include bone-ligament-bone transfers, tenodesis, partial fusions and carpectomies.
Innovative procedures using wrist arthroscopy, offer minimally invasive fixation without full exposure of carpal bones; however, the success of the technique and its impact on the reduction on the range of carpal movement is as yet not well known. In this work, the performance of Corella tenodesis technique to repair the SL ligament is evaluated for a wrist type II by numerical methods. Human wrist can be classified based on the lunate morphology: type I for lunate that articulates with radius, scaphoid, capitate and triquetrum, and type II which has an extra surface to articulate with the hamate. A finite element model was constructed from CT-scan images, the model includes cortical and trabecular bones, articular cartilage and ligaments. Three scenarios were simulated representing healthy wrist, SL ligament sectioning and the Corella technique. The performance of the technique was assessed by measure the SL gap in dorsal and volar side as well as the SL angle to be compared to cadaveric studies. In intact position, the SL gap and the SL angle predicted by the numerical model is 2.8 mm and 44.8º, these values are consistent to the standard values reported in cadaveric experiments (2.0 ± 0.8 mm for SL gap and 45.8 ± 9.7 for SL angle). Virtual surgeries may help to understand and evaluate the performance of the techniques at clinical application.

S8.4 BUILDING AN IN SILICO MODELOF MICRO-CRACK PROPAGATION IN BOVINE CORTICAL BONE

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Formation of micro-cracks occurs in bone due to daily activities. Through a mechanism of self-repair, these micro-cracks are detected, and the damaged areas are restored, avoiding further propagation. The Scissors Model suggests that the osteocyte processes that cross the micro-cracks break as consequence of the cyclic displacements of the micro-crack faces, due to fatigue, and this triggers the remodelling processes. A fresh bovine tibia bone was cut in sections oriented 20º from the transversal direction. The cortical bone was sliced using a circular saw and shaped to the dimensions: 20 x 10 x 1 (mm) and the surfaces were polished. μCT images were obtained from all the samples (μCT 40, Scanco Medical, Brüttisellen, Switzerland). From the DICOM files, the geometries were reconstructed and meshed using tetrahedrons, in ICEM CFD. The Elasticity Modulus (E) was determined in Bonemat, by applying an empirical relationship Elasticity-Density from the literature. The parts were then imported into ANSYS APDL to simulate micro-crack propagation in bone. This model will be validated with further experimental work where the micro-crack will be initiated in the prepared samples and propagated due to fatigue loading, and the osteocyte processes will be visualized in the Scanning Electron Microscope (SEM). This investigation aims to study how cyclic loading in bones and failure of osteocyte processes can trigger target the mechanism of bone remodelling. The resulting model can later contribute for the investigation of treatments for bone diseases such as osteoporosis and the response of bone to the presence of orthopaedic implants.

S8.5 FEMORAL HEAD SIZE INCREASE AND ITS EFFECTS IN THE PELVIC BONE STRESSES IN TOTAL HIP ARTHROPLASTY

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In order to reduce the risk of dislocation larger femoral heads in total hip arthroplasty (THA) are being used by surgeons in recent years. The standard head size of 28 mm used in 73% of all hip procedures in 2003 was used in only 29% in 2016; whereas head sizes of 32 mm and 36 mm combined, were used in 70%. The increase
of head size effectively reduces the thickness of the acetabular cup, altering the load transfer. Herein, this research work investigates the effect of increasing the femoral head size on the stresses of the periacetabular bone at two selected regions: A1 (superior) and A2 (anterior). Three Finite Element models were developed from CT scan data of a hemipelvis implanted with a cemented all-polyethylene acetabular cup with a 50 mm outer diameter and inner diameter to accommodate three head sizes: 28 mm, 32 mm and 36 mm. The peak reaction force at the hip joint during one leg stand for an overweight patient with a body weight of 100 Kg was simulated for head sizes investigated. We found that highest average von Mises stress was 5.7 MPa and occurred in the cortical bone of region A1 which is located within Zone 1 boundaries (Charnley & DeLee); whereas a lower stress of 4.0 MPa occurred at region A2. In the two regions the stresses were the same for the three head sizes. Periacetabular bone was found to be insensitive to the increase of femoral head diameter in cemented THA.

S8.6 PIN-ON-DISC: CARTILAGE, BONE AND PE AGAINST 3DISC MATERIALS

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A pin-on-disc tribometer test with a rotating disc and a sector-wise loaded pin was used to determine friction coefficients for different material pairings. The four pin materials porcine cartilage, subchondral bone of the porcine cartilage, UHMWPE, vitamin E enhanced, crosslinked UHMWPE (VEPE) in combination with the three-disc materials zirconia toughened alumina ceramic (ZTA), CoCr, carbon-fibre-reinforced carbon (CrC) were tested. Stepwise loading was employed with the forces 10 N, 5 N, 2 N and 1 N. Test duration was 1 h. Diluted calf serum according ISO 14242-1 was used to determine the friction coefficients. The surface topography of all pins was examined using optical profilometry before and after the rotation tribometer tests. No wear related modifications of the surface roughness parameters could be found. The coefficients of friction (COF) were lowest for the cartilage pins against all three-disc materials, with steady-state values between 0.01 and 0.02 for the highest applied load (10 N). Friction of subchondral bone yielded COF in the range 0.2 … 0.6, depending on the counterpart material. The two polyethylene materials behaved similar in this friction test with COF of about 0.1. The Ra roughness values of the different pins reflect the COF results: Ra of subchondral bone was one order of magnitude higher than Ra of the cartilage. This is in-line with the COF-values of bone being one order of magnitude higher than those of cartilage. These results will be discussed in view of the use of the disc materials as orthopaedic hemi-prostheses.

S9.1 INNATE IMMUNE SENSORS AND SELECTIVE AUTOPHAGY IN PERIPROSTHETIC JOINT INFECTION

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Immune response in periprosthetic joint infection (PJI) is diverse. Resident macrophage and/or wandering monocyte are superb guardians to sense microbial attacks, take invaders and alarm the danger. Neutrophils are refined but momentary fighters to kill microbes with projectile weapons as well as predation. The swift action is usually effective at the forefront to prevent expansion of infectious foci. However, such characteristics often evokes overshooting via self-defeating of pus, thus leading to crucial soft tissue damage in the acute phase. Intervention of monocyte/macrophages follow and act as wise organizers. In addition, stromal fibroblasts also act in front for host defence. They equip innate immune sensors (TLRs, NLRs), which can sense dangers and trigger off inflammatory response, but also is usually self-regulated. These sensors not only interact each other,
but also have possible contribution to selective autophagy (xenophagy and lysophagy) in PJI. In this presentation, overview of pathology in PJI will be summarized with a special attention to innate immune sensors (TLRs and NLRs), and selective autophagy.

**S9.2 ROLE OF MOLECULAR DIAGNOSIS IN PERIPROSTHETIC JOINT INFECTION**

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While stable long-term clinical results have been achieved in total joint arthroplasty, periprosthetic joint infection (PJI) has been actualized as difficult issue in this decade. For accurate diagnosis, it is important to establish standard criteria such as MSIS criteria, and it is prevailing now. As an issue involving PJI, however, the existence of viable, but non-culturable (VNC) bacteria must be noticed. It is difficult to identify the VNC state infection, because microbiologic culture result shows negative and other markers tend to be negative. Here, molecular diagnosis based on polymerase chain reaction (PCR) has certain role as potential diagnostic tools for such VNC infection. We have applied a real-time PCR system for the diagnosis of PJI, which is able to detect methicillin-resistant Staphylococcus (MRS) and distinguish gram-positive from gram-negative bacteria. The prominent advantage is that PCR is the singular way to identify MRS in such culture negative cases. Recent development of full-automatic PCR system may improve the time efficiency for routine application. In this presentation, we will show the overall sensitivity and specificity of our PCR system for diagnosing PJI and discuss the current problem and future prospect.

**S9.3 ONE- OR TWO-STAGE REVISION OF INFECTED KNEE REPLACEMENTS: IS A RANDOMISED CONTROLLED TRIAL FEASIBLE?**


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Patients with knee prosthetic joint infection (PJI) frequently receive one- or two-stage revision. To explore the feasibility of a randomised controlled trial (RCT) comparing methods, we analysed a UK registry, interviewed patients and surgeons, systematically reviewed literature, held a consensus meeting, and assessed progress of an RCT in hip PJI. In 2014, in England and Wales, knee PJI was treated with one- or two-stage procedures in 19% and 71% of patients respectively. Between 2007 and 2014, use of one-stage procedures doubled and, in major centres, up to 42% of treatments were one-stage. We conducted in-depth interviews with 16 patients with knee PJI and 11 surgeons performing one- or two-stage revision. Patients considered randomisation acceptable with appropriate counselling and, depending on infecting organisms and health status, surgeons would randomise treatments. In meta-analysis, two-year re-infection rates in 10 one-stage series (423 patients) and 108 two-stage series (5,129 patients) were 7.6% (95%CI 3.4,13.1) and 8.8% (7.2,10.6) respectively. In a series of patients with knee PJI, surgeons from 2 major centres considered 6/15 patients eligible for either treatment, with 4 more potentially eligible after treatment of soft tissue infection. In an ongoing RCT of surgical treatment of hip PJI, 116 patients have been randomised at 14 centres in 3 years. Randomising patients with PJI is feasible but, as knee PJI is uncommon, a multicentre RCT would be required. Based on WOMAC score outcome and appropriate assumptions on eligibility and acceptability, 170 patients would need to be randomised over 4 years at 14 major centres.
S9.4 ANTIBIOTIC NANOSPHERE COATED BILAYER SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATIONS

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There has been a significant increase in the demand of polymeric scaffolds with promising affects in bone regeneration. However, inflammation is still a problem in transplantations to overcome with local antibiotic therapy. In this study, it is aimed to develop a functional POSS nanocage reinforced chitosan scaffold (CS/POSS) coated with drug loaded chitosan composite nanospheres to provide a controlled antibiainytic delivery at the defect site. Gentamicin and vancomycin were selected as model antibiotic drugs. Drug loaded nanospheres were fabricated with electrospray method and characterized in terms of morphology, hydrodynamic size, surface charge, FT-IR, in vitro drug release, antimicrobial activity and cytotoxicity. CS/POSS scaffolds were fabricated via lyophilisation and characterized with mechanic, swelling test, SEM and micro CT analyses. Positively charged nanospheres with uniform morphology were obtained. High drug encapsulation efficiency (80-95%) and sustained release profile up to 25 days were achieved with a cumulative release of 80-90%. In addition, the release media of the nanospheres (in 6 hours, 24 hours and 25 days of incubation period) showed a strong antimicrobial activity against S.aureus and E.coli, and did not show any cytotoxic effect to 3T3 and SaOS-2 cell lines. CS/POSS scaffolds were obtained with high porosity (89%) and 223.3±55.2μm average pore size. POSS reinforcement increased the compression modulus from 755.7 to 846.1Pa for 10 % POSS addition. In vitro studies of nanosphere coated bilayer scaffolds have showed high cell viability. Besides ALP activity results showed that POSS incorporation significantly increased the ALP activity of Saos-2 cells cultured on the scaffold. In conclusion, these composites can be considered as a potential candidate in view of its enhanced physico-chemical properties as well as biological activities for infection preventive bone tissue engineering applications.

S9.5 EVALUATION OF THE CAPACITY OF AN ANTIBIOTIC-ELUTING SCAFFOLD TO TREAT INFECTION IN A RABBIT MODEL OF CHRONIC OSTEOMYELITIS


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Chronic osteomyelitis (OM) is a progressive, inflammatory infection of bone caused predominantly by S. aureus and requires treatment through surgical debridement and systemic antibiotic administration. We have previously reported the fabrication of an antibiotic-eluting scaffold which is responsive to microbial activity for the treatment of OM. Herein, we ventured to assess the capacity of this antibiotic-eluting scaffold to treat infection in a rabbit model of chronic OM. Infections were established in the radii of New Zealand White rabbits using inoculations of 2x10^6 CFUs S. aureus JAR 060131 over a period of 4 weeks. Following surgical debridement (6mm), rabbits underwent treatment for a period of 8 weeks until euthanasia. The treatment groups were; 1) empty, 2) antibiotic-eluting scaffold (collagen/hydroxyapatite scaffold loaded with vancomycin) and 3) commercially available antibiotic-eluting fleece (Septocoll E®, collagen fleece loaded with gentamicin). During the treatment period, all groups received systemic antibiotics (Cefazolin 25mg/kg) administered subcutaneously twice daily for 4 weeks. Inoculation of the radius resulted in the development of a sequestrum containing S. aureus, demonstrating the successful establishment of OM. After the 8-week treatment period, 4/5 rabbits in the empty group were still infected, indicating that systemic antibiotic administration following debridement was insufficient to treat the infection. Fewer rabbits in both the
antibiotic-eluting scaffold group (2/4) and the antibiotic-eluting fleece group (1/3) were infected. This work demonstrates that the implantation of an antibiotic-eluting biomaterial into a defect following debridement enhances bacterial clearance in conditions of chronic OM.

S9.6 METHOD OF IMPREGNATION WITH THE ANTIBIOTIC OF THE HEAD OF THE FEMUR, TAKEN FROM PATIENTS AFTER ARTHROPLASTY

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Recently in traumatology various methods of impregnation biodegradable implants and allografts with antibiotics are widely used. Among them the soaking, shaking and ionophores are common used. We aimed to choose the optimal method of impregnation with the antibiotic of the head of the femur, taken from patients after arthroplasty. We studied 6 femoral heads after hip replacement. Head №1 the iohexol (Omnipaque) was injected through circular ligament and through the neck of the femur. Head №2 through the circular ligament, head №3 through the neck of the femur, head №4 through the circular ligament and through the neck of the femur, head №5 through 4 pre-drilled channels a brilliant green solution was injected. The head №6 was soaked in a brilliant green solution. Head №1 assessed by radiology. All the heads, treated with brilliant green, were cut in half to assess the degree of impregnation. On the X-ray image of head №1 the contrast agent has spread enough. In osteotomy, the impregnation with brilliant green head №2, №3, №4, №5 was seen in 3-4 mm around the needle passage place. Head №6 the bone was not impregnated. Despite the fact that the radiograph showed a sufficient spread of contrast agent, on the sections of the head, treated with brilliant green, showed the spread of liquid 3-4 mm around the needle passage place. This indicates that the impregnation of large bone is not effective.

S10.1 MARINE ORGANISMS FOR BONE REPAIR

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Current strategies for bone repair have accepted limitations and the search for synthetic graft materials or for scaffolds that will support ex vivo bone tissue engineering continues. Bioprospecting has led to increased interest in potential applications for marine organisms and their by-products and biomimetic strategies have led to the investigation of naturally occurring porous structures as templates for bone growth. As a rich source of mineralising porous organisms, our seas and oceans could provide new directions for bone tissue engineering that may enhance in vivo and ex vivo bone formation.

S10.2 NATURAL BIOSILICA AS AN INDEX FOR BONE HEALING

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Diatoms are unicellular microalgae whose cell walls are composed of remarkably uniform, hierarchical micro/nanopatterned, amorphous biosilica that cannot be replicated synthetically. Each species hosts its own unique morphology which is identically replicated generation-to-generation. There are currently estimated to be over 200,000 different diatom species, each with their own unique shape and morphology. This offers a huge array of surface topographies, particle sizes and shapes, each with the same silica precursor. Our research to date has shown that diatom-biosilica is non-cytotoxic to J774.2 macrophages and hBMSC cells and does not invoke an immunological response or organ toxicity (kidney, spleen and liver) when tested in a murine model. Before testing diatom-biosilica in vivo in an animal fracture model, methods to incorporate the frustules into the defect are being investigated. Two methods have been developed 1) using a bioresorbable hydrogel and 2) 3-D printed polymer-biosilica scaffolds. Both methods have shown promising results with enhanced mechanical properties with the addition of the diatom-biosilica. Work is ongoing to further map and quantify the role of surface topography and chemical cues on cell fate through the systematic in vitro studies of different species of diatom-biosilica.

**S10.3 HIGH THROUGHPUT SCREENING STRATEGY FOR DRUG DISCOVERY: SCREENING MARINE NATURAL PRODUCTS AS ANTI-INFLAMMATORY AND PRO-OSTEGENIC BIOACTIVITY**

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Human mesenchymal stem cells are considered the golden standard for clinical application in regenerative medicine for their multilineage differentiation potential, best candidates to treat diseases such as osteoarthritis and osteogenesis imperfecta. In the past few years several molecules have been described to induce the hMSCs differentiation into osteo cell progenitors, mainly discovered by screening of single metabolites bioactivity. However, hMSCs osteogenic differentiation potential is still poor, and the discovery of differentiation inducers with high efficiency is needed. Thanks to automated processes, High Throughput Screening (HTS) strategies shorten the metabolites bioactivity investigation timeline, allowing testing of many molecules simultaneously. In this work, reliable assays for natural products bioactivity investigation detection were developed using HTS methodologies and validated by testing 15 purified compounds derived by marine fungi and sponges. The HTS cytotoxicity investigation using HepG2 cells allowed to test in a single experiment, 15 metabolites in 4 concentrations ranging from 1 to 20μM. Low cytotoxicity was detected for metabolites concentrations from 1 to 10μM and so set as treatment concentrations to be tested in further assays. Anti-inflammatory bioactivity was tested on THP1 cells triggered by LPS. Five out of 15 metabolites showed to prevent the LPS induced THP1 inflammatory activation by lowering the TNF-α production. The metabolites pro-osteogenic potential was investigated using hMSCs: their differentiation was evaluated by calcium mineralization after 10 days differentiation. Pro-osteogenic molecules were not detected in this screening, but the method validation represents a powerful tool for future natural product and synthetic molecules libraries screenings.

**S10.4 EXPOSURE TO EXTRACORPOREAL SHOCK WAVES INDUCES FORMATION OF NEW MINERALIZED TISSUE IN ZEBRA MUSSELS INSIDE AS WELL AS OUTSIDE OF THE FOCUS ZONE**


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A substantial body of evidence supports the use of extracorporeal shock wave therapy (ESWT) for fracture non-unions in human medicine. However, the success rate (i.e., radiographic union at six months after ESWT) is only approximately 75%. Detailed knowledge regarding the underlying mechanisms that induce bio-calcification after ESWT is limited. The aim of the present study was to analyse the biological response within mineralized tissue of a new invertebrate model organism, the zebra mussel *Dreissena polymorpha*, after exposure with extracorporeal shock waves (ESWs). Mussels were exposed to ESWs with positive energy density of 0.4 mJ/mm² or were sham exposed. Detection of newly calcified tissue was performed by concomitantly exposing the mussels to fluorescent markers. Two weeks later, the fluorescence signal intensity of the valves was measured. Mussels exposed to ESWs showed a statistically significantly higher mean fluorescence signal intensity within the shell zone than mussels that were sham exposed. Additional acoustic measurements revealed that the increased mean fluorescence signal intensity within the shell of those mussels that were exposed to ESWs was independent of the size and position of the focal point of the ESWs. These data demonstrate that induction of bio-calcification after ESWT may not be restricted to the region of direct energy transfer of ESWs into calcified tissue. The results of the present study are of relevance for better understanding of the molecular and cellular mechanisms that induce formation of new mineralized tissue after ESWT. Specifically, bio-calcification following ESWT may extend beyond the direct area of treatment.

**S10.5 INTERMITTENT TERIPARATIDE ENHANCED BONE STRENGTH OF FEMORAL NECK VIA CHANGES OF BONE MORPHOLOGY AND MICROARCHITECUTRE IN OVARIECTOMIZED RATS**

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Bone strength is influenced by bone quality besides its density. This study aimed to evaluate the effects of teriparatide on changes of bone strength as well as trabecular and cortical bone microstructures at femoral neck in female ovariectomized (OVX) rats. Eighteen female Wister rats were divided into three groups: the sham control, OVX and treatment (Tx) groups. All of them were sacrificed after 3-month intermittent teriparatide intervention in Tx group. All left femurs were removed and scanned using micro-CT and followed by mechanical test for each femoral neck. Regarding micro-CT, four trabecular parameters including bone volume fraction (BV/TV), trabecular thickness (TbTh), trabecular separation (TbSp), and trabecular number (TbN) and three cortical parameters including volumetric bone mineral density (vBMD), cortical cross-sectional area (CtAr) and cortical thickness (CtTh) were measured at femoral neck region. All data were analyzed and was presented as median ± SEM. The mean bone strength of femoral neck significantly decreased in OVX group when compared to the control group (p < 0.05) and was significantly restored in Tx group (p < 0.01). Regarding the trabecular parameters, the BV/TV and TbTh significantly decreased in OVX group while compare to Tx group. However, no significant difference was observed in TbSp and TbN between the groups. Regarding the cortical parameters, CtTh was significantly greater in Tx group than that in OVX group (p<0.01). As our findings, intermittent teriparatide can improve the deteriorated bone strength of femoral neck due to ovarian deficiency via changing both trabecular microarchitecture and cortical morphology.

**S10.6 PULSED ELECTROMAGNETIC FIELDS INCREASE OSTEOGENETIC COMMITMENT OF MSCs VIA THE mTOR PATHWAY: AN IN VITRO STUDY**

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Pulsed electromagnetic fields (PEMFs) have been considered a potential treatment modality for fracture healing. As bone fracture healing and osseointegration share the same biological events, the application of PEMF stimulation to facilitate the osseointegration process of orthopedic implants has been suggested. However, the mechanism of their action remains unclear. Mammalian target of rapamycin (mTOR) signaling may affect osteoblast proliferation and differentiation. This study aimed to assess the osteogenic differentiation of mesenchymal stem cells (MSCs) under PEMF stimulation and the potential involvement of mTOR signaling pathway in this process. PEMFs were generated by a novel miniaturized electromagnetic device (MED). Potential changes in the expression of mTOR pathway components, including receptors, ligands and nuclear target genes, and their correlation with osteogenic markers and transcription factors were analyzed. PEMF exposure increased cell proliferation, adhesion and osteogenic commitment of MSCs. Osteogenic-related genes were over-expressed following PEMF treatment. Our results confirm that PEMFs contribute to activation of the mTOR pathway via upregulation of the proteins AKT, MAP kinase, and RRAGA, suggesting that activation of the mTOR pathway is required for PEMF-stimulated osteogenic differentiation. In summary, the findings of the present study revealed that MED-generated PEMFs stimulate osteogenic differentiation and the maturation of the adipose tissue-derived MSCs via activation of the mTOR pathways. Even though further research is required to determine an optimal stimulation timing and flux density both in-vitro and in-vivo, this study results may serve as a source for an adjuvant therapy to improve orthopedic implant stability, longevity and enhance fracture healing.

S11.1 HUMAN AMNIOTIC MEMBRANE FOR NOVEL APPLICATIONS IN REGENERATIVE MEDICINE

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The human amniotic membrane (hAM) contains cells of stem cell characteristics with low immunogenicity and anti-inflammatory properties and has for centuries been applied in the clinics especially for ophthalmology and wound care. It has recently been shown to be promising for novel applications such as tissue engineering and regenerative medicine. Towards these novel applications, we have demonstrated the potential of hAM in toto to differentiate towards bone, cartilage, Schwann like cells and recently also a producer of surfactant. We have further investigated the relevance of the location of origin for the therapeutic potential of the membrane. We show that placental and reflected hAM differs distinctly in morphology and functional activity. The placental region has significantly higher mitochondrial activity, however lower levels of reactive oxygen species, which suggests that placental and reflected regions may have different potential for tissue regeneration. We have further investigated the suitability of hAM to support therapeutic cells and have improved its maintenance in vitro towards xeno-free conditions.

S11.2 DEVELOPMENT OF DECELLULARISED XENOGENEIC AND ALLOGENEIC BIOLOGICAL SCAFFOLDS FOR MUSCULOSKELETAL REPAIR

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The growth in the popularity of tissue engineering principles in the treatment of musculoskeletal disorders has been complemented greatly with research investment into tissue specific scaffolds. Biological scaffolds produced by means of decellularising native tissues have the advantage of providing the natural complex
hierarchical matrix and, in doing so, replicating the specific biomechanical and biological functions of the tissue in question. Decellularisation treatments are multi-faceted, vary considerably between different processes and may involve many lengthy treatment steps. Some of these bio-processes may cause undesirable structural changes to the extracellular matrix of tissues and, by association, their mechanical properties. Thus, it is of paramount importance to ensure that the properties of the scaffolds are not affected to the extent of reducing their integration, biomechanical performance and longevity. This talk consists of a body of work detailing investigations into bio-process optimisation, sterilisation strategies and the regenerative and functional capacity of decellularised xenogeneic and allogeneic tendon, ligament and bone scaffolds. In addition, on-going work concerning advanced pre-clinical assessment, stratification of these products to particular patient populations and the importance of the manufacturing value chain in their translation will be discussed.

**S11.3 GENERATION OF BONE MARROW MESENCHYMAL STROMAL CELL-DERIVED ECM FOR THERAPEUTIC APPLICATIONS AND BEYOND**

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Mesenchymal stem cells (MSC) have a well recognised potential for tissue repair. This potential is two pronged: they can differentiate into the functional cell types of the damaged tissues and they can support tissue recovery by secreting trophic factors, depositing an extracellular matrix (ECM) and dampening inflammation. Three-dimensional microscopy recently shown that MSCs in the bone marrow create an intricate proteo-cellular scaffold with the ECM forming an interconnected cellular continuum whose structure is guided by the deposited ECM. This proteo-cellular scaffold controls bone marrow functions from hematopoiesis to osteogenesis. In the current study we aimed to optimise ECM production under *in vitro* conditions by immortalised MSCs with the view that the generated ECM can be utilised for tissue repair. With immunocytochemistry we determined the deposition of bone marrow-characteristic ECM proteins: collagen I, III, IV, V, VI, laminin and fibronectin. While primary MSCs produced slightly higher amount ECM proteins than immortalised MSCs, the relative abundancy of the ECM proteins was very similar. In order to isolate the ECM, we optimised a decellularisation method based on gentle lysis with sodium-deoxycholate and DNase digestion. Immunostaining for collagen I, III, VI and fibronectin and labelling the nuclei with Hoechst-33342 confirmed removal of all cells while retaining the ECM in its original architecture. Ideally, the decellularised ECM retains associated cytokines and chemokines, such as CXCL12, important for attracting MSCs. To test this, we seeded Molm-13 leukemia cells on decellularised ECM as MSC-produced CXCL12- and other cytokines protect leukemia cells against chemotherapeutics. We found that the decellularisation process however removed these factors and thus for therapeutic purposes, the decellularised ECM would need to be re-loaded with the essential chemo/cytokines. Overall, we developed a system for decellularised ECM production by immortalised MSCs and the results warrant further exploration of this avenue.

**S11.4 EFFICIENT DECELLULARISATION OF EXTRACELLULAR MATRIX RICH CELL-DERIVED MATRICES**

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Macromolecular crowding (MMC) accelerates matrix deposition through excluded volume effect (EVE). Herein, we ventured to identify the optimal decellularisation protocol of MMC enhanced fibroblast cultures as a new cell formed platform model. Human dermal fibroblasts (hDF), human lung fibroblasts (hLF), and human mammary fibroblasts (hMF) seeded at 50,000 cells/cm² were cultured for 10 days without and with MMC (100 μg/mL carrageenan) and 100 μM L-ascorbic acid phosphate. Subsequently, the cultured cell layers were decellularised using various decellularisation protocols [i.e., ammonium hydroxide (NH₄OH), sodium deoxycholate (DOC), SDS-EDTA mixed buffer, and nonident P40 (NP40)]. SDS-PAGE, hydroxyproline assay, sGAG assay, SEM, histological staining (i.e., picrosirius red stain and H&E), immunocytochemistry (i.e., collagen I, III and fibronectin), PicoGreen® assay. SDS-PAGE with complementary density and hydroxyproline analysis for assessing collagen deposition, and sGAG assay for total sGAG content assessment demonstrated significantly increased ($p<0.001$) in the presence of MMC. SEM, histological and immunocytochemistry displayed enhanced ECM deposition, integrity, and maintenance of the matrix composition in the presence of MMC. PicoGreen® assay revealed efficient decellularisation with significant removal of DNA ($p<0.001$) in all matrices. MMC can be used effectively to accelerate ECM deposition by fibroblast from various tissue sources, to facilitate production of cell-derived matrix-rich constructs feasible as robust platform models.

S12.1 CELL THERAPY FOR IMMUNE MODULATION IN OSTEOARTHRITIS

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Inflammation has been associated with early degradative changes in articular cartilage and immune responses are key factor influencing normal tissue regeneration and repair. With synovitis a prominent feature in osteoarthritis (OA) and associated with the progressive degradation of articular cartilage, immune factors need to be factored into efforts to achieve efficient cartilage repair/regeneration. Recent efforts have focused on the use of autologous or allogeneic mesenchymal stem/stromal cells (MSCs) to modulate the inflammatory environment in the injured or osteoarthritic joint. Intraarticular injection of MSCs has modulated cartilage degradation in a variety of pre-clinical OA models. Results from early clinical trials have also shown effects on pain and function-associated outcome measures. Other cell types may also have some capacity for use as a therapy for OA. For example, primary allogeneic chondrocytes also seem to have some immune-privilege in the synovial joint and are immunomodulatory in a rat model. Although MSCs isolated from bone marrow that are induced to undergo chondrogenic differentiation do not retain these properties, MSCs isolated from the synovium or chondroprogenitors generated from cartilage itself may represent the future of cell therapy for OA.

S12.2 DONOR-HOST INTERACTIONS IN BONE TISSUE ENGINEERING: THE ROLE OF THE IMMUNE SYSTEM IN ENDOCHONDRAL OSSIFICATION

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Harnessing the potential of mesenchymal stem cell (MSC) mediated endochondral ossification for the repair of large bone defects represents a promising avenue of investigation as an alternative option to autologous
bone transplantation. To date, it has been shown that undifferentiated MSCs are somewhat immune-privileged. In order to induce bone formation from MSCs by endochondral ossification, it is usually necessary to first differentiate these cells chondrogenically. However, the status of differentiated cells is less clear than that of undifferentiated MSCs. Furthermore, the fate of implanted bone forming constructs in an allogeneic setting is not known. The potential to use allogeneic MSCs for large bone defect repair would offer opportunities to researchers to develop new therapies using more potent MSC sources and in a more readily available manner with regard to the patient. I will present our research investigating the interactions between chondrogenically primed MSCs and immune cell subsets, namely T cells and dendritic cells. Furthermore, I will discuss the ability of human paediatric MSCs to form bone in the in vivo allogeneic setting.

S12.3 CHRONOLOGICAL VERSUS BIOLOGICAL AGING: EXPERIENCE IN THE ADAPTIVE IMMUNITY IMPACTS BONE HOMEOSTASIS AND REGENERATION

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Industrialized countries experience a population aging. Elderly patients, due to the experienced immunity, have a constant pro-inflammatory milieu. Little is known on how adaptive immunity impacts the tissue homeostasis and regeneration. The standardized housing of lab animals is specific pathogen free (SPF). However, this housing condition hinders antigen exposure and thus an aging of the adaptive immune system. We hypothesized that exposure to antigens and a developing adaptive immunity will impact tissue homeostasis and regeneration in mice. Mice kept under SPF housing or non-SPF were examined towards their immune status via flow cytometry, bone structure via microCT and bone competence via biomechanical torsional testing. MSCs from these mice were analyzed regarding their differentiation potential and ECM production under various immune cell signaling. Bone regeneration was analyzed in vivo in a mouse osteotomy model. The memory and effector compartment of the adaptive immunity was significantly increased in mice under non-SPF housing. This housing led to an increased femoral cortical thickness and torsional stiffness (p<0.05), whereas the tissue mineral density was not affected. The differentiation potential of stem cells under the influence of an aged immune milieu was significantly reduced. Bone formation was highly affected by the immune status and availed of a naïve immune cell milieu. Adaptive immunity directly impacts bone tissue formation, by exhibiting a constant stress, leading to structural differences in bone tissue organization as well as mechanical competence. For experimental settings, it appears highly relevant if mouse models have had the chance to develop an experienced immune system.

S12.4 MACROPHAGE POLARISATION AND IMMUNO-MODULATION BY MURINE MESENCHYMAL STEM CELLS

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Human synovium harbours macrophages and T-cells that secrete inflammatory cytokines, stimulating chondrocytes to release proteinases like aggrecanases and matrix metalloproteinases (MMPs) during the development of Osteoarthritis (OA). Inflammation of the synovium is a key feature of OA, linked to several clinical symptoms and the disease progression. As a prelude to testing in an OA mouse model, we have used the tetracycline system (Tet) to modify mouse mesenchymal stem cells (mMSCs) to over-express viral
interleukin 10 (vIL10), an anti-inflammatory cytokine, to modulate the osteoarthritic environment and prevent disease development. MSCs isolated from the marrow of C57BL/6J mice expressed CD90.2, SCA-1, CD105, CD140a, and were negative for CD34, CD45 and CD11b by flow cytometry. Adenoviral transduction of MSCs carrying CMVIL10 and TetON as test, and untransduced, AdNull and TetOFF as negative controls was successful and tightly controlled vIL10 production was demonstrated by CMVIL10 and TetON MSCs using a vIL10 ELISA kit. Co-incubation of vIL10MSC CM with lipopolysaccharide activated bone-marrow derived murine macrophages (BMDMs) resulted in reduction of TNF-α, IL-6 levels and elevated production of IL-10 by ELISA and high iNOS release by Griess assay. Co-culture of active macrophages with TetON MSCs, resulted in polarisation of macrophage cell population from M1 to M2 phase, with decrease in pro-inflammatory MHC-II (M1 marker) and increase in regulatory CD206 (M2 marker) expression over time. The PCR profiler array on MSC CM treated BMDMs, also showed changes in gene expression of critical pro-inflammatory cytokines and receptors involved in the TLR4 pathway. The bicistronic TetON transduced MSCs proved to be most immuno-suppressive and therefore feasible as efficient anti-inflammatory therapy that can utilised in vivo.

S12.5 IMPROVED BONE FRACTURE HEALING BY CD4+ REGULATORY T CELLS IS STRICTLY DEPENDENT ON INDIVIDUAL EFFECTOR/REGULATORY T CELL RATIO

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Recently, we could illustrate how tightly the bone and the immune system are interconnected during normal homeostasis but even stronger during bone regeneration. Specifically, the patient’s individual ratio of CD8+ effector T cells (TEFF, already identified as potential unfavorable cells for successful healing) to CD4+ regulatory T cells (TREG, one counterpart to CD8+ TEFF in controlling intratissue inflammation) prior to injury/surgery appears to determine the healing outcome after fracture. We hypothesized that concentrating CD4+ TREG could serve as innovative therapeutic strategy to improve bone healing. We used an adoptive CD4+ TREG transfer in our well-established mouse osteotomy model. Before treatment, we identified the presurgery ratio of CD8+ TEFF/CD4+ TREG by flow cytometry to characterize the healing potential of individual animals. Thereafter, we performed an adoptive CD4+ TREG transfer to reshape inflammation for supporting osteotomy healing. Across all groups, healing outcome was analyzed after 21 days post-surgery by μCT. Whereas TREG were highly supportive in SPF mice, we observed a heterogeneous clustered healing outcome in the non-SPF mice: TREG responder (improved healing outcome; p = 0.038) and TREG non-responder (impaired healing outcome; p = 0.024). Interestingly, the pre-/peri-surgery ratio of CD8+ TEFF/CD4+ TREG was higher in the TREG non-responder (p=0.057). Thus, the amount of adoptively transferred CD4+ TREG was not sufficient to improve the healing outcome due to initial unfavorable high CD8+ TEFF/CD4+ TREG ratio. These results clearly show the importance of determining the individual immune status of each patient in the clinic before applying an immunotherapeutic approach.

S12.6 IN VITRO INFLAMMATORY RESPONSE EVALUATION OF PRE-DEGRATED BIRESORBABLE POLYMERS USED IN TRAUMA FIXATION AND TISSUE REGENERATION APPLICATIONS

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Research in orthopaedics is now moving away from permanent metallic implants, and looking towards the use of bioresorbable polymers (e.g. PLLA, PGA and related co-polymers) that, when implanted into the injured site, bioreabs as the tissue heals. However, reports of a delayed inflammatory response occurring in the late stages of polymer degradation has limited the wide scale use of these polymers. Few studies assess the long-term biocompatibility of these polymers and with an increasing market for bioresorbable materials it is anticipated that this will be a future issue. This work aims to develop a predictive tool that can be used to assess the delayed inflammatory response of poly(D,L-lactide-co-glycolide) (PDLGA) using in vitro tests. An elevated temperature accelerated test (47°C) was developed and utilised to induce predetermined amounts of degradation in PDLGA. This was used to mimic a range of clinically relevant in vivo implantation times up to 5-6 months. All pre-degradation work was performed under sterile conditions, in PBS solution. At predetermined time intervals, indicators of late stage inflammation will be assessed using an MTT cytotoxicity assay, an inflammation antibody array and an ELISA analysis for inflammatory factors, with mouse L929 fibroblasts, RAW264.7 and primary BMDM macrophages. It is hypothesised that at the later degradation time intervals signs of inflammatory factors will be observed. The methodologies developed in this work can be applied to the optimisation of polymer degradation profiles to minimise late-stage inflammatory repsonse and identification of beneficial additives in this regard.

S13.1 BIOLOGICAL ACTIVITY OF WEAR PARTICLES IN VIVO

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Improvements in arthroplasty design and materials led to superior lifetime of the implants. Nevertheless, aseptic loosening due to particulate debris is still one of the most frequent late reasons for revision of hip and knee replacements. The complex process of inflammation and osteolysis due to wear particles is not understood in detail so far. A cellular and receptor mediated response to wear particles results in a release of pro-inflammatory cytokines and induces an inflammatory reaction causing periprosthetic osteolysis. The overall cellular response is influenced by particle volume as well as characteristics. But there is still a lack of data concerning all signalling pathways that are involved. To answer some open questions appropriate in vivo models are shown closing the loop between wear simulation, particle analysis, generation of sterile particles and biological evaluation. Beyond that, new aspects of particle effects and deposits in retrieved human tissue are given.

S13.2 TRIBOLOGICAL STUDIES OF JOINT REPLACEMENTS

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Total joint replacement is a successful clinical intervention. However, aseptic loosening due to wear related particulate debris is still one of the most frequent reasons for late revision of total joint replacement. This lecture gives an overview about the application of methods to study wear and friction in total joint replacements (e.g. hip, knee, shoulder). This involves complex joint simulation conditions as well as analytical assessments. Regarding joint simulation the focus will be on ligament stabilized joints. New approaches will be shown and discussed. Furthermore, analytical methods to study the release of wear products in term of solid particles and soluble complexes like metal ions will be presented.
S13.3 FEASIBILITY OF PRESSURE MAT ANALYSES OF SIMPLE CLINICAL TESTS IN DETECTING INSTABILITY IN TOTAL KNEE ARTHROPLASTY: A PROOF OF CONCEPT EXAMINATION

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Instability accounts for approximately 20% of all revision total knee arthroplasty (TKA), however diagnostic tests remain crude and subjective. The aim of this examination was to evaluate the feasibility of pressure mat (SB Mat, TekScan) analyses of functional tasks to differentiate instability in a clinical setting. Five patients (M = 4; age = 69.80±7.05 years; weight = 79.73±20.12 kg) with suspected TKA instability were examined compared to five healthy controls (M = 1; age = 46.80±7.85 years; weight = 71.54±16.17 kg). Peak pressure and time parameters were measured during normal gait and two-minute bilateral stance. Side-to-side pressure distribution was calculated over 10-second intervals during the second minute. Pressure distributions were expressed relative to bodyweight (%BW). T-tests compared loading parameters between groups (significance level = \( p<0.05 \)). Analyses showed subtle differences in pressure distribution in unstable TKA patients versus healthy controls. Stance time during gait was indifferent. TKA patients tended to exhibit longer heel contact time (0.76 vs. 0.64 sec) and reduced weight acceptance (50.75% vs. 56.75%) on the operated versus non-operated limb. Side-to-side differences in toe-off forces were significantly more pronounced in TKA patients versus controls (9.25% vs. 3.75%; \( p=0.0088 \)). Uneven loading was significantly greater – favouring the non-operated limb – in TKA patients during bilateral stance compared to controls (\( p<0.05 \)). This feasibility work demonstrates subtle differences in limb loading and biomechanics during simple clinical tests in unstable TKA patients that might be undetectable to the naked eye. Pressure analyses may therefore be a useful diagnostic tool. These findings warrant further investigation.

S13.4 BIOTRIBOLOGICAL EVALUATION OF METAL ION RELEASE OF COBALT-CHROMIUM-MOLYBDENUM AND OF ZIRCONIUM NITRIDE MULTILAYER COATED KNEE IMPLANT: AN INTER-LABORATORY COMPARISON


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Total knee arthroplasty is a well-established treatment for degenerative joint disease, on the other hand metal ion release of cobalt or chromium and particle formation can trigger intolerance reactions. Biotribological examinations can help to assess the metal ion release in different settings. The purpose of this study was the evaluation of inter-laboratory differences in the metal ion concentration analysis. Samples were generated in a 3+1 station knee wear simulator (EndoLab GmbH, Thansau, Germany) with a medium size Columbus Knee System with or without AS multilayer coating. The wear simulation was performed under highly demanding activity (HDA) profile and samples were taken after 0.5, 5.0, 5.5. and 8.0 million cycles. The samples were blinded and sent to three different laboratories and the content of chromium, cobalt, molybdenum, nickel, and zirconium was assessed by inductively coupled plasma mass spectrometry (ICP-MS). The AS multilayer coating clearly reduced the release of chromium, cobalt and molybdenum. Mean levels were: Chromium 9329.78μg/l ± 985.44 vs 503.75μg/l ± 54.19, cobalt 10419.00μg/l ± 15.517.53 vs 2.60μg/l ± 1.35, molybdenum 2496.33μg/l ± 102.62 vs 2.46μg/l ± 2.31. Interestingly we found especially for nickel and zirconium big inter-laboratory differences in the metal assessment. There were up to 10-fold higher values in comparison of one
laboratory to another. The data demonstrate that results of metal ion assessment should be evaluated by interlaboratory comparison and should be critically interpreted.

**S13.5 THE LEVER ARM RATIO OF THE ROTATOR CUFF AND THE DELTOID MUSCLE IS RELATED TO THE DEVELOPMENT OF PSEUDOPARALYSIS OF THE SHOULDER – A COMBINED BIOMECHANICAL AND RADIOGRAPHIC ANALYSIS OF THE SHOULDER ABDUCTION MOMENT INDEX**

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Tear pattern and tendon involvement are risk factors for the development of a pseudoparalytic shoulder. However, some patients have similar tendon involvement but significantly different active forward flexion. In these cases, it remains unclear why some patients suffer from pseudoparalysis and others with the same tear pattern show good active range of motion. Moment arms (MA) and force vectors of the RC and the deltoid muscle play an important role in the muscular equilibrium to stabilize the glenohumeral joint. Biomechanical and clinical analyses were conducted calculating different MA-ratios of the RC and the deltoid muscle using computer rigid body simulation and a retrospective radiographic investigation of two cohorts with and without pseudoparalysis and massive RC tears. Idealized MAs were represented by two spheres concentric to the joints centre of rotation either spanning to the humeral head or deltoid origin of the acromion. Individual ratios of the RC /deltoid MAs on antero-posterior radiographs using the newly introduced Shoulder Abduction Moment (SAM) Index was compared between the pseudoparalytic and non-pseudoparalytic patients.

Decrease of RC activity and improved glenohumeral stability (+14%) was found in simulations for MA ratios with larger diameters of the humeral head which also were consequently beneficial for the (remaining) RC. Clinical investigation of the MA-ratio showed significant risk of having pseudoparalysis in patients with massive tears and a SAM Index <0.77 (OR=11). The SAM index, representing individual biomechanical characteristics of shoulder morphology has an impact on the presence or absence of pseudoparalysis in shoulders with massive RC tears.

**S13.6 NEW HYDROPHILIC COATINGS TO IMPROVE WEAR IN BEARING SURFACES FOR JOINT PROSTHESIS**

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In 2011, approximately 1.6 million total hip arthroplasties (THAs) were conducted in 27 of the 34 member countries in the Organization for Economic Cooperation and Development (OECD) However, approximately 10–15% of patients still require revision surgery every year. Therefore, new technologies are required to increase the life-span of the prosthesis from the current 10-15 years to at least 20-30 years. Our strategy focuses on surface modification of the bearing materials with a hydrophilic coating to improve their wear behaviour. These coatings are biocompatible, with high swelling capacity and antifouling properties, mimicking the properties of natural cartilage, i.e. wear resistance with permanent hydrated layer that prevents prosthesis damage. Clear beneficial advantages of this coating have been demonstrated in different conditions and different materials, such as UHMWPE, PEEK, CrCo, Stainless steel, ZTA and Alumina. Using routine tribological experiments, the wear for UHMWPE substrate was decreased by 75% against alumina, ZTA and stainless steel. For PEEK-CFR substrate coated, the amount of material lost against ZTA and CrCo was at least
40% lower. Further experiments on hip simulator adding abrasive particles (1-micron sized aluminium particles) during 3 million cycles, on a total of 6 million, showed a wear decrease of around 55% compared to uncoated UHMWPE and XLPE. In conclusion, CIDETEC’s coating technology is versatile and can be adapted to protect and improve the tribological properties of different types of surfaces used for prosthesis, even in abrasive conditions.

**S14.1 EFFECTIVENESS OF NEUROMUSCULAR ELECTRICAL STIMULATION (NMES) IN ASSISTING FUNCTIONAL RECOVERY FOLLOWING TOTAL KNEE ARTHROPLASTY**

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Total knee arthroplasty (TKA) is becoming more prevalent as the average age of the general population increases and is generally considered to be a very effective and successful surgery. However, functional recovery post-surgery can often be less than optimal. NMES is a beneficial therapy proven to improve haemodynamics and muscle strength and may be of great benefit in improving functional recovery in the acute phase post-TKA. The objective of the study was to assess functional recovery in the period immediately following TKA and hospital discharge in response to a home-based NMES programme. Twenty-six TKA patients were randomized into a NMES stimulation or placebo-controlled group. All participants were given a research muscle stimulator to use at home post-discharge for 90 minutes per day over a period of 5 weeks. In the stimulation group, application of stimulation resulted in an electrically activated contraction of the soleus muscle. Patients in the placebo-controlled group received sensory stimulation only. Outcome measures were physical activity levels, joint range of motion and lower limb swelling, which were measured pre-surgery and on a weekly basis post-discharge up until the sixth post-surgical week. 90 minutes per day NMES stimulation significantly increased the Activity Time (P = 0.029 week 1 post-discharge) and the number of Stepping Bouts (P < 0.05 weeks 1 to 4 post-discharge) in the early post-discharge phase. While there was a trend towards a greater knee flexion with use of NMES, this did not reach statistical significance (P = 0.722). No effect of NMES was observed on swelling (P > 0.05 for all measures). Compliance to the NMES therapy was measured by an on-board SIM card in the NMES device, with a 95% and 94% time compliance rate for the stimulation and placebo-controlled groups respectively. The results of this study suggest that NMES may be very useful in improving functional recovery through increasing physical activity levels in the early post-TKA discharge phase. The results of this study warrant further investigation into the use of an optimized NMES protocol whereby improvements in knee range of motion and swelling may also be observed.

**S14.2 MOBILITY PARAMETERS FROM ACTIVITY MONITORS FOR ORTHOPAEDIC OUTCOME ASSESSMENT**

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The relevance of physical activity (PA) for general health and the value of assessing PA in the free-living environment especially for assessing orthopaedic conditions and outcome are discussed. Available methods for assessing PA such as self-reports, trackers, phone apps and clinical grade monitors are introduced. An overview of devices such as accelerometers for research quality assessments is given and aspects for choosing
them such as wear location, usability or study population are reviewed. Basic principles to derive mobility parameters from the PA related sensor signals are presented. The symposium explains mobility parameters, their types, definitions, validity, analysis and those with particular relevance to assess orthopaedic conditions. The application of activity monitors is orthopaedic patient studies is demonstrated in various examples such as knee and hop osteoarthritis and total joint arthroplasty, in frail elderly subjects at fall risk or patients with shoulder pathologies.

**S14.3 A NOVEL MICRONEEDLE-BASED PLATFORM THAT ACHIEVES REPEATABLE INSERTION AND ROBUST ANCHORAGE TO SOFT TISSUE**

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Efficient, repeatable and reliable insertion of microneedles into skin is paramount to ensure efficacious drug and vaccine delivery, as well as effective microneedle-based biosensing. Through maintaining robust mechanical adhesion, this microneedle platform offers significant potential in therapeutic delivery and longitudinal wearable applications. Here, we have shown that an angled microneedle design, which is conducive to self-administration, has the potential to address key limitations in existing microneedle technology.

**S14.4 DYNAMIC 3D JOINT ANGLE MEASUREMENT USING INERTIAL SENSORS**

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3D measurement of joint angles so far has only been possible using marker-based movement analysis, and therefore has not been applied in (larger scale) clinical practice (performance test) and even less so in the free field (activity monitoring). 3D joint angles could provide useful additional information in assessing the risk of anterior cruciate ligament injury using a vertical drop jump or in assessing knee range of motion after total knee arthroplasty. We developed a tool to measure dynamic 3D joint angles using 6 inertial sensors, attached to left and right shank, thigh and pelvis. The same sensors have been used for activity identification in a previous study. To validate the setup in a pilot study, we measured 3D knee and hip angles using the sensors and a Vicon movement lab simultaneously in 3 subjects. Subjects performed drop jumps, squats and ran on the spot. The mean error between Vicon and sensor measurement for the maximum joint angles was 3, 7 and 8 degrees for knee flexion, ad/abduction and rotation respectively, and 9, 7 and 10 degrees for hip flexion, ad/abduction and rotation respectively. No calibration movements were required. A major part of the inaccuracy was caused by soft tissue effects and can partly be resolved by improved sensor attachment. These pilot results show that it is feasible to measure 3D joint angles continuously using unobtrusive light-weight sensors. No movement lab is necessary and therefore the measurements can be done in a free field setting, e.g. at home or during training at a sport club. A more extensive validation study will be performed in the near future.

**S15.1 NEW DEVELOPMENTS IN BIOLOGIC APPROACHES TO ARTICULAR CARTILAGE REGENERATION**

S. Chubinskaya
In least 12% of patients with symptomatic OA, the cause is joint injury that progressed over time to post-traumatic OA. Human adult articular cartilage has a limited innate ability to regenerate. Available treatment options are unable to restore native structure and function of hyaline cartilage. Agili-C (CartiHeal, Israel) is a first-in-class acellular scaffold consisted of two layers corresponding to cartilage and bone that is capable of attracting stem cells and guide a regenerative process in both tissues. Agili-C has been extensively tested in vitro in our laboratory using human normal cartilage and in vivo in preclinical and currently clinical studies. This scaffold consists of a natural crystalline aragonite, derived from corals, to which hyaluronic acid is added. It showed a great ability to induce regeneration of chondral and osteochondral lesions and attract chondrocytes and stems cells to fill the defect area. Cells remained viable over the course of the study (up to 2 months). Signs of the extracellular matrix formation were evident inside 3D structure of the scaffold. PG synthesis and gene expression of collagen type II and aggrecan were elevated by more than 2.5-fold in cartilage with the scaffold vs corresponding controls. Agili-C scaffold displays a potential in the treatment of focal chondral and osteochondral defects.

S15.2 OSTEOARTHRITIS: PHENOTYPES AND IMMUNOMETABOLIC ALTERATIONS

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For many decades, we have viewed osteoarthritis (OA) as a homogeneous disease characterised by “wear and tear”. However, this view has been challenged recently and it is now clear that OA is a heterogeneous and low-grade inflammatory disease with multiple aetiologies and phenotypes. Each of these different phenotypes may be identified and targeted differently, opening up multiple pathways for therapeutic intervention. Combining imaging and carefully selected panels of biochemical markers can achieve enhanced patient stratification and lead to better-designed clinical trials. Analyses of observational studies and clinical trial datasets are underway to understand better the phenotypes responsible for why people develop OA and why, prognostically, they have differences in terms of disease progression. The aim of this presentation is to discuss the underlying mechanisms involved in common OA phenotypes, with a particular focus on low-grade inflammation and metabolic alterations. Aberrant cellular metabolism has been implicated in the pathogenesis of OA and this talk will summarise the current state of knowledge on the role of impaired metabolism in the cells of the osteoarthritic joint and highlight areas for future research, such as the potential to target metabolic pathways and mediators therapeutically.

S15.3 THE LOCATION OF TIBIAL OSTEOPHYTES IS PATIENT-SPECIFIC IN SEVERE OSTEOARTHRITIS

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While osteophytes are a hallmark feature of knee osteoarthritis (OA), there is limited information regarding their location. In particular, it is unknown whether osteophytes develop in patient-specific locations or if there are consistent osteophyte locations among OA knees. This lack of data mainly stems from the fact that
osteoophytes have been mostly assessed with scores quantifying their size or severity but not their location. Given the important role that bone could play in OA development and the option it offers for OA treatment, there is a need to better understand the osteophyte locations. This study aimed to develop a method to compare osteophyte locations among knees and determine the overlapping ratio. CT arthrogram of 11 medial-compartment OA tibias (Kellgren-Lawrence grade ≥ 3) were segmented to locate the osteophytes and a bone matching technique was used to report the osteophyte locations of the 11 knees on a single reference tibia. This newly proposed method was highly reproducible (intra-operator ICC = 0.89). When used to compare the 11 tibias, it showed that more than 60% of the overall subosteophytal area, defined as the reference bone area covered by at least one osteophyte from one knee, was common to less than two tibias. Moreover, less than 20% of the overall subosteophytal area was common to five or more tibias. The results of this study suggest that osteophyte locations are specific to each knee. Future work should determine the relationships with mechanical loading, as this could explain the high inter-patient variability.

S15.4 L2 BONE QUALITY IN OSTEOPOROSIS: BIOMED 1 REVISITED.

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As a part of the European Union BIOMED I study “Assessment of Bone Quality in Osteoporosis,” Sixty-nine second lumbar vertebral body specimens (L2) were obtained post mortem from 32 women and 37 men (age 24–92 years). Our initial remit was to study variations in density of the calcified tissues by quantitative backscattered electron imaging (BSE-SEM). To this end, the para-sagittal bone slices were embedded in PMMA and block surfaces micro-milled and carbon coated. Many samples were re-polished to remove the carbon coat and stained with iodine vapour to permit simultaneous BSE imaging of non-mineralised tissues - especially disc, annulus, cartilage and ligament - uncoated, at 50Pa chamber pressure. We have now studied most of these samples by 30-μm resolution high contrast resolution X-ray microtomography (XMT), typically 72 hours scanning time, thus giving exact correlation between high resolution BSE-SEM and XMT. The 3D XMT data sets were rendered using Drishti software to produce static and movie images for visualisation and edification. We have now selected a set of the female samples for reconstruction by 3D printing - taking as examples the youngest, post-menopausal, oldest, best, worst, and anterior and central compression fractures and anterior collapse with fusion to L3 - which will be attached to the poster display. The most porotic cases were also the most difficult to reconstruct. A surprising proportion of elderly samples showed excellent bone architecture, though with retention of fewer, but more massive, load-bearing trabeculae.

S15.5 OSTEOPOROSIS INFLUENCE ON STRUCTURAL AND MECHANICAL PROPERTIES OF HUMAN HUMERAL HEADS

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Osteoporosis is a worldwide disease with a high prevalence in elderly population; it results in bone loss and decreased bone strength that lead to low-energy fractures. Since antiresorptive treatments could lead to long-term adverse effects, the ERC BOOST project aims to propose a biomimetic 3D-printed scaffold reproducing the architecture and chemistry of healthy bone. In this study, the structural parameters of healthy bone were studied in order to reproduce them through 3D printing; furthermore, structural and mechanical differences between healthy and osteoporotic (OP) bones were assessed. Healthy and OP humeral heads discarded during
surgical interventions (following ethical approval by Istituto Ortopedico Rizzoli-Italy) were tomographically analysed to obtain bone structural parameters. Successively, 8 mm diameter biopsies were harvested from the heads and underwent compression and nanoindentation tests to investigate macroscopic and microscopic mechanical properties, respectively. XRD measurements were performed on bone fragments. OP bone samples exhibited inferior mechanical properties to their less interconnected and more anisotropic structure, with thinner trabeculae and larger pores. On the other hand, nanoindentations performed on OP trabeculae showed increased Young Modulus compared to healthy samples probably due to their increased hydroxyapatite crystal size, as revealed by XRD. Osteoporosis causes the weakening of the trabecular structure that leads to a decrease of bone mechanical properties. However, OP trabeculae are stiffer due to increased dimensions of hydroxyapatite crystals.

**S15.6 THE ROLE OF INTEGRIN αvβ3 IN OSTEOCYTE MECHANOTRANSDUCTION DURING ESTROGEN DEFICIENCY**

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The expression of the mechanosensor, integrin αvβ3, is reduced in osteoporotic bone cells compared to controls. MLO-Y4 osteocytes experience altered mechanotransduction under estrogen deficiency and it is unknown whether this is associated with defective αvβ3 expression or signalling. The objectives of this study are to (1) investigate αvβ3 expression and spatial organisation in osteocytes during estrogen deficiency, and (2) establish whether altered responses of osteocytes under estrogen deficiency correlate to defective αvβ3 expression and functionality. MLO-Y4 cells were cultured as follows: Ctrl (no added estradiol), E+ (10nM 17β-estradiol for 5 days), and Ew (10nM 17β-estradiol for 3 days and withdrawal for 2 days). Cells were cultured with/without 0.5μM IntegriSense750 (αvβ3 antagonist). Laminar oscillatory fluid flow of 1Pa at 0.5Hz was applied for 1hr. αvβ3 content was quantified using an ELISA. The location and quantity of αvβ3 and focal-adhesions was determined by immunocytochemistry. Estrogen withdrawal under static conditions led to lower cell and focal-adhesion area (p<0.05), compared to E+ cells. Fluid flow led to higher αvβ3 content (p<0.05) in all groups, compared to static counterparts, with αvβ3 blocking altering this response. Fluid flow on Ew cells had the highest αvβ3 levels (p<0.05), but αvβ3 did not localise at focal-adhesions sites. Cell morphologies were similar after treatment with the αvβ3 antagonist to the Ew group. These results suggest there are fewer functional focal-adhesion sites at which αvβ3 integrins localise to facilitate mechanotransduction. To further understand these results, we are analysing osteocyte mechanotransduction by quantifying PGE2 and gene expression (COX-2, RANKL, OPG, SOST).

**WS1.1 LEADERSHIP IN UNIVERSITIES IN THE 21st CENTURY: A PERSONAL VIEW**

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I still remember as a green 16-year-old being completely seduced by Newman’s portrait of a university – the ideal of a liberal education. I was completely charmed not only by Newman’s seductive prose – but by the humanising ideals of the effects of an excellent education. The picture was compelling and inspirational to the daughter of a small farmer whose parents were forced to leave school at 12 years of age to go and earn a living. I was sitting in the “lap of luxury” in a boarding school for girls, whose excellent principal generated a huge respect for, and absolute belief in, the right to and the ability to gain from a rigorous and serious education –
which for me at that time in the 1970s extended at least to the end of secondary schooling – a luxury no one in my family had access to in the previous generation. What are universities for? Many authors have considered this issue since Newman’s time – in recent times for example Boyd (1979), Graham (2005), Collini (2012). They all, in different ways suggests the need not only to respond to societal / economic needs, but also the need for a more balanced, holistic conception of university activity. Leaders of universities in the 21st century must try to articulate this, seek greater understanding of it. We must lobby government for greater recognition, understanding and support for the university’s role not only for the present but also for the future. Contingency, vulnerability, adaptability, recognising the provisional nature of knowledge (and control); the caring versus the careless – all of this implies the need for diversity of disciplines, gender and experiences among university leadership in both the national and the international arena.

**WS1.2 FEMALE LEADERSHIP IN STEM**

C. Spillane
Ireland

**WS1.3 THE VALUE OF DIVERSITY IN GROWING A COMPANY**

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The Electrospinning Company designs, develops and manufactures biomaterials for use in regenerative medical devices. Since 2012, Ann has led the growth of the company from start-up to supplier of innovative, clinical-grade product to an FDA-approved medical device, evolving the business model and adding capabilities in innovation, manufacturing, quality and alliance management. Ann will share some of the highs and lows of the journey from her perspective as a female leader of a diverse team.

**WS2.1 THE DESIGN, DEVELOPMENT, AND MANUFACTURE OF BIOMATERIALS: THE PRACTICE AND VALUE OF BIOMIMICRY**

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Biomimicry is defined as the design and production of materials, structures, and systems that are modelled on biological entities and processes. Within the medical device sector, biomimicry uses an ecological standard to judge the “rightness” of biomaterial components and devices. After 3.8 billion years of evolution, nature has learned what works, what is appropriate, and what lasts. Biomimicry is a new way of viewing and valuing nature, and it introduces an era based not on what we can extract from the natural world, but on what we can learn from it. Original design manufacturing biomaterial projects that leverage the practice of biomimicry will be discussed. Both natural and synthetic polymer platforms will be reviewed for soft tissue and hard tissue applications. Given the complexity of musculoskeletal tissue structures, the key challenge is identifying the most appropriate materials and forms for recapitulating the native function in a tissue scaffold design. The general field of biomimicry will be reviewed along with specific examples in the regenerative medicine sector.
WS2.2 DESIGNING A COMMERCIAL BIOMATERIAL FOR A SPECIFIC UNMET CLINICAL NEED – AN ADHESIVE ODYSSEY

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There are clinical situations in fracture repair, e.g. osteochondral fragments, where current implant hardware is insufficient. The proposition of an adhesive enabling fixation and healing has been considered but no successful candidate has emerged thus far. The many preclinical and few clinical attempts include fibrin glue, mussel adhesive and even “Kryptonite” (US bone void filler). The most promising recent attempts are based on phosphorylating amino acids, part of a common cellular adhesion mechanism linking mussels, caddis fly larvae, and mammals. Rapid high bond strength development in the wetted fatty environment of fractured bone, that is sustained during biological healing, is challenging to prove both safety and efficacy. Additionally, there are no “predicate” preclinical animal and human models which led the authors to develop novel evaluations for an adhesive candidate “OsStic™” based on calcium salts and amino acids. Adhesive formulations were evaluated in both soft (6/12 weeks) and hard tissue (3,7,10,14 & 42 days) safety studies in murine models. The feasibility of a novel adhesiveness test, initially proven in murine cadaver femoral bone, is being assessed in-vivo (3,7,10,14 & 42 days) in bilateral implantations with a standard tissue glue as the control. In parallel an ex-vivo human bone model using freshly harvested human donor bone is under development to underwrite the eventual clinical application of such an adhesive. This is part of a risk mitigation project bridging between laboratory biomaterial characterisation and a commercial biomaterial development where safety and effectiveness have to meet today’s new medical device requirements.

WS2.3 CAREER CASE: FROM CARTILAGE REGENERATION RESEARCHERS TO ENTREPRENEURS IN A MEDICAL DEVICE START-UP

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In 2009, a multidisciplinary team of orthopaedic surgeons, material scientists, and cell biologists created a consortium focused on developing novel biomaterials for cartilage regeneration. After years of hard work across scientific boundaries, the team discovered a solution that could benefit a large number of patients. However, the research team was faced with a question on how to proceed. Whether to continue the scientific path of unravelling the mysteries of cartilage regeneration or to focus on bringing the invention from bench to bedside? The latter would mean commercialisation of the invention, and for the scientists, taking a completely new career path. Taking this turn would mean risking the team members’ scientific career, since running a start-up would inevitably mean lesser publications and other scientific merits in the forthcoming years. On the other hand, there was the potential to help a vast amount of patients. The team decided that the invention, a biodegradable weight-adaptive medical device for cartilage regeneration, was too promising to be left aside, so they made the choice to transform from academic researchers to entrepreneurs. Thus, Askel Healthcare Ltd was founded in March 2017. For a start-up operating in medical device sector, the company has a unique feature: the founding team is all-female. Not intentionally, but by a mere “side effect” of gathering the best talents to get the job done. The team continues to foster its strong scientific background, which is a true asset for a company focusing on tackling the big unmet medical need of cartilage regeneration.

WS3.1 GMP MANUFACTURE OF MESENCHYMAL STROMAL CELLS FOR CLINICAL TRIALS
Mesenchymal Stromal Cells (MSC) have been proposed as a potential therapy for a broad range of diseases including those affecting the musculoskeletal system. MSCs have received market authorization for treatment of graft versus host disease and fistulizing Crohn’s disease. In addition, there are clinical trials underway for diseases affecting all organ systems. GMP manufactured cells are required for these clinical trials and suitable facilities with regulatory approval are thus crucial for the translational process. In this presentation I will review the process whereby such a facility has been constructed at NUI Galway and discuss challenges in operations and sustainability. Researchers at REMEDI and spin out company Orbsen Therapeutics are currently involved in 7 clinical trials using MSCs, 4 of which are EU wide consortia funded by the EU Commission. The presentation will also discuss issues such as source of MSCs, cell sorting, use of bioreactors and xeno-free processes.

WS3.2 IMMUNOGENICITY OF ALLOGENEIC MSC: MORE TO THE STORY

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Blood transfusion, organ and bone marrow transplantation and allogeneic tissue grafting create the potential for significant immunological challenges through the introduction of non-genetically identical major (HLA) and minor histocompatibility antigens (“allo-antigens”) into the body. Strategies to avoid the complications of immune responses against allo-antigens (transfusion reactions, rejection and graft versus host disease) include HLA matching, immunosuppressive therapies and immune tolerance promoting protocols. In the case of allogeneic mesenchymal stem/stromal cells (allo-MSC), it was initially believed that their combined properties of low HLA expression and inherent immune modulatory functions would render them invisible to the host immune system and, therefore, capable of being permanently accepted without further interventions. For clinical indications such as bone and tendon repair, in which permanent engraftment of allo-MSC or MSC-derived tissue constructs is particularly desirable, this model of “immune privilege” seemed almost too good to be true – and indeed, a decade of experimental research in this area has now convincingly demonstrated that allo-MSC typically elicit cellular (T-cell) and humoral (B-cell/antibody) immune responses in immunocompetent hosts – raising concern about their safety and long-term efficacy in human conditions. However, questions related to the immunogenicity of allo-MSC have evolved beyond a simple yes/no scenario to involve interesting observations and concepts about the potency, diversity, duration, functional characteristics and even potential clinical benefits of immunological responses to allo-MSC. In this presentation, I will summarise and critically evaluate current understanding of allo-MSC immunogenicity under experimental and clinical trial conditions with an emphasis on the implications for orthopaedic therapeutics.

WS3.3 WHICH IS BETTER FOR AUGMENTING INTRA-SYNOVIAL TENDON REPAIR – MESENCHYMAL STEM CELLS OR CELL-FREE SCAFFOLDS?

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Intra-synovial tendon injuries affect compressed tendon within a synovial environment (e.g., Rotator cuff tears of the shoulder) and frequently demonstrate ‘failed healing’. Current therapeutic methods for tendon tears (intra-synovial corticosteroid medication and surgical debridement) offer poor outcomes and new strategies for enhancing repair are needed. We have therefore evaluated two different approaches involving the use of mesenchymal stem cells and scaffolds. Bone marrow- and synovial-derived stem cells were capable of adhering to cut surfaces of tendon in vitro and modulating the release of extracellular matrix into the media. However, when administered in vivo into the digital flexor tendon sheath in naturally-occurring deep digital flexor tendon tears in horses and in an experimental model in sheep, neither cell type was capable of healing the tendon defect. Superparamagnetic iron oxide particle labelling of the implanted cells imaged using MRI and histologically revealed that cells only engraft into the synovium. In contrast a non-cellularised bilayered electrospun and woven polydioxanone scaffold, when used in the same experimental sheep model via a modified open approach and sutured over the created defect resulted in no local or systemic signs of excessive inflammation 3 months after implantation. All the tendon lesions healed with only a mild local inflammatory reaction and minimal-to-mild adhesion formation. Significant proliferative fibroblast infiltration was observed within and immediately adjacent to the implanted scaffold. The cellular infiltrate was accompanied by an extensive network of new blood vessel formation within the new tissue. In conclusion, the use of a scaffold to cover the defect appears to be a more successful strategy to repair intra-synovial tendon defects than intra-synovially injected mesenchymal stem cells. It remains to be tested whether the combination of the two techniques might offer an even better healing response.

WS4.1 INNOVATION IN OPEN ACCESS PUBLISHING

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This talk will initially give a brief overview of the motivations behind open access publishing and explain the practicalities of the different business models from an author’s point of view. The talk will then discuss open access policy, particularly in Europe, and how the publishing landscape is constantly changing, with new initiatives and mandates being introduced all the time. Innovation in peer review such as transparent peer review and registered reports will be outlined and evaluated with examples from the BMC journals portfolio. The talk will then explain some of the funding options available to authors for open access publishing, and introduce the Springer Nature funding support service, which is available to anyone wishing to find out their options. Finally, the importance of data sharing will be discussed, as will the relatively new area of open access books.

WS4.2 WRITING FOR IMPACT: GETTING YOUR RESEARCH INTO TOP TIER JOURNALS

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You have a great research question or an idea for an innovation that will change your field. You have worked tirelessly to develop the project and are excited with the outcome. Now it is time to disseminate your findings to the world. This talk will give some insight into how to maximise the impact of your writing to reach the largest possible audience. It will discuss what makes a great paper, and provide pointers for navigating the
editorial process, from your initial interactions with the editor to handling the sometimes-difficult process of peer review.

**WS4.3 THE RELIABILITY AND READABILITY OF ONLINE RESOURCES ON CONGENITAL TALIPES EQUINOVARUS**

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Congenital talipes equinovarus (CTEV), also known as club foot or talipes is a common congenital disorder. Parents are using the Internet more and more as a source of information about health care. Unfortunately, the quality of health care information on the Internet varies. This study looked at information available to parents using two instruments for judging the equality of information on the internet. The top five search engines were searched on Google. Three of these were also included in the top 50 sites in Ireland so these 3 sites were used. The phrases CTEV and club foot were searched from all 3 platforms. Websites were then scrutinized using the HON code and the DISCERN tool. 54 organic sites were found for the 3 search engines using the key word club foot. For the key word CTEV 55 matches were returned for the three search engines. 4 websites displayed the HON code. Using the discern tool CTEV websites had a mean score of 60 with a standard deviation of 17. While club foot had a mean score of 56.8 with a standard deviation of 13. Max score 80. Large volumes of information are available to parents on the Internet. Often parents find comfort in sharing experiences and feel empowered by learning about their children’s illnesses. However, information provided on the internet can also be ambiguous and disingenuous. Practitioners should be aware of a number of key websites that parents can be directed towards.

**WS4.4 INFOGRAPHICS – THE FUTURE OF RESEARCH DISSEMINATION**

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The widespread dissemination of high-quality research facilitates keeping up to date with evidence-based practice, but the vast quantity can be overwhelming to physicians and surgeons. Information graphics, abbreviated to infographics, convey information using visualisations and images in an engaging manner. This format of presenting research format is preferable to 80% of clinicians when compared to text articles, and the long-term retention of information has been shown to be improve by a factor of 6.5 when methods were compared. Journal club was audited in our institution over 3 months. A multi-choice questionnaire was constructed weekly so as to test the attendees’ recall of the research presented on a weekly basis. After five weeks, infographics were introduced, and the attendees’ recall was assessed again on a weekly basis at the end of each journal club. The introduction of infographics to journal club saw improved test results from the journal club attendees. Not only was information retention improved, but the duration of journal club reduced following the intervention. Research can be disseminated efficiently using infographics in place of conventional journal club presentations. Satisfaction rates among clinicians, both with information retention and journal club duration, demonstrate the benefit of their use in teaching hospitals.

**WS5.1 IDENTIFYING YOUR AUDIENCE**

A. Fitzpatrick
Tailoring your message to the people you are trying to engage will make it easier for them to listen, absorb and act on your message. Audiences can vary in a lot of ways such as gender, age, socio economic status, and ethnicity. Workshop attendees will learn how to identify specific audiences and deliver the topic of their research accordingly. This will help development of key messages to communicate research depending on what information would be interesting to the audience, the amount of science education the audience has, and how the audience will use the information afterwards.

**WS5.2 CREATING IMPACTFUL PUBLIC ENGAGEMENT**

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Being able to communicate what your research entails quickly and effectively will ensure that you get the most important points across in a limited amount of time. Workshop attendees will develop a concise, compelling introduction to their research that can be communicated in a short message. This skill is beneficial in multiple scenarios, particularly when introducing yourself in an interview or a networking event. Additionally, this will help develop more effective ways of communicating through social media, press releases and conversations with people who do not have a scientific background.
S16.1 ELASTIC BIOMATERIALS AND ACCELERATED BONE REPAIR

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Robust repair relies on blood flow. This vascularization is the major challenge faced by tissue engineering on the path to forming thick, implantable constructs. Without this vasculature, oxygen and nutrients cannot reach the cells located far from host blood vessels. To make viable constructs, tissue engineering takes advantage of the mechanical properties of synthetic materials, while combining them with extracellular matrix proteins to create a natural environment for the tissue-specific cells. Tropoelastin, the precursor of the elastin, is the extracellular matrix protein responsible for elasticity in diverse tissues, including robust blood vessels. We find that tropoelastin contributes a physical role in elasticity and also substantially to the biology of repairing tissue. The emerging model from a range of our in vivo studies is that tropoelastin encodes direct biological effects and has the versatility to promote repair. We have discovered that tropoelastin substantially improves healing by halving the time to repair bone in small animals and large animal preclinical models; tropoelastin elicits this response with early stage neo-angiogenesis, recruitment of endogenous cells with consistently accelerated repair. This potency is marked by the concerted appearance of blood vessels, tissue and phased cellular contributions that work together to accelerate repair.

S16.2 SYSTEMICALLY ADMINISTERED WOUND-HOMING PEPTIDE ACCELERATES WOUND HEALING BY ACTIVATING SYNDECAN-4 DEPENDANT CELL MIGRATION PATHWAY

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CAR (CARSKNKDC) is a systemically administered wound-homing peptide that specifically recognizes angiogenic blood vessels and extravasates into sites of injury. CAR peptide requires heparan sulfate proteoglycans (HSPGs) for its cell penetrating activity. Syndecan-4 (SDC4) is a HSPG and binding to it triggers the wound re-epithelialization process. We have discovered that CAR peptide has the inherent ability to promote wound healing; wounds close and re-epithelialize significantly faster in CAR treated mice than in control groups (PBS and mutant peptide, i.e. mCAR injections). To delineate the molecular mechanism by which CAR accelerates wound healing, we focused on the requirement of HSPG binding for CAR peptide function. We demonstrate that CAR peptide endocytosis and its stimulation of keratinocyte cell migration are both dependent on SDC4. Finally, we show that the systemic administration of CAR peptide stimulates wound re-epithelialization only in WT mice, but not in SDC4 knockout (KO) mice. As SDC4 has very restricted expression in skin wounds, we propose that CAR peptide activates SDC4 function to promote re-epithelialization. CAR peptide may provide an entirely new way of enhancing wound healing, and perhaps tissue regeneration in general. This therapeutic approach is systemic, yet target organ- and cell-specific, and dependent on the naturally occurring SDC4 dependent migratory pathway that is crucial for tissue regeneration.

S16.3 DEVELOPMENT OF COLLAGEN/HYALURONIC ACID-TYRAMINE (COLL/THA) COMPOSITE HYDROGELS WITH TUNABLE GELLING KINETIC AND THA CONTENT FOR THE TREATMENT OF NUCLEUS PULPOSUS

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Collagen and hyaluronic acid are two major components of intervertebral disc (IVD). They give resistance and hydration to Nucleus Pulposus. In this study, we assessed the impact of Collagen (COLL) and Hyaluronic acid-Tyramine (THA) contents on the mechanical properties and the structure of composite hydrogels. For this purpose, a range of composites were obtained using a 4 mg/mL collagen concentration and different COLL/THA ratios from 8:1 to 1:5 (w/w). Composite gelling was performed by pH increase, triggering collagen fibrillogenesis and oxidative coupling of tyramine moieties in THA catalyzed by H₂O₂ and horseradish peroxidase (HRP). To modulate the THA gelling kinetic, different HRP concentrations (0.05; 0.1 and 0.5 U/mL) were used. Composites with a low THA content exhibited a fibrillar structure and possessed mechanical properties close to those of pure collagen hydrogels (200 Pa). From the ratio 1:1, the storage modulus increased to reach c.a 1200 Pa for the ratio 1:5. From the ratio 1:2, the fibrillar structure disappeared and sheets, characteristic of THA hydrogels, were observed. The HRP activity dramatically impacted the physical properties. A rapid THA gelling associated with a high THA content tended to destabilize collagen fibrils and promoted the formation of covalent bond between collagen and THA. On the opposite a slow gelling kinetic favored collagen fibril formation up to the COLL/THA ratio 1:2. Taken together, these results show that a slow gelling and an 8 mg/mL THA concentration are the appropriate conditions to obtain biomimetic biomaterials for the treatment of Nucleus Pulposus.

S16.4 MULTILAYER COLLAGEN-BASED SCAFFOLD AS DELIVERY VEHICLES OF BIOACTIVE MOLECULES FOR THE BONE-TO-TENDON INTERFACE REGENERATION

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The enthesis is a specialised zonal tissue interface between tendon and bone, essential for adequate force transmission and composed by four distinct zones (tendon, fibrocartilage, mineralized fibrocartilage and bone). After injury, the native structure is often not re-established and a mechanically weaker fibrovascular scar is formed. Traditionally used monotherapies have failed to be effective, posing the need for multi-cargo localized delivery vehicles. We hypothesize that multilayer collagen-based scaffolds can serve as delivery vehicles for specific bioactive molecules with tenogenic, chondrogenic and osteogenic potential to enhance the functional regeneration of the enthesis. Three-layer scaffolds composed by a tendon-like layer of collagen type I, a cartilage-like layer of collagen type II and a bone-like layer of collagen type I and hydroxyapatite were fabricated by an iterative layering freeze-drying technique. The scaffolds were cross-linked with varying concentration of 4-arm polyethylene glycol (4s-PEG) and the biological and mechanical properties were assessed. Each layer was functionalized with platelet-derived growth factor, insulin growth factor, heparan sulfate or bone morphogenetic protein 7 and their tenogenic, chondrogenic and osteogenic potential on bone-marrow derived stem cells was investigated in vitro. Scaffolds cross-linked with 1 mM 4s-PEG showed 60% free amines reduction respect to non-cross-linked scaffolds, were stable in collagenase over 24 hours and had a compression modulus of 30 kPa. The bioactive molecules had a sustained release profile (approximately 50 ng/mL) over 5 days as a function of cross-linking. Preliminary in vitro studies confirmed the chondrogenic potential of heparin sulfate and insulin growth factor by the increase of proteoglycans.

S16.5 SELF-SETTING AND INJECTABLE HYALURONIC ACID HYDROGELS WITH BIOINSPIRED PROPERTIES FOR SKELETAL TISSUE ENGINEERING

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Tissue engineering is a promising approach to regenerate damaged skeletal tissues. In particular, the use of injectable hydrogels alleviates common issues of poor cell viability and engraftment. However, uncontrolled cell fate, resulting from unphysiological environments and degradation rates, still remain a hurdle and impedes tissue healing. We thus aim at developing a new platform of injectable hyaluronic acid (HA) hydrogels with a large panel of properties (stiffness, degradation...) matching those of skeletal tissues. Hence, HA with different molecular weights were functionalized with silylated moieties. Upon injection, these hydrogels formed through a sol-gel chemistry within 5 to 20 minutes in physiological conditions, as demonstrated by rheological characterization. By varying the crosslinking density and concentration, we obtained hydrogels spanning a large range of elastic moduli (E = 0.1-20 kPa), similar to those of native ECMs, with tunable biodegradation rates (from 24 hours to > 50 days) and swelling ratios (500 to 5000% (w/w)). Cell viability was confirmed by Live/Dead assays and will be completed by in vivo subcutaneous implants in mice to study the foreign body reaction and degradation rate. We further developed hybrid HA/biphasic calcium phosphate granules hydrogels and demonstrated a strong mechanical reinforcement (E = 0.1 MPa) and a faster relaxation behaviour (τ1/2 < 400s), with similar degradation rates. Ongoing in vitro differentiation assays and in vivo implantations in a rabbit femur model will further assess their ability to drive bone regeneration. Collectively, these results suggest that this hydrogel platform offers promising outcomes for improved strategies in skeletal tissue engineering.

S16.6 THE ROLE OF HYALURONIC ACID IN VISCOELASTIC PROPERTIES OF EQUINE PATHOLOGICAL SYNOVIAL FLUID.

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The rheological properties of synovial fluid (SF) are largely attributed to the presence of high molecular weight hyaluronic acid (HA). In normal SF, HA has been shown to be an anti-inflammatory molecule able to increase the viscosity and promote endogenous production of HA. The aim of the present report was to investigate the possible effect of HA concentration in rheological properties (elastic modulus, G’ and viscous modulus, G”) of osteoarthritic equine SF. For this purpose, SF from intercarpal, metacarpophalangeal and distal interphalangeal joint was aspirated by aseptic arthrocentesis from 60 Warmblood horses. For determining HA concentrations in equine SF samples, a commercially available ELISA kit was used. Additionally, full rheological sample characterization was carried out with an AR-G2 rheometer (TA Instruments Ltd., UK) in order to measure the elastic G’ and viscous G” moduli, at horse’s body (37.5 ºC) temperature. The ANOVA findings revealed statistically significant main effects of the factors Joint Type (p = 0.001), and main effects of covariates Age (p = 0.019) and HA (p < 0.001) on the mean values of logG” and logG’ measurements. Interpreting the coefficients of the covariate HA, a positive correlation of HA was detected on the response logG” and logG’ measurements. Collectively, these data illustrate the role of HA in equine pathological SF.

S17.1 CONTROLLING OSSIFICATION USING CONDENSED PHOSPHATES

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Phosphate metabolism is central to the bone formation pathway. Phosphate is shuttled through the cell membrane to the mitochondria, where it is polymerised to form adenosine triphosphate. Once exocytosed the ATP may then be cleaved to form pyro and orthophosphates, the balance of which can determine whether mineralisation occurs or not. We are developing a range of materials at the University of Birmingham that have been formulated so that they can influence this balance, with the potential either to drive or prevent mineralisation from occurring. This talk will describe how we have used this process to develop materials that can be used to stimulate bone formation around an implant or to prevent the formation of pathological bone. It will also talk about the steps that we have taken to move these therapies towards clinical trial.

S17.2 ENHANCED METHODOLOGIES TO ENGINEER THE BONE-BIOMATERIAL INTERFACE

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The nature of the initial interaction between calcium phosphate (CaP) thin films and osteoblasts can be mediated by the outermost surface properties of that material. As such, the phase, crystallinity, stoichiometry, composition and morphology of the CaP surfaces are seen as key parameters that must be accurately controlled in order to influence their potential biofunctionality with respect to osteoblasts. Hydroxyapatite \[\text{HA} = \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\] has been extensively studied due to the structural and chemical similarities demonstrated with the main inorganic constituent of bone tissue and teeth. However, it is well documented that biological hydroxyapatite, which forms the mineral phases of calcified tissues, differ from pure and synthetically produced HA. Biological apatite is comprised of a mixture of calcium phosphate phases and trace elements, e.g., strontium, zinc, magnesium and silicon. As such, when designing CaP biomaterials for clinical use (both bulk materials and coatings) one proposed route would be to introduce multiple ionic substitutions into HA in order to mimic the complex chemistry of human bone and thereby improve the biological performance of such materials, both \textit{in vitro} and \textit{in vivo}. This presentation will explore a novel approach to depositing substituted and co-substituted CaP systems onto a range of different substrates types, namely metal and polymers. In particular, this presentation will examine how the surface properties of bioinert polymers, such as Poly(etheretherketone) (PEEK) accurately controlled in order to provide an enhanced \textit{in vitro} performance. The presentation will also look at how resorbable magnesium implants can also be manipulated to provide both enhanced bioactivity and to provide a route to control how they resorb in a physiological environment.

S17.3 NOVEL 2D NANOMATERIALS-REINFORCED NANOCOMPOSITES FOR ORTHOPAEDIC APPLICATIONS

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The enhancement of current bone cement properties is a challenging issue that has been the focus of much research. Developing bone composites with high level of cytocompatibility, mechanical and antibacterial properties is a challenging task. We overcome this challenge by designing a nanocomposite that contain two-dimensional (2D) nanosheets. To develop our novel bone cement nanocomposite, 2D nanosheets were
synthesized, mixed in different ratios, and then added to the PMMA matrix. The results reveal that the incorporation of 2D nanosheets into the PMMA matrix leads to increase in the antibacterial properties of the bone cement composite against E. coli bacteria. In addition, the 2D nanosheets improve the compression strength of the bone cement nanocomposite significantly. We also show that nanosheets increased the bioactivity of the bone cements. Finally, MTT assay results indicate that PMMA as a control sample has the lowest cytocompatibility, however, our novel nanocomposites have the highest amount of cytocompatibility. Thus, the current study suggests that 2D nanosheets are potential filler components for the next generation of PMMA bone cement nanocomposites. The findings of this work reveal that the excellent performance of the proposed bone composite can result in a paradigm shift in design of state-of-the art bone cement composites.

S17.4 X-RAY: AN ALTERNATIVE TECHNOLOGIE TO IMPROVE POLYETHYLENE PROPERTIES AS AN ORTHOPEDICAL IMPLANT MATERIAL

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Crosslinking has been already used for about 80 years to enhance the longevity of polyethylene cables. The polymer alteration has been achieved with peroxide, silane or irradiation. The medical devices industry discovered the benefit of this technology for its tribological applications like hip or knee bearings in the 2000s as crosslinking improves considerably the abrasion resistance of the material. The more current methods used are Gamma and Beta irradiation. On the basis of economical (rising prices of Cobalt), environmental (the radioactive source can not be turned off), technological (low dose rate) drawbacks for Gamma respectively low penetration for Beta irradiation we decided to investigate an alternative technology: the X-Ray irradiation, which provides a homogeneous crosslinking in a relatively short time. We analyzed the wear, mechanical, thermal, oxidative and network properties of two vitamin E doped UHMWPE: first crosslinked with E-Beam, second with X-Ray. There wasn’t any significant difference between the X-Ray and the E-Beam crosslinked material.

S17.5 UNDERSTANDING THE NETWORK FORMATION, SURFACE MORPHOLOGY, AND CELL VIABILITY OF MOLDED HYDROGELS IN VARIOUS CONCENTRATIONS OF A CEOSSLINKING SOLUTION

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Hydrogels are hydrated 3-dimensional (3D) polymer networks that can be chemically or physically crosslinked. Interest in the use of hydrogels for tissue engineering applications has been growing in the past few decades due to their excellent biocompatibility and biodegradability . One of the major drawbacks of the use of hydrogels in such applications is their lack of structural strength. To address this, in this work, we have combined two hydrogel types, namely gelatin and alginate. In this work, a 1 ml volume of gelatin alginate hydrogel was molded in each well of a 24 well-plate and crosslinked with different concentrations of calcium chloride (CaCl₂) (20, 40, 60, 80, and 100 mM) to investigate the influence of concentration on hydrogel properties and cell viability. The hydrogel was characterized using Fourier transform infrared (FTIR) spectrometry, environmental scanning electron microscopy (ESEM), and an Alamar blue assay to assess the chemical structure, the surface morphology, and the epithelial cell viability of the hydrogel, respectively. The FTIR analysis shows that network formation improved with increasing concentration; decreased ion-polymer
interactions have been noted for concentrations ≤ 60 mM. This appears to be in agreement with ESEM images that show an evolution from a smooth, featureless surface to the appearance of surface pore structure for concentrations ≥ 80 mM. Perhaps as ion concentration increases and network formation improves, the effect is evidenced as surface porosity; low concentrations result in swelling and a smooth surface. In terms of cell viability, viability has been found to increase with increasing concentration. The cell viability is 90% at 100 mM CaCl₂, in contrast to 50% for a concentration of 20 mM after 9 days of incubation. It is possible that the reduced viability can be attributed to the high proportion of uncrosslinked polymer chains at low concentrations. Overall, these results provide useful information about the role of crosslinking concentration on hydrogel properties, knowledge that may be applied to 3D bioprinting.

S17.6 ASSESSING THE PROPERTIES OF COLLAGEN TYPE II SCAFFOLDS AS A FUNCTION OF SPECIES, TISSUE AND GENDER

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Porcine and fish by-products in particular are rich sources for collagen, which is the main component of the extracellular matrix (ECM). Although there are studies investigating different collagen derived from various tissue sources for the purpose of creating biomaterials, the comparison of biophysical, biochemical and biological properties of type II collagen isolated from cartilaginous tissues has yet to be assessed. In addition, it has been shown from previous studies that sex steroid hormones affect the collagen content in male and female animals, herein, type II collagens from male and female porcine cartilage were assessed in order to investigate gender effects on the property of collagen scaffolds. Moreover, type II collagen has a supportive role in articular cartilage in the knee joint. Therefore, the aim is to assess the properties of type II collagen scaffolds as a function of species, tissue and gender for cartilage regeneration. Type II collagen was extracted from male and female porcine trachea, auricular, articular cartilage and cartilaginous fish through acid-pepsin digestion at 4°C. SDS-PAGE was conducted to confirm the purity of extracted collagen. Collagen sponges were created via freeze-drying. Scaffold structure and pore size were evaluated by scanning electron microscopy (SEM). Thermal stability was assessed by differential scanning calorimetry (DSC). Sponges were seeded with human adipose derived stem cells to assess chondro-inductive potential of collagen sponges after 7, 14 and 21 days of culture. In conclusion, collagen sponges support the proliferation and differentiation of human adipose derived stem cells to different extents.

S18.1 CELLULAR AND MOLECULAR PROCESSES DURING HUMAN ACHILLES TENDON HEALING

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Tendon pathologies represent an unresolved clinical challenge where the patients suffer from pain and impaired mobility. One of the most frequently ruptured tendons is the Achilles tendon and primarily seen in recreational and professional athletes. A study from Sweden reported a significant increase in the incidence of Achilles tendon ruptures of 17% in men and 22% in women due to the demographic changes and the higher sportive activity of older adults (Huttunen TT Am J Sports Med 2014). The re-rupture rate is between 2-10%, and the patients suffer from an impairment over a long time accompanied with incapability to work. The
A detailed knowledge on the cellular and molecular processes underlying human Achilles tendon healing is necessary to develop new treatment strategies and judge therapeutic success. The analysis of human Achilles tendon samples at different time points post rupture and the comparison to intact and degenerated tendon tissue provides important information on the healing process.

S18.2 THE EFFECT OF ACHILLES TENDON RUPTURE ON HUMAN MUSCLE-TENDON UNIT FUNCTION

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Muscle and tendon have an adaptive, symbiotic biomechanical relationship that is drastically altered following acute tendon injury. Such injuries, like Achilles tendon rupture (ATR), do not only lead to impairments in the resultant tendinous tissue, but also to irrecoverable atrophy in the connected muscle in series. As a result, a new relationship between muscle and tendon is established after ATR, leading to lasting functional deficits in the lower limb. It remains unclear how these develop, particularly since this imbalance may be influenced by the dependent relationship of the two tissues to each other. A further confounding factor is that tendon and muscle tissues adapt on different time scales in response to mechanical loading, such as those introduced during rehabilitation. Thus, it is warranted to perform assessments not only of the overall muscle-tendon unit, but also its constituent tissues. This presentation will discuss findings from both short-term and long-term follow-ups of ATR patients, with a focus on the recovery of gait and changes in the muscle-tendon unit tissues following ATR repair. Both the influence of the rehabilitation process and suggestions for future research directions will be additionally presented.

S18.3 PULSED ELECTROMAGNETIC FIELD MODULATES THE INFLAMMATORY ENVIRONMENT INDUCED BY INTERLEUKIN-1β ON HUMAN TENDON-DERIVED CELLS

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Unresolved inflammatory processes in tendon healing have been related to the progression of tendinopathies. Thus, the management of tendon injuries may rely on cell-based strategies to identify and modulate tendon inflammatory cues. Pulsed electromagnetic field (PEMF) has been approved by FDA for orthopedics therapies and has been related to a reduction in pain and to improve healing. However, the influence of PEMF in tendon healing remains largely unknown. Human tendon resident cells (hTDCs) were cultured in an inflammatory environment induced by exogenous supplementation of IL-1β and their response assessed after exposure to different PEMF treatments. This study demonstrates that IL-1β induced up-regulation of pro-inflammatory factors (IL-6 and TNFα) and extracellular matrix components (MMP-1, -2, -3) whereas reduces the expression of TIMP-1, suggesting IL-1β as a candidate inflammation model to study hTDCs response to inflammation cues. Moreover, in both homeostatic and inflammatory environments, hTDCs respond differently to PEMF treatment suggesting that cells are sensitive to magnetic field parameters such as strength (1.5 – 5mT), frequency (5-17Hz) and duration (10-50% duty cycle, dc). Among the conditions studied, PEMF treatment with 4mT/5Hz/50%dc suppresses the inflammatory response of hTDCs to the IL-1β stimulation, as evidenced by the decreases amount of IL-6, TNFα and downregulation of MMP-1, -2, -3 and COX-2, IL-8, IL-6, TNFα.
genes. These results demonstrate the potential of PEMF, in particular 4mT/5Hz/50%dc PEMF in treating tendon inflammation suppressing the inflammatory stimulation induced by IL-1β, which may be beneficial for tendon healing strategies.

**S18.4 MOLECULAR CONTROL OF TENOCYTE PHENOTYPE THROUGH MATRIX-MEDIATED MECHANOTRANSDUCTION**

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During *in vitro* sub-culturing, tenocytes lose their phenotype which ultimately affects their functioning. As spindle-shaped fibroblasts, tenocytes have a unique thin elongated phenotype and they possess more spread-out shape through phenomena named dedifferentiation1. Given the link between cell shape and cell function, in this study, we first aimed to dedifferentiate tenocytes through *in vitro* sub-culturing in order to have a model system for dedifferentiation. For this, we isolated human flexor tendon cells from healthy female flexor digitorum longus and seeded at 5000 cells/cm² cell density, passaged every two days for six passages. In order to assess cell phenotype, we fixed with 4% paraformaldehyde and stained with phalloidin and DAPI to visualize the actin cytoskeleton and DNA respectively. We noted that in each passage, cells lost their spindle-shaped phenotype and became more pancake-shaped. At passage 1 and 2, the main cell phenotype is spindle-shaped. However, as the cells are further passaged, the phenotype of the cell population becomes more heterogeneous and at passage 5 and 6, they already display a more spread-out shape. Based on these results, we further hypothesized that they can be re-differentiated through matrix-mediated mechano-transduction and regain their morphology and function. For this aim, we generated decellularized tendon from porcine Achilles tendon and setup a mechanical loading system where we can provide mechanical loadings at physiological levels. This system will provide a new approach on *in vitro* tenocyte culturing.

**S18.5 A MULTI-FACTORIAL TOOLBOX TOWARDS TENOCYTIC PHENOTYPE MAINTENANCE**

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Cellular therapies play an important role in tendon tissue engineering with tenocytes being described as the most prominent cell population if available in large numbers. *In vitro* expansion of tenocytes in standard culture leads to phenotypic drift and cellular senescence. Maintenance of tenogenic phenotype *in vitro* can be achieved by recapitulating different aspects of the tendon microenvironment. One approach used to modulate *in vitro* microenvironment and enhance extracellular matrix (ECM) deposition is macromolecular crowding (MMC). In addition, as tendon has been described to be a relatively avascular and hypoxic tissue and low oxygen tension can stimulate collagen synthesis and cross-linking through the activation of hypoxia-inducible factor 1-alpha (HIF1-α), we venture to assess the synergistic effect of MMC and low oxygen tension on human tenocyte phenotype maintenance. SDS-PAGE and immunocytochemistry analysis demonstrated that human tenocytes treated with MMC at 2 % oxygen tension showed increased synthesis and deposition of collagen type I. Moreover, immunocytochemistry for the tendon-specific ECM proteins collagen type III, V, VI and fibronectin illustrated enhanced deposition when cells were treated with MMC at 2 % oxygen tension. In
addition, western blot analysis revealed increased expression of tendon-specific protein Scleraxis, while a detailed gene analysis illustrated upregulation of tendon-specific genes and downregulation of trans-differentiation genes again when cells cultured with MMC under hypoxic conditions. Collectively, results suggest that the synergistic effect of MMC and low oxygen tension can accelerate the formation of ECM-rich substitutes, which stimulates tenogenic phenotype maintenance.

S18.6 CORRELATIONS OF LONGITUDINAL CHANGES IN DYNAMIC JUMPING BIOMECHANICS AND PATELLAR TENDON QUANTITATIVE IMAGING MEASURES IN ELITE COLLEGIATE BASKETBALL PLAYERS

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This investigation of elite male collegiate basketball players aims to determine 1) the change in 3D dynamic functional variables across a single season and 2) correlate cross-season changes in functional variables with changes in clinical and quantitative ultrasound measures. Eleven male college basketball players (mean age 19, range 18-21 years) from a single team underwent baseline patellar tendon shear wave (SW) elastography and dynamic function at the start of the season (Visit1) and at a late-season time point (Visit2). Players reported their VISA-P scores every two weeks across their 24-week season. Each athlete performed a box-ground-box jump five times while 3D lower extremity kinematic and kinetic variables were collected. Functional measures included for landing (LAND) and take-off (TOFF) phases: knee valgus angle, valgus torque, and peak limb force. Knee valgus angular impulse and ground contact time were also measured. Paired t-tests and Pearson correlation coefficients (r) compared Visit1 and Visit2 variables and assessed the strength of linear dependency, respectively. The mean change in VISA-P score was 15.18 (±8.55). No functional variables were different across the season. Clinical, quantitative ultrasound and functional variables were moderately correlated with take-off valgus moment, landing force, take-off force and contact time. Other correlations were low (<0.4). Our analyses have shown moderate correlations between important clinical, quantitative imaging and function measurements. These correlations reflect the changes that occur between relevant time points and which relate internal structure and external function.

S19.1 DELIVERY OF SELF-ASSEMBLING OSTEOGENIC NANOPARTICLES VIA A THERMO-RESPONSIVE NANOFIBRE REINFORCED HYDROGEL

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Ceramics such as hydroxyapatite are routinely used in fracture repair. However, their effects could be significantly improved as its bioavailability is incredibly poor (issues including low solubility, anionic charge, tendency to agglomerate). Nanoscale hydroxyapatite are gaining much interest, demonstrating increased effectiveness when compared to their micro-sized counterpart. In this study, we have developed a bioactive cargo–polymer-based system that allowed for the sustained, localised non-viral delivery of hydroxyapatite nanoparticles using an amphipathic peptide as a capping agent. The nanoparticles were delivered from a polycaprolactone nanofibre reinforced novel Alg-co-PNIPAAm thermoresponsive hydrogel. The bioactive cargo–polymer-based system was characterised in terms of its physiochemical properties, in vitro properties and in vivo performance using a subcutaneous mouse model. From this study, we have demonstrated that osteogenesis and bone regeneration were significantly increased when our novel capping agent was used to
limit the particle size distribution and optimised the physiochemical characteristics of nanoscale hydroxyapatite (i.e. reducing risk of agglomeration and increasing its bioavailability). Additionally, the dual functionality of the thermoresponsive hydrogel as a scaffold for bone regeneration and as a vehicle for the sustained, local delivery of hydroxyapatite nanoparticles over an extended period was successfully demonstrated.

S19.2 MODULATING MACROPHAGE BEHAVIOUR AT THE BIOMATERIALS-TISSUE INTERFACE FOR ENHANCED OSTEOGENESIS AND OSSEOINTEGRATION

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All types of regenerative materials, including metal implants, porous scaffolds and cell-laden hydrogels, interact with the living tissue and cells. Such interaction is key to the settlement and regenerative outcomes of the biomaterials. Notably, the immune reactions from the host body crucially mediate the tissue-biomaterials interactions. Macrophages (as well as monocytes and neutrophils), traditionally best known as defenders, accumulate at the tissue-biomaterials interface and secrete abundant cytokines to create a microenvironment that benefits or inhibits regeneration. Because the phenotype of these cells is highly plastic in response to varying stimuli, it may be feasible to manipulate their activity at the interface and harness their power to mediate bone regeneration. Towards this goal, our team have been working on macrophage-driven bone regeneration in two aspects. First, targeting the abundant, glucomannan-recognising receptors on macrophages, we have devised a series of glucomannan polymers that can stimulate macrophages to secrete pro-osteogenic cytokines, and applied them as coating polymer of mesenchymal stem cells-laden hydrogels. Second, targeting the toll-like receptors (TLRs) on macrophages, we have screened TLR-activating polysaccharides and picked up zymosan (beta-glucan) to be modified onto titanium and glass implants. We evaluated both the efficacy of integration and safety of immune stimulation in both in vitro and in vivo models. Our future exploration lies in further elaborating the different roles and mechanisms of macrophages of various types and origins in the regenerative process.

S19.3 ADIPOSE-DERIVED STROMAL VASCULAR FRACTION SHOWS MARKED BONE REGENERATIVE POTENTIAL ON A XENOHYBRID BONE SCAFFOLD


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Intra-articular infusions of adipose tissue-derived stem cells (ASCs) are a promising tool for bone regenerative medicine, thanks to their multilineage differentiating ability. One major limitation of ASCs is represented by the necessity to be isolated and expanded through in vitro culture, thus a strong interest was generated by the adipose stromal vascular fraction (SVF), the non-cultured fraction of ASCs. Besides the easiness of retrieval, handling and good availability, SVF is a heterogeneous population able to differentiate in vitro into osteoblasts, chondrocytes and adipocytes, according to the different stimuli received. We investigated and compared the bone regenerative potential of SVF and ASCs, through their ability to grow on SmartBone®, a composite xenohybrid bone scaffold. SVF plated on SmartBone® showed better osteoinductive capabilities than ASCs. Collagen I, osteocalcin and TGF-β markedly stained the new tissue on SmartBone®, microCT analysis indicated a progressive increase in mineralised tissue apposition by quantification of newly formed trabeculae...
(3391 ± 270.5 vs 1825 ± 133.4, p < 0.001); an increased secretion of soluble factors stimulating osteoblasts, as VEGF (153.5 to 1278.1 pg/ml) and endothelin 1 (0.43 to 1.47 pg/ml), was detected over time. In conclusion, the usage of SVF, whose handling doesn't require manipulation in an in vitro culture, could definitively represent a benefit for a larger use in clinical applications. Our data strongly support an innovative idea for a bone regenerative medicine based on resorbable scaffold seeded with SVF, which will improve the precision of stem cells implant and the quality of new bone formation.

**S19.4 HETEROGENEOUS AND CD271-ENRICHED MSCS SHOW DIFFERENTIAL OSTEOGENIC POTENTIAL WHEN CULTURED ON APATITE-WOLLASTONITE 3D SCAFFOLD**

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Mesenchymal stromal cells (MSCs) are widely used in clinical trials for the treatment of many bone defects. Apatite-wollastonite glass ceramic (A-W) is an osteoconductive biomaterial shown to be compatible with MSCs. This is the first study comparing the osteogenic potential of two MSC populations, heterogeneous plastic adherence MSCs (PA-MSCs) and CD271-enriched MSCs (CD271-MSCs), when cultured on A-W 3D scaffold. The paired MSC populations were assessed for their attachment, growth kinetics and ALP activity using confocal or scanning electron microscopy and the quantifications of DNA contents and p-nitrophenyl (pNP) production. While the PA-MSCs and CD271-MSCs had similar expansion and tri-lineage differentiation capacity during standard 2D culture, they showed different proliferation kinetics when seeded on the A-W scaffolds. PA-MSCs displayed a well-spread attachment with more elongated morphology compared to CD271-MSCs, signifying a different level of interaction between the cell populations and the scaffold surface. PA-MSCs also fully integrated into the scaffold surface and showed a stronger propensity for osteogenic differentiation on the A-W scaffold as indicated by higher ALP activity than CD271-MSCs. Furthermore, A-W scaffold seeded uncultured bone marrow mononuclear cells (BM-MNCs) demonstrated a higher proliferation rate and greater ALP activity compared to freshly isolated CD271-enriched BM-MNCs. Our findings suggest that enrichment of CD271-positive population is not beneficial for osteogenesis when the cells are seeded on A-W scaffold. Furthermore, unselected heterogeneous MSCs or BM-MNCs are more promising for A-W scaffold-based bone regeneration, providing novel insight with potential clinical implications in regenerative medicine for bone defects using an innovative tissue engineering approach.

**S19.5 HUMAN AMNIOTIC MEMBRANE FOR GUIDED BONE REGENERATION OF CALVARIAL DEFECTS IN MICE AND IMPROVEMENT OF ITS PRESERVATION PROCEDURE**

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The human amniotic membrane (hAM) may be helpful as a support for bone regeneration. To assess its potential for bone repair, a wide heterogeneity of preservation methods of hAM has been studied. The objectives of this study were: i) to assess bone regeneration potential of fresh versus cryopreserved hAM, and ii) to characterize hAM depending on four preservation methods. hAM was used either fresh (F-hAM), cryopreserved (C-hAM), lyophilized (L-hAM) or decellularized and lyophilized (DL-hAM). First, critical calvarial bone defects were performed in mice. Defects remained empty or were covered by F-hAM or C-hAM. Then, the cytotoxicity of the four preservation methods of hAM was assessed in vitro on human bone marrow mesenchymal stem cells (hBMSCs), and, their biocompatibility was evaluated in vivo in a rat subcutaneous model. X-Rays analysis showed that no calvarial defect was regenerated ad integrum. Bone
 regeneration was slightly enhanced by C-hAM. In vitro, the decellularization and the lyophilization process did not confer any cytotoxicity of the tissue compared to other preservation methods. In vivo, L-hAM and DL-hAM were easier to handle. Histological analysis of explanted samples from the rat indicated a slight to moderate inflammatory reaction with hAM. One month after surgery, a complete resorption of F-hAM and C-hAM implants occurred, whereas L-hAM and DL-hAM were still observed. C-hAM has a limited potential for GBR. L-hAM and DL-hAM are biocompatible without cytotoxic effects. These preservation methods should be suitable in the field of bone regeneration.

**S19.6 ESTABLISHMENT OF A MOUSE LARGE BONE DEFECT MODEL RECONSTRUCTED BY BONE TRANSPORT**


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Segmental bone transport (SBT) with an external fixator has become a standard method for treatment of large bone defect. However, a long time-application of devices can be very troublesome and complications such as nonunion is sometimes seen at docking site. Although there have been several studies on SBT with large animal models, they were unsuitable for conducting drug application to improve SBT. The purpose of this study was to establish a bone transport model in mice. Six-month-old C57BL/6J mice were divided randomly into bone transport group (group BT) and an immobile control group (group EF). In each group, a 2-mm bone defect was created in the right femur. Group BT was reconstructed by SBT with external fixator (MouseExFix segment transport, RISystem, Switzerland) and group EF was fixe d simply with unilateral external fixator (MouseExFix simple). In group BT, a bone segment was transported by 0.2 mm per day. Radiological and histological studies were conducted at 3 and 8 weeks after the surgery. In group BT, radiological data showed regenerative new bone consolidation at 8 weeks after the surgery, whereas high rate of nonunion was observed at the docking site. Histological data showed intramembranous and endochondral ossification. Group EF showed no bone union. In this study, experimental group showed good regenerative new bone formation and was similar ossification pattern to previous large animal models. Thus, the utilization of this bone defect mice model allows to design future studies with standardized mechanical conditions for analyzing mechanisms of bone regeneration induced by SBT.

**S20.1 NEW APPROACHES TO REDUCE BACTERIAL ADHESION ON POLYMETHYL METHACRYLATE (PMMA)**

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The discussion will focus on new approaches to reduce bacterial adhesion on the surface of polymethylmethacrylate (PMMA) in contact with bone, comparing the clinical and engineering point of view. One possibility is to encourage and speed up direct interaction with the bone, for example by adding a bioactive phase in the cement (hydroxyapatite, glass and bioactive glass ceramic). A widespread strategy is also the addition of different types of antibiotics (gentamicin, tobramycin vancomycin, etc.), although they are known to have some drawbacks: not complete release, resistant strain development. Another strategy could be represented by the PMMA-based composite cements loaded with a completely inorganic filler consisting of a bioactive glass doped with ions whose bioactivity mechanism is well-known and encompasses a chemical and biological interaction with tissues promoting osteoinduction. Bioactive glasses can be doped with antibacterial ions (silver, copper, etc.) preserving their biocompatibility and bioactivity and, at the same time, acquiring
antibacterial properties. Thus, it is possible to produce composite cements that combine the properties of the polymer matrix with those of the inorganic filler, overcoming the main problems associated with the use of antibiotics. An additional possibility is the addition of essential oils, vegetable oils with remarkable antibacterial properties.

**S20.2 INNOVATIVE PMMA-BASED BONE CEMENTS CONTAINING A SINGLE INORGANIC PHASE WITH BIOACTIVE AND ANTIMICROBIAL PROPERTIES**

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Post-surgical infections are still one of the most frequent adverse events in the prosthetic surgery. PMMA-based cements are widely employed in orthopaedic surgery as filler or prosthetic fixing device. The main problems associated with this material are poor bone integration and infection development. Aiming to avoid bacterial adhesion and to extend the longevity of implants, different solutions were proposed, both in terms of operative procedures and new materials development. Regarding the materials advancement, innovative PMMA-based composite bone cements, contemporaneously bioactive and antibacterial (without the use of antibiotics), were developed. The composites are based on a PMMA matrix containing a bioactive glass, doped with antibacterial ions (Ag⁺ or Cu⁺⁺); so, the same filler shows at the same time the ability of promoting bone ingrowth and an antibacterial effect. Composite cements were characterized in terms of morphology and composition, curing parameters and mechanical properties; *in vitro* tests were performed to verify the material ability to release antibacterial ions and to promote the precipitation of hydroxyapatite. Moreover, cytotoxicity and antimicrobial properties were verified. The cements characteristics were tested using different commercial matrix and different viscosities; therefore, the proposed formulations represent an innovative solution for a new family of antibiotic-free, bioactive and antibacterial cements.

**S20.3 PROFILE OF MINIMUM INHIBITORY CONCENTRATION OF STAPHYLOCOCCUS SPECIES IN ORTHOPAEDICS INFECTION**


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Infection is one of the most serious complications of orthopedic surgery, particularly in implant-related procedures. Minimum inhibitory concentration (MIC) for identified bacteria is an important factor for successful antibiotic treatment. We investigated the MIC of antibiotics in Staphylococcus species from orthopedic infections, comparing with isolates from respiratory medicine. Staphylococcus species isolated in our laboratory from January 2013 to July 2016 were retrospectively reviewed. The MIC of vancomycin (VCM), arbekacin (ABK), teicoplanin (TEIC), linezolid (LZD), and rifampicin (RFP) was reviewed. Differences in the MIC of each antibiotic in orthopedic and respiratory samples were determined. A total of 259 isolates were evaluated (89 orthopedic, 170 respiratory). Staphylococcus aureus was the most commonly identified species (58%). In comparison with orthopedic samples, the number of isolates with a VCM MIC <0.5 μg/ml in methicillin sensitive staphylococcus aureus (MSSA) was significantly higher in respiratory isolates, while a MIC of 2 μg/ml was significantly lower (P = 0.0078). The proportion of isolates with a VCM MIC of 2 μg/ml in methicillin-resistant coagulase-negative staphylococci (MRCNS) was significantly higher in orthopedic isolates than that seen in respiratory isolates of methicillin-resistant staphylococcus aureus
(MRSA; P < 0.001). When comparing MRCNS and other orthopedic Staphylococci, the rate of RFP MIC >2 μg/ml in MRCNS isolates was significantly higher (P = 0.0058). The MIC of VCM in Staphylococcus species from orthopedic infection was higher than that of respiratory samples, particularly in MRCNS from implant-related samples. MRCNS showed a significantly higher rate of resistance for RFP versus other orthopedic isolates.

S20.4 BIOLOGICAL INTEREST OF CU-DOPED CALCIUM PHOSPHATE BIOCERAMICS FOR BONE TISSUE ENGINEERING

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Biphasic calcium phosphates (BCP) are the most frequently used materials because of their mineral analogy with bio-mineral part of bones. Their chemical synthesis can be modulated by doping, in order to respond to the biological needs. We present here the biological responses induced by copper ions in solution, to characterize its cytotoxicity and antibacterial activity. We also investigate the antibacterial property of Cu-doped BCP (Ca10 Cu0.1 (PO4)6 (OH)1.8 O0.2) on a strain of clinical interest: S. aureus, compared to undoped BCP. The sol-gel route has been used to prepare the BCP ceramics. Human BMC (Bone Marrow Cells) were obtained from metaphysal cancellous bone collected during hip arthroplasty and used for cytotoxicity evaluations. A strain of Staphylococcus aureus isolated from an osteoarticular infection after total knee arthroplasty was used to evaluate antibacterial activities. Results indicate that 3 ppm of copper ions leads to the death of all cultured bacteria in 24 hours and 25 ppm caused the death of all cells in 15 days. Regarding BCP, the undoped bioceramics increased the bacterial growth compared to a control without bioceramic. After 16 hours of contact, the copper ions released by the Cu-doped BCP induced a significant decrease of the bacterial concentration, indeed no viable bacteria were found. These materials seem to be a promising alternative for the preparation of multifunctional bone substitutes.

S20.5 LASER MANUFACTURING OF MULTI-FUNCTIONAL AND ANTI-BACTERIAL SURFACES FOR ORTHOPAEDIC APPLICATIONS

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With an ever-increasing aging population, total hip and knee arthroplasty is projected to increase by 137% and 601%, respectively, between the period; 2005-2030. Prosthetic Joint Infection (PJI) occurs in approximately 2% of total joint replacements (TJRs) in the U.S. PJI is primarily caused by adherence of bacteria to the surface of the prosthesis, ultimately forming an irreversibly attached community of sessile bacteria, known as a biofilm, highly tolerant to antibiotic treatment. Often the only resolution if the ensuing chronic infection is surgical removal of the implant – at high cost for the patient (increased morbidity), and for healthcare resources. Strategies to prevent bacterial adherence have significant potential for medical impact. Laser surface treatment using an automated continuous wave (CW) fiber laser system has shown promise in producing anti-adherent and bactericidal surfaces. Work presented here aims to investigate the effect of this approach on orthopaedic metals as a proof of concept, specifically Ti-6Al-4V (kindly supplied by Stryker Orthopaedics, Limerick). A coupon was surface treated using a laser (MLS-4030; Micro Lasersystems BV, Driel). Samples were incubated in Müller Hinton Broth (MHB) inoculated with meticillin resistant Staphylococcus aureus (MRSA; ATCC 43300) for 24h before Live/Dead staining (BacLight™ solution; Molecular Probes) and inspection by fluorescence microscopy (GX-M-L3201 LED; GX Optical). Images were analysed using ImageJ
software (NIH) and a significant reduction (p > 0.05, n=24) in total biofilm coverage and Live/Dead ratio was observed between the laser treated and as received surfaces. This data demonstrates the anti-adherent, and indeed bactericidal, effect of Laser-surface treatment.

S20.6 RISK FACTORS ASSOCIATED WITH REVISION FOR PROSTHEtic JOINT INFECTION FOLLOWING PRIMARY KNEE REPLACEMENT: EVIDENCE FROM ENGLAND AND WALES

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Periprosthetic joint infections (PJIs) are uncommon but are devastating complications of total knee replacement (TKR). We analysed the risk factors of revision for PJI following primary TKR and their association with PJI at different post-operative periods. Primary TKRs and subsequent revision surgeries performed for PJI from 2003-2014 were identified from the National Joint Registry (NJR). Multilevel piecewise exponential non-proportional hazards models were used to estimate the effect of the investigated factors at different post-operative periods. Patient, perioperative and healthcare system characteristics were investigated and data from the Hospital Episode Statistics for England were linked to obtain information on specific comorbidities. The index TKRs consisted of 679,010 primaries with 3,659 subsequently revised for PJI, 7% within 3 months, 6% between 3-6months, 17% between 6-12months, 27% between 1-2years and 43% ≥2 years from the index procedure. Risk factors for revision for PJI included male sex, high BMI, high ASA grade and young age. Patients with chronic pulmonary disease, diabetes and liver disease had higher risk of revision for PJI, as had patients who had a primary TKR for an indication of trauma or inflammatory arthropathy. Surgical procedure, fixation method, constraint and bearing type influenced the risk of revision for PJI. Their effects were period-specific. No or small associations were found with the operating surgeon grade, surgical volume and hospital surgical volume. These findings from the world’s largest joint replacement registry show a more complex picture than the meta-analyses published to date with specific time-dependent effects for the identified risk factors.

S21.1 ARTICULAR CARTILAGE PROGENITOR CELLS

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Cells with stem/progenitor characteristics can be isolated from articular cartilage and may have utility in cartilage repair and regeneration therapies. Unlike other adult cell types with differentiation capabilities, clonal chondroprogenitors differentiate into cartilage that resembles stable cartilage rather than endochondral cartilage. We have isolated a large series of chondroprogenitor clones from normal human articular cartilage from individuals of one to forty-five years of age and characterized them with known and novel markers. The clones were isolated separately from different zones of the articular cartilage. As first reported by others, the cloneable cells were mainly found in the upper zones. However, there are clones with chondroprogenitor status in the deeper zones, albeit at far lower frequency. These deep zone clones have different characteristics to those from the upper zones. We have used selected clones to re-engineer stable cartilage with use of the right environmental conditions (growth factors, oxygen level etc).

S21.2 WILL CHONDrOINDUCTIVE MATERIALS REVOLUTIONIZE CARTILAGE REGENERATION?
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One of the core tenets of our philosophy for tissue regeneration include the use of “raw materials,” where biomaterials themselves serve as both building blocks and bioactive signals. In recent years, a few groups around the world have gravitated toward cartilage matrix as a potentially chondroinductive material for cartilage regeneration. The major challenge to date in cartilage injury has been creating a biomaterial-only strategy that is capable of regenerating true hyaline-like cartilage without the addition of growth factors or exogenous cells. In the past few years, we have focused our efforts on establishing chondroinductivity in vitro, and in developing new materials synthesis strategies to provide ease of application for orthopedic surgeons in the operating room. By leveraging nanotechnology, we have developed a paste-like material constructed from cartilage matrix with encouraging mechanical performance post-crosslinking, and which avoids contraction after extended time. Looking to the future, we are working on next-generation approaches to chondroinductive materials. We have encouraging preliminary data which suggest the possibility of a chondroinductive response to a novel peptide sequence in vitro, which may be enhanced by simultaneous inclusion of adhesion peptides. Initial in vivo data in regeneration of rabbit femoral condyle cartilage defects may suggest promising regenerative capabilities with hydrogels based on these peptides. If indeed chondroinductive materials exist, and if they can be delivered easily, are safe, and can be provided at reasonable cost and with a reasonable regulatory strategy, chondroinductive materials may hold the potential to revolutionize cartilage regeneration.

S21.3 BIOPRINTING FOR BONE AND CARTILAGE REGENERATION

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Our musculoskeletal system has a limited capacity for repair. This has led to increased interest in the development of tissue engineering strategies for the regeneration of musculoskeletal tissues such as bone, ligament, tendon, meniscus and articular cartilage. This talk will review our attempts to use biomaterials and mesenchymal stem cells (MSCs) to bioprint functional articular cartilage and bone grafts for use in bone and joint regeneration. It will begin by describing how 3D bioprinting can be used to engineer biological implants mimicking the shape of specific bones, and how these bioprinted tissues mature into functional bone organs upon implantation into the body. Next, it will be demonstrated that different musculoskeletal injuries can be regenerated using 3D bioprinted implants, including large bone defects and osteochondral defects. The talk will conclude by describing how we can integrate biomaterials and MSCs into 3D bioprinting systems to engineer scaled-up tissues that could potentially be used regenerate entire diseased joints.

S21.4 LONG TERM OUTCOMES OF MATRIX INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION (MACI) INFORM THE IMPORTANCE OF FUNCTIONAL BARRIER STRUCTURE OF OSTEOCHONDRAL UNIT

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Symptomatic articular cartilage defects are one of the most common knee injuries, arising from acute trauma, overuse, ligamentous instability, malalignment, meniscectomy, osteochondritis dissecans. Surgical treatment options include bone marrow–stimulating techniques such as abrasion arthroplasty and microfracture, osteochondral mosaicplasty, corrective osteotomy, cartilage resurfacing techniques and tissue engineering techniques using combinations of autologous cells (chondrocytes and mesenchymal stem cells), bioscaffolds, and growth factors. Matrix induced autologous chondrocyte implantation (MACI) is considered the most surgically simple form of autologous chondrocyte implantation. Our group has involved in the development of MACI since 2000 and has led to the FDA approval of MACI as the first tissue engineering product for cartilage repair in 2016. In this article, we have documented the characterisation of autologous chondrocytes, the surgical procedure of MACI and the long term clinical assessment (15 years) of patients with treatment of MACI. We have also reported the retrospective survey in patients with MACI in Australia. Our results suggest that MACI has gained good to excellent long term clinical outcome and probably can delay total knee replacement. However, restoration of hyaline-like cartilage by MACI may be interrupted by the osteoarthritic condition of the joint in patients with progressed osteoarthritis. In addition, because articular cartilage and subchondral bone are considered a single functional unit that is essential for joint function, many cartilage repair technologies including MACI and microfractures have failed short to address the functional barrier structure of osteochondral unit. Further studies are required to develop tissue engineering osteochondral construct that is able to fulfil the function of articular cartilage-subchondral bone units.

S21.5 MECHANOSENSITIVE miR CLUSTERS REGULATED AFTER LOADING OF HUMAN ENGINEERED CARTILAGE

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Dynamic loading is necessary for the preservation of native cartilage, but mechanical disuse is one major risk factor for osteoarthritis (OA). As post-transcriptional regulators, microRNAs (miRs) represent promising molecules to quickly adjust the cellular transcriptome in a stimulus-dependent manner. Several miR clusters were related to skeletal development, joint homeostasis and OA pathophysiology but whether miRs are associated with mechanosensitivity and regulated by mechanotransduction is so far unknown. We aimed to investigate the influence of mechanical loading on miR expression and to identify mechanosensitive miR clusters characteristic for non-beneficial loading regimes which may serve as future tools for improved diagnosis or intervention during OA development. Loading regimes leading to an anabolic or catabolic chondrocyte response were established based on an increase or decrease of proteoglycan synthesis after loading of human engineered cartilage. miR microarray profiling at termination of loading revealed only small changes of miR expression (7 significantly upregulated miRs) by an anabolic loading protocol while catabolic stimulation produced a significant regulation of 80 miRs with a clear separation of control and compressed samples by hierarchical clustering. Overall regulation of 8/14 miR was confirmed by qRT-PCR with mean amplitudes of up to 2.5-fold for catabolic loading. Cross-testing revealed that 2 miRs were upregulated by both loading conditions and 6 were specifically elevated by the catabolic loading regime. Conclusively, this study defines the first mechanosensitive miR cluster associated with non-beneficial compressive cyclic loading of human engineered cartilage which can now be tested for its diagnostic potential in healthy versus OA-affected human cartilage.

S21.6 COMPOSITE BASED ON CHITOSAN AND HYDROXYAPATITE ASSOCIATED WITH PLATELET-RICH PLASMA FOR BONE AND CARTILAGINOUS RENEGERATION OF FEMORAL TROCHLEA IN RABBITS

The aim of this study was to evaluate the trochlear bone and cartilaginous regeneration of rabbits using a composite based on platelet rich plasma (PRP), chitosan and hydroxyapatite. The study was approved by the ethics committee of the Federal University of Campina Grande under number 72/2017. Surgical holes measuring four millimetres in diameter were performed in rabbit trochleae, one surgical hole in each animal remained empty and another one was filled with the composite. Clinical-orthopaedic and radiographic evaluations were carried out for 60 days, after which the animals were euthanized for histomorphometric evaluations. Clinical-evaluations exhibited lameness of two members of the treatment (T) group and one member of control (C) group. The radiographic evaluation of T group exhibited absence of subchondral bone reaction (33%); nonetheless, presence of moderate subchondral bone reaction was more frequently reported in group C with 67%. Microscopic evaluation revealed the presence of tissue neoformation, composed of dense connective tissue. Microscopic findings were similar in both groups, with a difference in the amount of neoformed tissue, which was confirmed after the morphometric analysis, revealing a significant difference in the quantity of newly formed tissue at the bone / cartilage / implant interface in the T group. The results indicate that the composite based on chitosan, hydroxyapatite and PRP enhanced bone and cartilage healing.

**S22.1 DESIGN OF NOVEL COMPOSITE BIOMATERIALS FOR BONE REGENERATION WITH SELF-HEALING AND LOAD-BEARING CAPACITY**

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Calcium phosphate ceramics and bioactive glasses are frequently used in orthopedic surgery to stimulate the regeneration of bone tissue due to their superior compatibility to bone tissue. Nevertheless, the brittleness and lack of self-healing behavior of bioceramics are still considered as serious drawbacks. Therefore, these bioceramics have been combined with organic biomaterials for several decades. Since the 1990s, the emergence of nanotechnology has accelerated the progress with respect to the development of organic-inorganic nanocomposites of improved functionality compared to conventional composite biomaterials. This presentation focuses on the development of injectable (nano)composites with self-healing and/or load-bearing capacity. To this end, the affinity between polymeric and inorganic components was tuned by modifying non-covalent interactions between both composite components. Specifically, we exploited reversible interactions between hydrogel matrices and inorganic nanoparticles (traditional nanocomposites), hydrogel nanoparticles and inorganic nanoparticles (colloidal nanocomposites), as well as fibers and bioceramic matrices (fiber-reinforced cement composites). The resulting composite biomaterials were mechanically strong and self-healing, which may open up new avenues of research on the applicability of self-healing and load-bearing composite biomaterials for regenerative medicine.

**S22.2 DESIGNING COMPOSITE BIOMATERIALS TO CONTROL BIOLOGICAL AND MECHANICAL FEATURES IN BONE TISSUE ENGINEERING**

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The development of functional biomaterials scaffolds for bone tissue engineering applications includes the control of specific biological and mechanical parameters that are involved in the growth of bone tissue in a way that mimics the physiological process of healing bone defects. Here, we report on the development of composite scaffolds made from biodegradable natural and synthetic biomaterials with characteristic architectural features, functionalized with the osteoinductive growth factor bone morphogenetic protein BMP-2, and evaluating their osteogenic response in static and dynamic cell culture systems. The results show that scaffold design with advanced technologies combined with appropriate biochemical and mechanical stimulating factors, results to an enhanced proliferative and osteogenic/chondrogenic differentiation response of cells cultured on the developed scaffolds, and thus controlling the new tissue formation and reconstruction.

S22.3 PERSONALISED BIOACTIVE IMPLANT MADE BY STEREOLITHOGRAPHY FOR ORBITAL FLOOR FRACTURE REPAIR

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Orbital floor (OF) fractures are commonly treated by implanting either bioinert titanium or polyethylene implants, or by autologous grafts. A personalized implant made of biodegradable and osteopromotive poly(trimethylene carbonate) loaded with hydroxyapatite (PTMC-HA) could be a suitable alternative for patients where a permanent implant could be detrimental. A workflow was developed from the implant production using stereolithography (SLA) based on patient CT scan to the implantation and assessment its performance (i.e. implant stability, orbit position, bone formation) compared to personalised titanium implants in a repair OF defect sheep model. Implants fabrication was done using SLA of photo-crosslinkable PTMC mixed with HA [1-3]. Preclinical study: (sheep n=12, ethic number 34_2016) was conducted by first scanning the OF bone of each sheep in order to design and to fabricate patient specific implants (PSI) made of PTMC-HA. The fabricated PSI was implanted after creating OF defect. Bone formation and defect healing was compared to manually shaped titanium mesh using time-laps X-ray analyses, histology (Giemsa-Eosin staining) and sequential fluorochrome staining over 3-months. Additionally, the osteoinductive property of the biomaterials was assessed by intramuscular implantation (IM). In this study, we showed that the composite PTMC-HA allowed for ectopic bone formation after IM implantation, without requiring any biotherapeutics. In addition, we could repair OF defect on sheep using SLA-fabricated PTMC-HA with a good shape fidelity (compared to the virtual implant) and a better bone integration compared to the titanium mesh. This study opens the field of patient-specific implants made of degradable and osteoinductive scaffolds fabricated using additive manufacturing to replace advantageously autologous bone and titanium implants.

S22.4 OSTEOCONDUCTIVE MICROARCHITECTURE REALIZED BY ADDITIVE MANUFACTURING

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The current gold standard bone substitute is still autologous bone, despite the fact that its harvest demands for a second operation site, causes additional pain, discomfort, potential destruction of the grafting site, and is limited in supply. Since newly developed clinical approaches like transplantation of cells are invasive and costly, and osteoinduction by bone morphogenetic proteins is expensive and is associated with mild to severe side effects, the optimization of osteoconduction appears as promising option to realize bone substitute-based
bone tissue engineering. In the 90ties of the last century, the holy grail of pore size for scaffolds in bone tissue engineering was set between 0.3 and 0.5 mm. More recent, papers from others and us indicated that the optimal microarchitecture for bone tissue engineering scaffolds in terms of pore size, constrictions, rod thickness, or rod distance is still unknown. Additive manufacturing appears as an ideal tool to study those diverse microarchitecture options since it can generate scaffolds where size and location of pores and connections between pores can be tested. For the production of our test scaffolds, we applied laser sintering of titanium and lithography-based additive manufacturing of ceramics. Histomorphometry of calvarial defects in rabbits revealed that bone formation was significantly increased by scaffolds with pore diameters in the range of 0.7-1.2 mm. Scaffolds with pores of 1.5 and 1.7 mm perform significantly worse. Therefore, pore diameters in osteoconductive bone substitutes should be 1.0-1.2 mm and thus much bigger than previously suggested. In essence, osteoconductive microarchitectures of degradable bone substitutes can be realized by lithography based additive manufacturing and this methodology appears as a promising tool for the production of personalized bone tissue engineering scaffolds to be used in cranio-maxillofacial surgery, dentistry, and orthopedics.

S22.5 SYNTHESIS AND CHARACTERISATION OF POLY(VINYL ALCOHOL) HYDROGEL CRYOGENIC SPHERES FOR BIOMEDICAL APPLICATIONS

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Despite poly(vinyl alcohol) (PVA) hydrogel-based drug delivery systems have been extensively studied in the last years, so far there is no research investigating hydrogels in microspherical shape. In the present study, hydrogels for drug delivery systems were obtained from different formulations of poly(vinyl alcohol), poly(acrylic acid), ciprofloxacin and hydroxyapatite (Hap) aqueous solutions and shaped into spheres through dripping the solution into liquid nitrogen at extremely low temperatures. Hydrogels were then strengthened by freeze-thaw cycles. Characterisation of the samples produced aimed to evaluate the thermal (DSC), chemical (EDS), morphology (SEM), drug release properties of the hydrogel and to investigate the influence of each compound on PVA and their biocompatibility. Samples were able to maintain a spherical shape after the freeze-thawing cycles, also, cross-section of these samples revealed different internal structures depending on the components incorporated into the PVA, EDS revealed quantities of Ca and P into these hydrogels due to the HAp and the incorporation of drug, poly(acrylic acid) and hydroxyapatite increased both the melting point and the glass transition temperature of PVA. Ciprofloxacin release exhibited a burst release for approximately two hours, then stabilising the drug release to a maximum of 96.82%. PAA has acted as a release retardant and the burst release was significantly delayed. PAA chains helped encapsulating the drug and reinforced the three-dimensional structure of the hydrogel, hampering ciprofloxacin to be delivered, the total of drug release was 92.11%. Cells mortality rate (MTT) shows that PVA substrates is non-toxic for NRK cells after 24 hours of exposure.

S22.6 FABRICATION OF A SILVER NANOPIRATE-COATED COLLAGEN MEMBRANE WITH CONTAINED ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES

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Orthopaedic infection with bacteria leads to high societal cost and is detrimental to the life quality. Particularly, deep bone infection leading to osteomyelitis results in an inflammatory response whereby localized bone destruction occurs. Current treatments like antibiotic-containing polymethylmethacrylate (PMMA) still has the high risk of bacterial resistance. Taking advantages of silver which has antibacterial and anti-inflammatory effect and bioactive collagen, we fabricated a silver nanoparticle (AgNP)-coated collagen membrane by sonication and sputtering. SEM showed good deposition of AgNPs on collagen membrane by both coating methods. The optimal coating concentration was finalized by assessing optimal antibacterial effect against cytotoxicity and finally collagen membrane coated with 1mg/mL AgNPs solution was selected. We also found that the coated collagen membrane demonstrating short-term cytotoxicity within 24 hours with damage to the cell membrane, which was evidenced by MTS and LDH release test, but had no significant influence (p > 0.05) thereafter. The amount of released AgNPs from coated collagen membrane had negligible cytotoxicity (p > 0.05). Confocal laser scanning microscope displayed similar cell morphology in both coated and uncoated collagen membrane. ELISA and qPCR presented the decreased secretion and expression (p < 0.001) of IL-6 and TNF-alpha. Upregulated expression (p < 0.001) of osteogenesis markers (RUNX2, ALP, and OPN) could be found and this might be attributed to the modified collagen fibre surface coated by AgNPs. Collectively, the osteogenesis induced by AgNPs demonstrates a promising application in orthopaedic surgery for its use both as an antimicrobial agent, and to enhance bone regeneration.

S23.1 TENDON DEGENERATION AND REPAIR - LESSONS FROM TENDON DEVELOPMENT AND AGEING

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There is a growing socio-economic need (i.e. “ageing society”) for effective and reproducible strategies to repair musculoskeletal tissue. In particular, acute tendon injury and chronic tendinopathies remain clinically challenging and novel treatment modalities are urgently needed. Tendons resemble a connective tissue rich in highly organized collagen fibers, displaying a remarkably high tensile strength. However, partly due to the low number of cells and their more or less avascular nature tendons heal relatively slowly. Ultimately, tendon regeneration encompasses the full restoration of the biological, biochemical and biomechanical properties, which are often impaired by endogenous healing cascades. Usually, a connective scar tissue forms at the injury site and the replaced tissue does not function adequately at high strain levels, increasing the chance of re-rupture. Despite significant advancements in tissue regeneration and engineering strategies, the clinical impact for the regeneration of tendon remains limited. For the development of novel methods to repair tendons we need to pin down the molecular and cellular mechanisms amenable to modulate endogenous (or exogenous) cell behaviour towards functional tissue regeneration. By comparing the gene expression profile of Achilles tendon tissue harvested from young-mature and old mice we demonstrate profound changes in the expression of ECM-related proteins and a previously unknown role of Secreted protein acidic and rich in cysteine (Sparc; also known as BM-40 or osteronectin) in tendons. Sparc levels in tendons are critical for proper collagen fibril maturation and its age-related decrease, together with a change in ECM properties potentially drives adipogenic differentiation of tendon stem and progenitor cells (TDSPCs) and consequently lipid accretion in tendons. Generally, the fate of stem/progenitor cells is largely determined by stimuli from the stem cell niche. In tendons, we describe a novel cellular barrier, most likely preventing the leakage of blood-borne products into the tendon proper. We propose that this “blood-tendon barrier” is part of the stem cell niche in tendons controlling TDSCP fate, preventing erroneous differentiation. By investigating the developmental programs driving tendon tissue formation and on the other hand the mechanisms contributing to the senescence of tendons, ultimately resulting in decreased quality of tendons in the elderly, novel targets for clinical intervention potentially can be discovered.
S23.2 MECHANISMS OF TENDON GENERATION, DEGENERATION AND REGENERATION

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Tendon and ligament tissues are fascinating in their simplistic appearance of tissue architecture coupled with outstanding biomechanical properties. In the last decade, the mechanisms governing their development, degenerative disease progression and step-wise repair process are becoming better understood. In this talk, I will present an overview of our basic research work on these following points. (i) Tendon generation: I will discuss our finding on the role of growth and biomechanical factors influencing tendon stem/progenitor cells; (ii) Tendon degeneration: I will provide evidences how disturbed cell-cell and cell-matrix contacts are involved in loss of tissue integrity; (iii) Tendon regeneration: I will present in vivo data on the application and performance of various cell populations in tendon repair.

S23.3 ENTHESIS REGENERATION WITH TOPOGRAPHICALLY DESIGNED SCAFFOLDS AND GROWTH FACTORS

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The structure and extracellular matrix composition of the interface are complex and allow for a gradual mechanical stress transfer between tendons and bone. In this study, biphasic silk fibroin scaffolds designed to mimic the gradient in collagen molecule alignment present at the interface. The scaffolds had two different pore alignments: anisotropic at the tendon side and isotropic at the bone side. Total porosity ranged from 50-80% and the majority of pores were <100-300 μm. Young’s modulus varied from 689-1322 kPa. In addition, human adMSC were cultured on the scaffolds to evaluate the effect of pore morphology on cell proliferation and gene expression. Biphasic scaffolds supported cell attachment and influenced cytoskeleton organization depending on pore alignment. In addition, the gene expression of tendon, enthesis and cartilage markers significantly changed in each region of the scaffolds. We functionalized those scaffolds with heparin and explored their ability to deliver TGF-β2 and GDF5. TGF-β2 and pore anisotropy synergistically increased the expression of tendon/ligament markers and collagen I protein content. The combined delivery of TGF-β2 and GDF5 enhanced the expression of cartilage markers and collagen II protein content on substrates with isotropic porosity, whereas enthesis markers were enhanced in areas of mixed anisotropic/isotropic porosity.

S23.4 RGTA BASED MATRIX THERAPY IN REGENERATIVE MEDICINE: BACKGROUND AND RECENT DEVELOPMENTS IN TENDINOPATHIES

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Matrix therapy is a newly coined name emphasizing the importance of the extracellular matrix in regenerative medicine. Heparan sulfates (HS) are key elements of the extracellular matrix (ECM) scaffold which store and protect most growth factors/cytokines controlling the cell migration and differentiation required for healing
processes. We have engineered biodegradable nano-polymers (alpha 1-6 polyglucose carboxymethyl sulfate) mimicking (RGTA®) to replace destroyed HS in the damaged ECM scaffolding and to protect cytokines produced by healthy neighbouring cells, thereby restoring the ECM microenvironment and tissue homeostasis and, if needed, provide a homing niche for cell therapy. This matrix therapy approach has considerably improved the quality of healing in various animal models, including muscle and tendon, with reduction or absence of fibrosis resulting in a regeneration process. Over 50 000 patients have been treated in the last years for skin and corneal wounds with dedicated products based on this technology. A randomized controlled trial was performed on 22 racing French Standardbred Trotters (ST) horses to evaluate the efficacy of another polymer, OTR4131 Equitend®, to treat tendinopathies. We evaluated the effect versus placebo on acute superficial digital flexor tendonitis over 4 months by clinical and ultrasonographic measures and their racing performances followed up over the 2 years after treatment. A significant reduction on tendon cross section area was measured in treated animals, racing was 2-3 times more often than placebo, with 3.3 times fewer recurrences and pre-injury performance level was maintained. This study may pave the way for development in humans.

S23.5 PULSED ELECTROMAGNETIC FIELDS (PEMFs) STIMULATION FOR ACHILLES TENDINOPATHY: AN IN VIVO MODEL

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Tendon-related pathologies such as tendinopathy represent a relevant clinical and socioeconomic issue. The most innovative and conservative therapeutic approaches are meant to stimulate the intrinsic healing capability of the tissue. In this study, the use of pulsed electromagnetic fields (PEMFs) was investigated in a rat model of Achilles tendinopathy as a potential therapy. Achilles tendinopathy was chemically induced in eighty-six Sprague Dawley rats by injecting collagenase Type I within the tendon fibers. Fifty-six of them were stimulated with PEMFs (8 hours/day, 1.5 ± 0.2 mT; 75 Hz), divided in different experimental groups basing on the starting-time of PEMFs exposure (after 0, 7, 15 after Collagenase injection) and its duration (7, 15 or 30 days). Thirty animals were left unstimulated (CTRL group). According to the different time points, explanted tendons were evaluated through histological and immunohistochemical analyses in terms of matrix deposition, fiber re-organization, neovascularization and inflammatory reaction. The most effective PEMF stimulation was demonstrated in the 15 days of treatment. However, when PEMF were applied immediately after the collagenase injection, no significant therapeutic results were found. On the contrary, when PEMF were applied after 7 and 15 days from the collagenase injection, they promoted the deposition of extracellular matrix and tendon fiber re-organization, reducing both the inflammatory reaction and vascularization, with significant differences compared to the CTRL group (p<0.05). Therefore, these results suggest an effective activity of PEMFs stimulation that provides a satisfying restoration of the damaged tissue, although the most performing protocol of application still needs to be identified.

S23.6 PULSED ELECTROMAGNETIC FIELD (PEMF) EFFECTS ON SOFT TISSUE REPAIR


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PEMF is currently approved by the FDA for adjunctive treatment of lumbar/cervical spine fusion and for treatment of long-bone non-unions. Soft tissues are a potential new therapeutic application for PEMF due to
pre-clinical studies showing a reduction of inflammatory markers following PEMF exposure. The aim was therefore to investigate the structural/functional effects of PEMFs on tendon-to-bone and tendon-to-tendon healing in a rotator-cuff (RC) and Achilles tendon (AT) repair model, respectively. RC study: Adult male rats (n=280), underwent bi-lateral supraspinatus tendon transections with immediate repair followed by cage activity until sacrifice (4, 8, and 16 weeks). Non-controls received PEMF for 1, 3, or 6 hours daily. AT study: Male rats underwent acute, complete transection and repair of the Achilles tendon (FULL, n=144) or full thickness, partial width injury (PART, n=160) followed by immobilization for 1 week. Sacrifice was at 1, 3, and 6 weeks. Outcome measures included passive joint mechanics, gait analysis, biomechanical assessments, histological analysis of the repair site and mCT (humerus) assessment (FULL only). RC study: Significant increases in modulus, stiffness, bone mineral content and improved collagen organization was observed for the PEMF groups. No differences in joint mechanics and ambulation were observed. AT study: A decrease in stiffness and limb-loading rate was observed for the PEMF groups for the FULL groups, whereas an increase in stiffness with no change in range-of-motion was seen for the PART groups. The combined studies show that PEMF can be effective for soft tissue repair but is dependent on the location of application.

S24.1 EXTRACELLULAR MICROENVIRONMENT DICTATES THE FATE OF MESENCHYMAL STROMAL CELLS

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The stem cell fraction of a cell population is finely tuned by stimuli from the external microenvironment. Among these stimuli, a decrease of extracellular pH (pHe) may occur in a variety of physiological and pathological conditions, including hypoxia and inflammation. Also in bone, the maintenance of acid-base balance is fundamental for skeleton homeostasis. Bone cells are extremely sensitive to the effects of interstitial pH. Acidosis inhibits mineral deposition by osteoblasts and activates osteoclast-mediated bone resorption. Moreover, acidosis is associated with inflammation, and in case of bone injury, local short-term acidosis is a crucial regulator of the healing process. Evidence of the role of acidosis as an enhancer of MSC stemness and for their activation as sensors and switcher of inflammation will be discussed.

S24.2 MESENCHYAL STEM CELLS FROM ADIPOSE TISSUE REPRESENT AN ASSET FOR ORTHOPAEDIC REGENERATIVE

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Human mesenchymal stem cells (MSCs) are multipotent stem cells with the ability to differentiate into mesoderm-type cells such as osteoblasts, chondroblast, tenocytes etc. They can be retrieved by different sources, but the number of cells obtained suggested the adipose tissue as a primary harvest site of MSCs. Cells can be harvested using the Coleman procedure, obtaining stromal vascular fraction (SVF), enriched with MSCs, after collagenase digestion. The availability of SVF storage has been envisioned for multiple treatments of the degenerated tissue. Indeed, the use of SVF has been introduced into clinical trials for tissue regeneration for orthopaedic patients. Difficulties of a selective delivery of SVF locally have been previously discussed. Thus, the use of biological scaffolds in order to better localize SVF in the tissue site has been studied. The methodological evolution for the use of SVF in the best possible biological conditions is a milestone for good clinical results.
S24.3 SKELETAL CELL BASED STRATEGIES FOR BONE REPAIR - OPPORTUNITIES AND CHALLENGES

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Advances in our understanding of skeletal stem cells and their role in bone development and repair, offer the potential to open new frontiers in bone regeneration. However, the ability to harness these cells to replace or restore the function of traumatised or lost skeletal tissue as a consequence of age or disease remains a significant challenge. We have developed protocols for the isolation, expansion and translational application of skeletal cell populations with cues from developmental biology informed by in vitro and ex vivo models as well as, nanoscale architecture and biomimetic niche development informing our skeletal tissue engineering approaches. We demonstrate the importance of biomimetic cues and delivery strategies to directly modulate differentiation of human adult skeletal cells and, central to clinical application, translational studies to examine the efficacy of skeletal stem and cell populations in innovative scaffold compositions for orthopaedics. While a number of challenges remain multidisciplinary approaches that integrate developmental and engineering processes as well as cell, molecular and clinical techniques for skeletal tissue engineering offer significant promise. Harnessing such approaches across the hard tissue interface will ultimately improve the quality of life of an increasing ageing population.

S24.4 SUSTAINED RELEASE OF TARGETED THERAPY WITH A REPLENISHABLE IMPLANT RESERVOIR

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The clinical translation of regenerative therapies, whether in the form of mesenchymal cells, macromolecules or small molecules, is hampered by several factors: the poor retention and short biological half-life of the therapeutic agent, the adverse side effects from systemic delivery, and difficulties with the administration of multiple doses to a target site. We report the development and application of a therapeutic reservoir device that enables sustained and repeated administration of small molecules, macromolecules and cells directly to organs and tissues of interest via a polymer-based reservoir connected to a subcutaneous port. In a myocardial infarct rodent model, we show that repeated administration of cells over a four-week period using the reservoir provided functional compared to a single injection of cells and to no treatment. Recent advances of the system include a multi-port and multi-reservoir system that can be tailored to cargo and application need. The pre-clinical use of our therapeutic reservoir as a research model may enable insights into regenerative orthopaedic therapy, particularly those therapies that require multi-dose approaches.

S24.5 THE ROLE OF CELL DEATH IN MESENCHYMAL STEM CELL THERAPY FOR OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative disease with a strong inflammatory component. Intra-articular (IA) injections of mesenchymal stem cells (MSCs) modulate local inflammation, although the lack of engraftment suggests that they undergo apoptosis. The aim of this study is to investigate the fate of IA-delivered MSCs in an animal model of OA and to assess the role of apoptosis in vitro. Collagenase-induced OA (CIOA) was performed on C57BL/6 mice and 2×10^5 GFP+ MSCs were IA-injected in the animals. 3 days later, knee joints were digested into a single-cell suspension and MSCs retrieved by cell sorting. Conditioned medium (CM) of retrieved cells was tested on murine macrophages and cytokine secretion was measured. Apoptosis of MSCs was induced in vitro with staurosporine (STS) and evaluated by Annexin V/Sytox Blue staining; activation of caspases was measured by FLICA assays. Murine lymphocytes were cocultured with apoptotic MSCs and their proliferation measured by quantification of Cell Trace Violet. 1.63% of injected cells were retrieved and proliferated in culture. Their CM significantly modulated activation of macrophages, with greater effects from OA-induced MSCs. STS induced apoptosis with activation of Caspase 3/7. Apoptotic MSCs significantly prevented the proliferation of murine lymphocytes. MSCs can be administered and retrieved from murine knees. Retrieval yield is low, consistent with previous studies. MSCs were licensed from the OA joint to produce an immunosuppressive milieu that modulated macrophages ex vivo. In vitro, apoptosis increased the immunomodulatory potential of MSCs. This suggests that apoptosis may contribute to the therapeutic effects of MSCs in OA.

S24.6 ASSESSMENT OF NOVEL MACROMOLECULAR CROWDERS TO PRODUCE CELL SHEETS FOR SCAFFOLD-FREE MUSCULOSKELETAL TISSUE REGENERATION


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Cell-based scaffold-free tissue equivalents present a limited clinical translation as consequence of the delayed extracellular matrix (ECM) deposition due to the prolonged production time in vitro. Different combinations of media supplements such as ascorbic acid, growth factors, oxygen tension among others can modulate the cell fate or the ECM synthesis. New research lines are focusing on the use of macromolecular crowders (MMCs) as media supplement for cell sheet production due to their ability to increase ECM deposition by volume exclusion effect, pro-collagenases alosteric regulation, matrix self-assembly by confinement and diffusion limitation (most probably, modulating the interaction between the ECM, MMPs and TIMPs). Herein, different molecular weights and concentrations of a natural potential MMC (Crowder-A) have been tested in equine adipose-derived stem cell (eADSC) and human dermal fibroblast (hADF) cultures in comparison with other commonly used crowders such as carrageenan and the Ficoll™ cocktail 70 KDa and 400 KDa. The eADSCs were characterized according to the current criteria for horse MSCs. Tri-lineage and FACS analysis showed eADSC osteogenic and adipogenic potentials and the presence of the markers CD29, CD44, CD90. The screening of the aforementioned Crowder-A was performed in cultures of 15,000 cells / cm² for the eADSCs and 25,000 cells / cm² for the hADFs during 3, 5, and 7 days. Non-MMC conditions were used as negative controls. Collagen type I was analysed by SDS-PAGE. Other collagen types were studied by immunocytochemistry assays. Significant increase of some ECM components was observed in some concentrations and molecular weights of the Crowder-A.
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Osteoarthritis (OA), characterised by pain, disability and joint degeneration, is common and has no cure. Prevalence of severe radiographic knee OA is 19% in over 45’s and 50% in over 75’s in the US and Europe. Abnormal joint loading, or injury, increase risk of OA. We have discovered that glutamatergic signalling is mechanically regulated and glutamate receptors (GluR) drive inflammation, degeneration and pain representing potential drug targets in osteoarthritic joints. Joints from OA and knee injured patients, and rodent models of arthritis, show increased synovial fluid glutamate concentrations and abundant GluR expression. Since AMPA/kainate GluRs regulate IL-6, a critical mediator of arthritic degeneration, we tested protective effects of the AMPA/KA GluR antagonist, NBQX in animal models of arthritis. In rodent antigen induced arthritis, and osteoarthritis (meniscal transection and anterior cruciate ligament rupture), NBQX reduced joint swelling, degeneration and pain, exceeding anti-degenerative effects of other drugs tested similarly. 3D osteocyte/osteoblast co-cultures and human bone samples taken from patients undergoing high tibial osteotomy joint realignment surgery, revealed underlying cellular mechanisms mediated by bone cells. Related drugs, already used in humans for epilepsy and migraine, represent a repurposing opportunity and are effective in our models of arthritis.

S25.2 THE ROLE OF SUBCHONDRAL BONE DAMAGE AND BONE MARROW LESIONS IN POST-TRAUMATIC OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common musculoskeletal disease in the EU and is characterized by cartilage degeneration, pain and restricted movement. Post-Traumatic OA (PTOA) is a specific disease subset that occurs subsequent to traumatic injury, such as ACL rupture and makes up 12% of the overall disease burden. Our current understanding PTOA is that initial injury affects multiple tissues, and many/all contribute to overall ‘joint failure.’ MRI scans show that subchondral bone marrow lesions (BMLs) are present in 80% of ACL rupture cases in the immediate aftermath of joint injury. Their presence indicates an acute consequence in subchondral bone. It has also been suggested that BMLs overlap with, or directly represent, bone microdamage. Microdamage is known to induce osteoclast-mediated remodelling in bone. Therefore, the inhibition of subchondral bone remodelling, particularly in the early phase post-injury, may be a candidate therapeutic approach for preventing PTOA. Finally, the contiguous link between subchondral bone and articular cartilage, can allow transport of small molecules across this boundary, this suggests that bone/cartilage crosstalk is likely to be a key factor in PTOA development after injury. This presentation will summarize recent advances in our understanding these phenomena in both animal and human studies.

S25.3 MICROENVIRONMENTAL REGULATION OF OSTEOARTHRITIC CHONDROCYTE FUNCTION

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Onset and progression of osteoarthritis (OA) is affected by a plethora of factors, including joint injury, obesity, aging, and heredity. This multi-factorial etiology obstructs our understanding of driving molecular mechanisms, which likely comprise an interplay between systemic and local factors. Next to biomechanical...
factors and cytokines, the course of OA appears to be altered by microenvironmental oxidative stress: cumulative evidence now suggests a prominent participation of cell signalling mediated by nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a master regulator of cellular protective processes, in this process. Nrf2 activation through phosphorylation of mitogen-activated protein kinases (MAPKs) regulates Nrf2 target genes, like hemeoxygenase-1 (HO-1), superoxide dismutase 2 (SOD2), or NAD(P)H Quinone Dehydrogenase 1 (NQO1) in OA chondrocytes. Maintaining high levels of HO-1 appears to be beneficial against OA development. Experimental manipulation of putative antioxidant response element (ARE) binding sites alters the in vitro expression of key transcription factors of chondrocyte markers in promoter-reporter assays. Potentially, Nrf2 is involved in autophagy, intermediary metabolism and unfolded protein response. RNAi-mediated depletion of Nrf2 further significantly abrogated anti-inflammatory and chondroprotective effects and epigenetics link transcriptional pathways of ‘N-factors’, Nrf2 and NFATs, to micro-RNA signalling. Current findings thus reveal novel mechanisms regulating extracellular matrix synthesis by chondrocytes. A further understanding of these pathways and their regulation will lead to important novel targets to slow OA progression.

S25.4 MICRORNA AND HISTONE ASSEMBLY REGULATION OF OSTEOARTHRITIS

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Chondrocyte dysfunction is attributable to the development of osteoarthritis (OA). Deregulation of chondrogenic regulators and deleterious factors, e.g. proteinases, Wnt signalling components, and autophagy repressors lowers chondrogenic activities and ultimately deteriorates cartilage homeostasis. Emerging evidence is that epigenetic pathways, including non-coding microRNAs and histone remodelling switch on/off the expression of joint-deleterious factors. MicroRNAs reduces the expressions of mRNAs through binding to the 3'-untranslation regions of targets. The levels of microRNAs, e.g. miR-29a, miR-128a in serum, synovial fluid, synovium, and cartilage are correlated with the occurrence of OA. Mice overexpressing/deficient microRNAs of interest show minor responses to OA progression. Besides, acetylation and methylation statuses of histones regulate the factors detrimental to chondrocytes through altering the interactions between histones and promoters. Histone deacetylases and demethylases, e.g. HDAC4, SIRT1, and EZH2 contribute to the modification reactions of histones, which modulate cartilage matrix metabolism. An intricate nature is that reciprocal actions between microRNAs and histone deacetylase/demethylase are indispensable in chondrocyte survival and function. Administrations with specific inhibitor/agonists for microRNAs and histone deacetylases/demethylase enable joints to show minor responses to articular injury, which mitigate the pathogenesis of OA. This talk highlights the biological roles and therapeutic advantage of epigenetic microRNAs and histone remodelling in OA.

S25.5 DNA METHYLATION INHIBITOR REGULATES BROWN AND WHITE MARROW ADIPOCYTE REDISTRIBUTION IN OSTEOPOROTIC BONE

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Fatty marrow and bone loss are prominent pathologic features of osteoporosis. DNA hypermethylation shifts mesenchymal stem cells towards adipocytes impairing bone formation. Brown adipocytes produce growth factors advantageous to osteogenesis, whereas white adipocytes secrete pro-inflammatory cytokines
deleterious to bone homeostasis. We assess DNA methylation inhibitor action to brown and white adipocyte formation in marrow fat of osteoporotic skeletons. Osteoporotic skeletons in mice were induced by glucocorticoid, ovariectomy or ageing. Marrow adipose volume and bone structure were quantified using OsO4 contrast-μCT imaging. Brown and white adipocytes were probed using immunostaining, RT-PCR and primary bone-marrow mesenchymal stem cell cultures. Abundant marrow fat and spare trabecular bone existed in osteoporotic skeletons. Osteoporosis increased expressions of general adipogenic markers PPARγ2 and FABP4 and white adipocyte markers PGC-1α and UCP-1 and osteogenic markers Runx2 and osteocalcin were significantly decreased. Number of UCP-1 immunostaining-positive brown adipocytes also reduced in osteoporotic bone. In vitro, DNA methylation inhibitor 5’-aza-deoxycystidine significantly increased brown adipocyte formation and osteogenic differentiation and mitigated dexamethasone-induced white adipocyte formation in mesenchymal stem cells. 5’-aza-deoxycystidine control of brown adipogenesis and white fat formation appeared to be regulated by increasing Wnt3a/β-catenin and reducing Dkk1. Disintegrated brown adipocyte and white fat cell differentiation contribute to osteoporosis pathogenesis. Maintaining DNA hypomethylation promotes Wnt signalling and brown adipocyte differentiation facilitating osteogenic differentiation. This study shed a new light to the contribution of brown adipocytic cells to bone metabolism during osteoporosis.

S25.6 PRINTING OSTEOARTHRITIS MODELS FOR DRUG TESTING


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This paper reports on a proof of concept project funded by the UK National Council for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), with the aim of developing an in vitro model to recapitulate the human osteoarthritic joint, based on a multiple human cell type co-culture system, for research and drug development in OA. The targets were: (i) the development of a cell culture platform that could produce a mixed stable cell culture of cell types that represent the key components of the human joint: synoviocytes – type I and type II; osteoblasts; osteoclasts; chondrocytes/cartilage or cartilage-like matrix; adipocytes; and immune cells. (ii) demonstration of cell phenotype stability and viability for at least 72 hours. In order to establish the cell culture platform we have developed an eight-channel cell printer, capable of accurately and reliably printing the required cell types to create osteochondral and synovial cell types within a transwell system. Two different sets of cells have been developed and processed using the cell printer: a set based on using an immortalised hTERT MSC line to create osteoblasts, chondrocytes and adipocytes, with commercial cells lines providing the other cell types, and a set obtained from tissue excised during orthopaedic surgery. This gives both a repeatable set of cells with which to undertake mode of action studies, and a bank of cell sets which will be representative of different stages of osteoarthritis. The co-cultures have been immunohistochemically assessed in order to demonstrate maintenance of phenotype.

S26.1 CROSS-TALK BETWEEN MUSCLE AND CARTILAGE: THE MYOKINE IRISIN ATTENUATES OSTEOARTHRITIS-RELATED CARTILAGE DEGENERATION

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Irisin is a hormone-like myokine released from skeletal muscle during exercise. It has also been reported that irisin levels in serum and synovial fluid of knee osteoarthritis (OA) patients were negatively correlated with
OA severity. We hypothesized that irisin might play a role in the cartilage homeostasis mediated by physical activity. Therefore, this study aims to explore the cross talk between skeletal muscle and cartilage tissues in human with OA mediated by the myokine irisin. Human articular OA chondrocytes were isolated, expanded and cultured in micro-mass 3-D culture system. Pellets were cultured with or without r-Irisin, and then activated by protein inhibitors of p38-MAPK signalling pathway. After one week the amount of GAG content was evaluated. Quantitative gene expression of Coll-X and Coll-II was performed. WB was utilized to detect expressions of p38-MAPK signalling pathway and Coll-X and Coll-II. In the current study, chondrocytes cultured in r-Irisin showed a significant higher GAG/DNA content compared to control (p<0.05). Moreover, r-Irisin promoted a significant increase of the expression collagen type II and decrease of collagen type X in (p<0.05). This OA chondrocytes recovery was abrogated by the p38 MAPK and ERK signalling pathways. Our observation suggests that Irisin targets chondrocytes promoting GAG content, increasing Collagen Type II and decreasing Collagen type X gene expressions. The observed OA chondrocyte recovery mediated by irisin is obtained through the inactivation of p38/ERK MAP kinase signalling cascades in vitro. This is the first study that demonstrates a cross-talk between muscle and cartilage mediated by irisin.

S26.2 THE ROLE OF ORTHOBIOLOGICS IN TREATING OSTEOCHONDRAL LESIONS

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The goal of surgery for osteochondral lesions is to regenerate the damaged cartilage with ideally hyaline cartilage. The current gold standard treatment is bone marrow stimulation (BMS) by microfracture. In reality however BMS typically results in the generation of fibrocartilage. Orthobiologics including bone marrow aspirate, platelet rich plasma and hyaluronic acid products have been shown to promote cartilage healing and potentially increase the formation of hyaline cartilage in treated lesions. However the role of these products, the timing of their administration and frequency of application are still not clearly defined and their routine use is still not recommended. These issues and future directions for research and future clinical application will be discussed.

S26.3 BIZONAL CARTILAGE CONSTRUCTS DEVELOPING BOTTOM ZONE–RESTRICTED IN VIVO MINERALIZATION

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Engineered cartilage is poorly organized and fails to recapitulate physiologic organization in a hyaline upper and a mineralizing bottom zone deemed important for proper function. Objective was to grow bizonal human cartilage constructs in which \textit{in vivo} mineralization is self-restricted to the bottom zone. Self-assembling biomaterial-free cell discs were generated from mesenchymal stroma cells and allowed to accumulate proteoglycans and collagen-type II over 3 weeks. \textit{In vitro} mineralization of the cell discs with four mineralization media for up to 8 weeks showed that calcification was supported in all media containing β-glycerophosphate. However, proteoglycans were retained only in media containing insulin. Bizonal cartilage constructs were made from 3-week non-mineralized cell discs overlaid with chondrocyte-seeded starPEG-heparin hydrogel or with a fibrin-gel layer to select the best design for upper zone development. Freshly prepared zonal constructs were implanted into subcutaneous pouches of immuno-deficient mice to compare \textit{in vivo} development. After 6 weeks \textit{in vivo}, both construct types were rich in collagen-type II in the upper zone and contained a mineralized bottom zone. However, solely for starPEG constructs, tissue volume of the upper
zone remained high and alkaline phosphatase, alizarin red, and collagen-type X staining were restricted to the bottom zone. StarPEG zonal constructs were superior to fibrin constructs due to self-restriction of mineralization and hypertrophic markers to the bottom zone. This innovative design of bizonal constructs offers the successful generation of an organized cartilage resembling the native cartilage with the chance for immediate use of autogenous chondrocytes in a one-step surgical joint intervention.

**S26.4 MODULATION OF INFLAMED SYNOVIIUM AND ITS MACROPHAGES IMPROVES CHONDROGENESIS OF BONE MARROW STROMAL CELLS**

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Joint injuries often result in inflammation and cartilage defects. When inflamed, the synovium secretes factors that prevent successful cartilage repair by inhibiting chondrogenic differentiation of progenitor cells. In particular the pro-inflammatory macrophages in the synovium are indicated to contribute to this anti-chondrogenic effect. Thus, we aimed to counteract the anti-chondrogenic effect of inflamed synovium by modulating synovial inflammation and its macrophages. Synovium tissue obtained from osteoarthritic patients undergoing a total knee replacement was cut into explants and cultured for 72 hours +/- 1 μM of the anti-inflammatory drug triamcinolone acetonide (TAA) (Sigma Aldrich). TAA significantly decreased gene expression of TNFA, IL1β and IL6, and increased expression of CCL18, IL1RA in synovial explants (all with p < 0.001). On the other hand, TAA significantly decreased the percentages of pro-inflammatory CD14+/CD80+ and CD14+/CD86+ macrophages in the synovium (both p < 0.001) as assessed by flow cytometry analyses. The percentages of anti-inflammatory CD14+/CD163+ macrophages, is significantly increased (p < 0.001) in TAA treated synovium. Conditioned medium (CM) from synovium explants downregulated the gene expression of cartilage matrix components collagen type-2 and aggrecan expression in chondrogenic MSCs. This chondrogenesis inhibiting effect was reduced by treating synovium with TAA during the production of the CM. Our findings indicate that reducing synovial inflammation might improve the joint environment for better cartilage repair, possibly by modulation of macrophage phenotypes.

**S26.5 THE FIBRILLAR NETWORK OF THE LAMINA SPLENDENS OF ARTICULAR CARTILAGE**

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High resolution imaging techniques such as atomic force microscopy, provide a platform to study the fibrillary architecture of biological tissues, but are not capable of imaging the internal microstructure of tissues in 3D. Conversely, multiphoton microscopes facilitate 3D imaging to study the spatial relationship of micro-components within tissues, but without the resolution of atomic force microscopy. The lamina splendens is the most superficial layer of articular cartilage. It is believed to play a crucial role in the health of the tissue. However, the precise form of this layer is uncertain as it has never been independently studied. Here, we use multiphoton microscopy and atomic force microscopy to demonstrate the anatomic form of the lamina splendens. The lamina splendens were peeled from the femoral condyles of healthy, adult sheep (n=20). Using atomic force microscopy, we show that the collagen and elastin form an interweaving fibrillary network at the surface of the lamina splendens and at the interface of the lamina splendens with the underlying cartilage. Moreover, using fluorescent stains; sulforhodamine B and acridine orange, multiphoton microscopy shows the heterogeneous distribution of collagen, elastin and chondrocytes throughout the depth of the lamina splendens.
Our results demonstrate the fibrillar and component level architecture of the lamina splendens. We believe our findings provide the backbone of knowledge to advance tissue engineering techniques that will lead to more promising strategies to treat cartilage pathologies, including osteoarthritis. Furthermore, our results provide a starting point to determine the role of the lamina splendens in cartilage pathology.

**S26.6 PHYSIOXIA MODULATION OF IL-1 INHIBITED CHONDROGENESIS**

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Mesenchymal Stem Cells (MSCs) are a candidate cell type for treating osteoarthritic focal defects. *In vivo*, cartilage and bone marrow reside under a low oxygen tension, between 2-7% oxygen or physioxia, that has been shown to enhance MSC chondrogenesis. However, chondrogenesis is inhibited in the presence of IL-1. Here, it was hypothesized that physioxia reduces IL-1 inhibited chondrogenesis. Human MSCs (Mean age, 32 years; \( n = 9 \)) were split equally for expansion under either 2% (physioxia) or 20% (hyperoxia) oxygen. Chondrogenic pellets (2 x 10^6 MSCs/pellet) were formed and cultured in the presence of 10 ng/ml TGF-b1 and in combination with either 0.1 or 0.5 ng/ml IL-1 under their respective expansion conditions. Pellets were assessed for their wet weight, GAG and collagen II content and evaluated histologically (Collagen X and MMP-13). Statistical analysis was performed using a Two-way ANOVA with Tukey post-hoc test, significant differences stated when \( p < 0.05 \). A significant dose-dependent IL-1 inhibition in chondrogenesis was observed for pellet wet weight and GAG content under hyperoxia (\( p < 0.05 \)). Physioxia alone significantly increased wet weight, GAG and collagen II content (\( p < 0.05 \)) compared to hyperoxia. A donor-dependant response was observed, whereby 80% of donors responded to physioxia and their analysis showed significant increases in wet weight and GAG content in the presence IL-1 (\( p < 0.05 \)). Furthermore, reduced hypertrophy marker expression (Collagen X and MMP-13) was observed under physioxia in the presence of IL-1. The molecular signalling mechanisms controlling these responses are to be investigated.

**S27.1 BIODEGRADABLE METALS WITH EXTREME PROPERTIES FOR INNOVATIVE BIOMATERIALS**

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Over the last 50 years, biomaterials, prostheses and implants saved and prolonged the life of millions of humans around the globe. The main clinical complications for current biomaterials and artificial organs still reside in an interfacial mismatch between the synthetic surface and the natural living tissue surrounding it. Today, nanotechnology, nanomaterials and surface modifications provides a new insight to the current problem of biomaterial complications, and even allows us to envisage strategies for the organ shortage. Advanced tools and new paths towards the development of functional solutions for cardiovascular clinical applications are now available. In this talk, the potential of nanostructured metallic degradable metals to provide innovative solutions at medium term for the cardiovascular field will be depicted. Focus will be on Fe-based biodegradable metals with exceptional resistance, ductility and elasticity, for pushing innovative vascular applications. The intrinsic goal of this talk is to present an extremely personal look at how biodegradable metals can impact materials, surfaces and interfaces, and how the resulting unique properties allowed biomedical functional applications to progress, from their introduction, to the promising future that biodegradable metals may or may not hold for improving the quality of the life of millions worldwide.
S27.2 BIODEGRADABLE ZN BASED ALLOYS DESIGNED FOR FUTURE ORTHOPEDIC APPLICATION

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In order to evaluate the feasibility of zinc alloys as future biodegradable bone implant materials, the mechanical properties, corrosion resistance, hemocompatibility, cell activity, proliferation and adhesion, in vivo animal implantation experiments have been employed. The experimental results show that the alloying element magnesium, calcium and strontium can significantly improve the mechanical properties of pure zinc, and further deformation processes can further improve the mechanical properties of zinc alloys. Alloying elements can effectively control the corrosion rates of zinc alloys, which are between the rates of magnesium alloys and iron alloys. Zinc and zinc alloys exhibit excellent hemocompatibility and the hemolysis rate is far lower than 5%. After adding alloying elements Mg, Ca and Sr, MG63 and ECV304 cell proliferation rate and activity increased significantly, while for VSMC cell, the influence of alloying elements effect is not obvious. Zinc alloy intramedullary pins can effectively promote the new bone formation, and after 2 months implanted in mice femur, they still maintained a relatively complete structure, indicating that they are able to provide enough mechanical strength and thus more conducive to bone tissue repair and healing.

S27.3 DESIGN AND CHARACTERIZATION OF SYNTHETIC BIODEGRADABLE FILMS FOR MUSCULOSKELETAL TISSUE ENGINEERING


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To repair soft tissue, it is vital to ensure that the biomaterial is able to mimic the complex elasticity of the native tissue. It has been demonstrated that substrate stiffness has a huge influence on cellular growth, differentiation, motility and phenotype maintenance. The goal of the present study is to characterize extensively a set of polymeric films with variable mechanical profiles. A range of synthetic biodegradable polymers was selected according to the physico-chemical intrinsic properties of aliphatic polymers. They have similar chemistry (absorbable polyesters made from lactic acid, glycolic acid, trimethylene carbonate, dioxanone & β-caprolactone), however show different mechanical and degradation properties. The films were manufactured by thermal presser and then characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FTIR). The mechanical properties of the films were assessed by uniaxial tensile tests in wet conditions and also by atomic force microscopy (AFM) to assess the material’s stiffness at a micro-level. In vitro assays were performed to assess the cell cyocompatibility, proliferation and differentiation potential of the films. The mechanical properties of the materials are within the range intended for musculoskeletal tissue repair. Biological assays showed good cell adhesion, cell proliferation and cell viability. Stem cells were able to differentiate into adipogenic, osteogenic, chondrogenic and tenogenic lineages. Overall the selection of polymers gave good options for a potential tissue repair scaffold. In the future, the combined effect of stiffness and topography will be assessed on cell phenotype maintenance.
S27.4 DECELLULARISED PORCINE PERITONEUM AS A MULTIFUNCTIONAL MATERIAL FOR TENDON TISSUE ENGINEERING

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Collagen materials are extensively used in regenerative medicine. However, they still present limitations such as a mono-domain composition and poor mechanical properties. On the other hand, tissue grafts overcome most of these limitations. In addition, the potential of tissue grafts in musculoskeletal tissue engineering has not been fully investigated. Herein, we ventured to assess the potential of a decellularised porcine peritoneum for musculoskeletal applications by comparing its characteristics with a commercial collagen scaffold employed in tendon. Results indicated that the porcine peritoneum had higher mechanical properties and a lower crosslinking ratio (p < 0.01). Furthermore, it presented a lower resistance to collagenase digestion, which suggests a faster remodelling in vivo of the tissue graft. Immunohistochemistry analysis showed a preserved and multicomponent structure in the porcine peritoneum contrary to the collagen matrix, confirming the multifunctional nature of the tissue graft. Regarding the cell-response assessment, tenocytes and ADSCs were able to grow on both materials, however, proliferation was enhanced by the porcine peritoneum (p<0.01). Immune response by THP-1 showed an acute inflammatory response by macrophages to the collagen matrix, contrary to that observed in the porcine peritoneum which triggered a mild reaction. The in-progress in vivo study in a rabbit tendon model will elucidate the potential of porcine peritoneum for tendon repair applications. The present study shows how the multifunctionality of the porcine peritoneum provides higher cytocompatibility than a mono-domain collagen matrix with human tenocytes and ADSC. Besides, its lower immune response in vitro suggests better remodelling after implantation.

S27.5 A NEW COMPOSITE BIOMATERIAL OF OSTEOINDUCTIVE NANOHYDROXYAPATITE (NHAP), SYNTHETIC POLYMER (PLA-PEG) AND BONE MORPHOGENETIC PROTEIN-2 (RHBMP-2) FOR BONE REGENERATION

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Sustained release of BMP-2 is reported to be able to reduce the required dose of BMP-2 for bone induction. Nanohydroxyapatite (nHAp) has an osteoinduction capability which is lack in conventional hydroxyapatite. In this study, we combined PLA-PEG with nHAp and investigated the bone regenerative capacity of the newly established composite material of rhBMP-2/PLA-PEG/nHAp in a rat model of spinal fusion. The PLA-PEG was liquidized in acetone and mixed with nHAp and rhBMP-2. The sheet-shaped BMP-2/PLA-PEG (5mg)/nHAp (12.5mg) composites were prepared while evaporating the acetone. The release kinetics of rhBMP-2 from the composite was investigated by ELISA. In vivo bone formation was investigated by posterolateral spinal fusion in rats (the dosage of rhBMP-2; 0μg/ 0.5μg / 3μg). Bone formation was assessed by μCT and histology at post-op. 8 weeks. The composite showed the burst-release in the initial 24 hours (69% of total release) and the subsequent sustained-release for 25 days. According to μCT and histology of the spinal fusion experiment for all groups the bone formation was observed. While no bony bridging was observed in 0 μg and 0.5 μg BMP groups; in 3 μg group bony bridging and fusion were achieved. We developed a new technology for bone regeneration with rhBMP-2/PLA-PEG/nHAp composite. The reduction in the required
dose of BMP-2 for bone induction was achieved. This result can be explained by the high bone induction ability of nHAp and sustainable release of BMP from PLA-PEG in the composite.

**S27.6 DIRECT METAL PRINTED BIODEGRADABLE POROUS MAGNESIUM SCAFFOLDS FOR ORTHOPAEDIC APPLICATIONS**

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The ideal bone substituting biomaterials should possess bone-mimicking mechanical properties; have of porous interconnected structure, and adequate biodegradation behaviour to enable full recovery of bony defects. Direct metal printed porous scaffolds hold potential to satisfy all these requirements and were additively manufactured (AM) from atomized WE43 magnesium alloy powder with grain sizes between 20 and 60 μm. Their micro-structure, mechanical properties, degradation behavior and biocompatibility was then evaluated *in vitro*. Firstly, post-processing values nicely followed design parameters. Next, Young’s moduli were similar to that of trabecular bone (i.e., $E = 700–800$ MPa) even after 28 days of simulated *in vivo*-like corrosion by *in vitro* immersion. Also, a relatively moderate hydrogen evolution, corresponding to a calculated 19.2% of scaffold mass loss, was in good agreement with 20.7% volume reduction as derived from reconstructed μCT images. Finally, only moderate cytotoxicity (i.e., level 0, <25%), even after extensive ISO 10993-conform testing for 72 h using MG-63 cells, was determined using WE43 extracts (2 way ANOVA, post-hoc Tukey's multiple comparisons test; $\alpha = 0.05$). Cytotoxicity was further evaluated by direct live-dead staining assays, revealing a higher cell death in static culture. However, intimate cell-metal contact was observed by SEM. In summary, while pure WE43 may not yet be an ideal surface for cell adhesion, this novel AM process allows for adjusting biodegradation through topological design. Our approach holds tremendous potential to develop functional and biodegradable implants for orthopaedic applications.

**S28.1 MESENCHYMAL STROMAL CELL THERAPY SUPPORTS DIABETIC FEMORAL FRACTURE HEALING**

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Long bone fractures in patients with diabetes mellitus (DM) are slow to heal, often resulting in delayed reunion or non-union. It is reasonable to postulate that the underlying cause of these DM-associated complications is a reduced population of bone marrow progenitor cells and/or their dysfunction. With the hypothesis that the administration of healthy, allogeneic adult bone marrow-derived mesenchymal stromal cells (MSCs) can enhance DM fracture healing, the aim of this endeavour was to assess the efficacy of MSC administration to support fracture repair using two doses. Here 250,000 or 500,000 human bone marrow-derived MSCs were locally introduced to femoral fractures in diabetic mice, and the quality of de novo bone assessed 8 weeks later. Preliminary bone bridging was evident in all animals; however, a large circumferential reparative callus was consistently retained indicating non-union. Micro-CT analysis elucidated consistent callus dimensions, bone mineral density, bone volume/total volume in all groups, but an increase in bone surface area/bone volume in cell-treated fractures. Moreover, greater amounts of mature bone were identified in fractures treated with a low dose of MSCs. Four-point bending evaluation of the mechanical integrity of the repairing fracture indicated a statistically significant improvement in flexure strength and flexure modulus in DM fractures treated with
250,000 MSCs as compared to controls. An improvement in total energy required for failure was observed in both groups that received MSCs. Therefore, the administration of non-DM bone marrow-derived MSCs supported the development of more mature bone in the reparative callus, resulting in greater mechanical integrity.

S28.2 COX-2 EXPRESSION IN OSTEOCLASTS PROMOTES FRACTURE HEALING

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Cyclooxygenase-2 (COX-2) activity is necessary for fracture healing to proceed normally. In most cell types, COX-2 is inductively expressed and acts in a coordinated pathway to produce prostaglandins, which affect many physiological processes including inflammation. In the fracture callus, however, COX-2 expression and the molecular and cellular processes affected by COX-2 activity remain poorly understood. Using LC-MS/MS and xMAP, we measured fracture callus prostaglandin and inflammatory cytokine levels. We found that inflammatory cytokines rapidly peaked after fracture before declining to normal levels by day 4 after fracture. However, callus prostaglandin levels did not peak until 4 days after fracture before returning to normal levels by day 10. We used immunohistochemistry to detected COX-2 expression in callus cells and found that COX-2 was expressed in callus chondrocytes and osteoclasts during endochondral ossification, including those osteoclasts at the callus chondro-osseous junction. Targeted deletion of the COX-2 gene (Ptgs2) in osteoclasts or in chondrocytes was found to delay fracture healing. Using cell-based experiments, we found that COX-2 expression could be induced in osteoclasts by osteopontin treatment, suggesting an integrin-dependent induction of COX-2 expression in osteoclasts. This was confirmed in vivo using mice lacking osteopontin or integrin β3. Immunohistochemistry also showed abundant osteopontin expression at the callus chondro-osseous junction. The results indicate that COX-2 expression in osteoclasts is controlled by integrin-dependent signalling, that COX-2 expression in osteoclasts and chondrocytes is necessary for fracture healing to proceed normally, and that COX-2 expression in chondro-osseous junction osteoclasts may be induced by osteopontin-dependent signalling by chondrocytes.

S28.3 A QUALITATIVE STUDY ON THE PERCEPTION OF DIABETES MELLITUS-RELATED OSTEOPATHY IN INDIVIDUALS LIVING WITH TYPE 1 DIABETES MELLITUS.

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Patients living with type 1 diabetes mellitus (T1DM) can develop early onset osteoporosis and are exposed to an increased risk of fracture. Skeletal health can be influenced easily with diet and exercise. However, diabetes mellitus (DM)-related osteopathy is not emphasized in the public information campaigns on the American Diabetes Association, Diabetes UK, Diabetes Ireland or International Diabetes Federation websites. This investigation aims to assess the perceptions of patients regarding living with T1DM and their baseline knowledge on DM-related osteopathy. A survey was administered to 102 consenting individuals living with T1DM in attendance at the Galway University Hospital Diabetes Centre. Of the respondents, 44% were female and 56% male (mean age of 43). Respondents had T1DM for a mean of 21 years. Participants were asked to identify DM-related complications, including bone thinning and bone fractures. Respondents were primarily concerned about developing DM-related blindness, kidney damage and amputations, but not osteopathy. 49% of respondents did not identify osteopathy as a potential DM-related complication, 28% of respondents related
DM with bone thinning and bone fractures, and 22% individuals only identified bone thinning or bone fractures. When asked for their primary source of DM-related information, endocrinologists and internet where identified. When comparable questions were asked of DM-related healthcare professionals, 56% did not recognize osteopathy as a complication of T1DM. This study demonstrated a low-level awareness of the impact living with T1DM has on bone health. The deployment of patient-interactive activities or educational modules may enhance the future health of individuals living with T1DM.

**S28.4 DIFFERENCES IN METAPHYSEAL AND CORTICAL BONE REGENERATION USING LOCAL DELIVERY OF BONE MORPHOGENIC PROTEIN-2 AND ZOLEDRONIC ACID: A STEP TOWARDS GUIDED TISSUE ENGINEERING**


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Metaphyseal fracture healing is important in joint-adjacent fractures and appears to differ from diaphyseal healing. We recently found that a biomaterial delivering bone morphogenic protein-2 (BMP-2) and zoledronic acid (ZA) healed the metaphyseal bone in a tibial defect but failed closing the cortical defect. In this study we added a BMP-2 soaked collagen membrane to study cortical healing from the muscle tissue surrounding the bone. We used SD rats and a 4.5 mm metaphyseal circular tibial defect. In group 1 (G1), a porous gelatin-calcium sulphate-hydroxyapatite (GCH) biomaterial containing rhBMP-2 and ZA was used to fill the defect (GCH+5 μg BMP-2+10 μg ZA). In group 2 (G2), we used a collagen membrane (2 μg BMP-2) to cover the GCH filled defect (GCH+3μg BMP+10 μg ZA). Group 3 (G3) was an empty control. Animals were sacrificed after 8-weeks and bone regeneration was evaluated with micro-CT and histology. In both G1 (P<0.001) and G2 (p<0.001) a significantly higher mineralized volume was found in the defect compared to empty G3. In G2 higher mineralized volume was found in the cortical region compared to both G1 (p<0.01) and G3 (p<0.001) as seen via micro-CT. Histologically, G1 and G2 showed islands of trabecular bone in the defect peripherally but only G2 showed cortical healing. G3 was empty in the middle but showed healed cortex. In conclusion, GCH can be used to deliver BMP-2 and ZA to promote metaphyseal bone growth. A membrane (CM) doped with low dose BMP-2 improved cortical regeneration.

**S28.5 SCLEROSTIN VACCINATION PREVENTS ESTROGEN LOSS-INDUCED OSTEOPOROSIS**

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Sclerostin (SOST) is an endogenous inhibitor of Wnt/β-catenin signalling pathway to impair osteogenic differentiation and bone anabolism. SOST immunotherapy like monoclonal antibody has been observed to control bone remodeling and regeneration. This study is aimed to develop a SOST vaccine and test its protective effects on estrogen deficiency-induced bone loss in mice. Gene sequences coded SOST peptide putative targeting Wnt co-receptor LRP5 were cloned and constructed into vectors expressing Fc fragment to produced SOST-Fc fusion protein. Mice were subcutaneously injected SOST-Fc to boost anti-SOST antibody. Bone mineral density, microstructure, and mechanical property were quantified using μCT scanning and material testing system. Serum bone formation and resorption markers and anti-SOST levels were measured using ELISA. SOST-Fc injections significantly increased serum anti-SOST antibody levels but reduced serum SOST concentrations. SOST-Fc vaccination significantly reduced estrogen deficiency-induced serum bone resorption markers CTX-1 increased serum bone formation marker osteocalcin. Of note, it significantly
alleviated the severity of estrogen-induced loss of bone mineral density, trabecular morphometric properties, and biomechanical forces of bone tissue. Mechanistically, SOSF-Fc vaccination attenuated trabecular loss histopathology and restored immunostaining of Wnt pathway like Wnt3a, β-catenin, and TCF4 in bone tissue along with increased serum osteoclast inhibitor OPG levels but decreased serum osteoclast enhancer RANKL concentrations. Taken together, SOST-Fc vaccination boosts anti-SOST antibody to neutralize SOST and mitigates the estrogen deficiency-induced bone mass and microstructure deterioration through preserving Wnt signalling. This study highlights an innovative remedial potential of SOST vaccine for preventing osteoporosis.

S28.6 PULSED ELECTROMAGNETIC FIELDS MEDIATE ANTI-INFLAMMATORY EFFECTS THROUGH ADENOSINE RECEPTORS PATHWAY IN JOINT CELLS

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Several studies explored the biological effects of low frequency low energy pulsed electromagnetic fields (PEMFs, Igea Biophysics Laboratory, Carpi, Italy) on human body reporting different functional changes. In the orthopedic field, PEMFs have been shown to be effective in enhancing endogenous bone and osteochondral repair, incrementing bone mineral density, accelerating the process of osteogenic differentiation and limiting cartilage damage. Much research activity has focused on the mechanisms of interaction between PEMFs and membrane receptors such as adenosine receptors (ARs). In particular, PEMF exposure mediates a significant upregulation of A2A and A3ARs expressed in various cells or tissues involving a reduction of most of the pro-inflammatory cytokines. In tissue engineering for cartilage repair a double role for PEMFs could be hypothesized: in vitro by stimulating cell proliferation, colonization of the scaffold and production of tissue matrix; in vivo after surgical implantation of the construct by favoring the anabolic activities of the implanted cells and surrounding tissues and protecting the construct from the catabolic effects of inflammation. Of particular interest is the observation that PEMFs, through the increase of ARs, enhance the working efficiency of the endogenous modulator adenosine, producing a more physiological effect than the use of exogenous drugs. This observation suggests the hypothesis that PEMFs could be considered a non-invasive treatment with a low impact on daily life. In conclusion, PEMFs represent an important approach in the pharmacological field providing excellent therapeutic results in various inflammatory diseases and in particular in the functional recovery of the damaged joint tissues.

S29.1 NANOSCALE APPROACHES TO MESENCHYMAL STEM CELL ENGINEERING

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In this presentation, the response of mesenchymal stem cells (MSCs) to nanoscale cues (e.g. topography, chemistry and vibrations) will be considered. In particular, control of MSC self-renewal and differentiation. A focus will be on a new bioreactor that has been developed, the nanokick, that delivers precise nanovibrational cues to MSC cultures in 2D and 3D, driving the cells to turn into mineralizing osteoblasts. Mechanotransductive signalling will be considered looking at ion channel mediated differentiation.

S29.2 DIGITIZING LIFE AT THE CELL-BIOMATERIALS INTERFACE

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Our lab uses computer-aided design to build in silico libraries of surface topographies, which we reproduce on polymeric chips and analyse for cellular responses using high content imaging and machine learning. In addition, we use transcriptomics and mass spectrometry to obtain a holistic view of biomaterial-mediated cellular responses and build gene regulatory networks thereof. This approach enables us to parameterize both the biomaterial properties as well as the cell response and to correlate them using computational tools. We think that this approach can be translated to other biomaterial platforms, such as polymer arrays, and foresee large scale crosstalk between them if we can standardize our methodology to describe the materials and to analyse the cells. To this end, we have started cBIT, the compendium for biomaterial-induced transcriptomics, an open-source database in which scientists can deposit and search material-induced transcriptomics data. The meta-analyses that cBIT enables, could lead to the identification of genes, pathways or expression profiles that can inform the design and development of new biomaterials. As such, by generating new information and simultaneously accumulating it in cBIT, we expect it is possible to one day predict cell responses to biomaterials.

S29.3 NOVEL INTEGRIN ALPHA 11-DEPENDENT PATHWAY INVOLVED IN TENDON STEM/PROGENITOR CELL (TSPC) AGING AND DEGENERATION

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Background: The exact pathways of collagen remodeling in tendon tissue are not well understood. Therefore, we have established a 3D collagen gel system and studied the remodeling capacity of two different TSPC lines: young, Y-TSPC and aged/degenerative, A-TSPC. We specifically investigated the involvement of integrin receptors in the remodeling process. Methods: Y- and A-TSPC were derived from human Achilles tendon. RT-PCR was used to assess the expression of collagen-binding integrins. Integrins a1 and a11 were silenced by lentiviral delivery of shRNA in the Y-TSPC. Control-shRNA, a1-shRNA and a11-shRNA virus was given for 24h and then cells were selected with zeocin for 10 days. The integrin knockdown (KD) efficiency was assessed by quantitative PCR and western blotting. Last, time-lapse recording of gel contraction of Y-TSPC+con, Y-TSPC+a1KD, Y-TSPC+a11KD, and A-TSPC were performed. Results: Integrin a1 and a11 were significantly downregulated in A-TSPC. Therefore, to mimic the A-TSPC we carried out a1 and a11 KD in Y-TSPC. PCR and western blot validated very efficient KD. Analyses of collagen contraction revealed that Y-TSPC+a11KD had significant reduction in collagen contractibility comparable to A-TSPC phenotype. Regarding integrin a1, we found that this receptor had no effect on the contraction rate of TSPC. Thus, to our knowledge we have now identified for the first time a novel role of a11 integrin in tendon matrix remodeling, and a follow up analyses of the exact downstream cascade are on the way.

S29.4 INFLUENCE OF HYPOXIC ENVIRONMENT ON MESENCHYMAL STEM CELL PRO-ANGIOGENIC PROFILE

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Mesenchymal stem cells (MSCs) are tissue-resident stroma cells capable of modulating immune cells through the secretion of paracrine factors. However, the comparison of MSCs potential, from different sources and submitted to hypoxia within a 3D scaffold, in secreting pro-healing factors has never been investigated. With a chemical composition similar to type I collagen, a major component of connective tissues retrieved in dental pulp, bone and umbilical cord, Hemocollagene® haemostatic foam presented porous and interconnected structure (> 90%) and a relative low elastic modulus of around 60 kPa. All these criteria meet basic requirements for tissue engineering based material. Herein, we assessed and compared the effect of hypoxia (3% O2) on the regulation and release of pro-angiogenic factors (VEGF, b-FGF and IL-8) from bone marrow (BM), Wharton’s jelly (WJ) and dental pulp (DP) derived MSCs cultured in Hemocollagene®. After 10 days of culture, qRT-PCR analysis showed an up-regulation of \textit{b-FGF} and \textit{VEGF} mRNA in BM- and WJ-derived MSCs, but not in DP-derived MSCs. Furthermore, hypoxia highly up-regulated IL-8 expression in WJ-derived MSCs and moderately in both BM and DP-derived MSCs. In contrast, ELISA analysis showed a higher amount of VEGF and IL-8 in supernatant provided from DP-derived MSCs culture compared to BM and WJ-derived MSCs. B-FGF was not detected whatever the experimental condition. In conclusion, MSCs derived from several tissues were able to release pro-angiogenic factors under hypoxic conditions. There was no clearly superior type of MSCs for therapeutic use, however DP-derived MSCs are likely to be more advantageous.

**S29.5 INJECTABLE HUMAN BONE-FORMING CELLS DERIVED FROM BONE MARROW MSC DISPLAY POTENT OSTEOGENIC PROPERTIES TO PROMOTE BONE REPAIR**

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Mesenchymal Stromal Cells (MSC) are promising therapies for fracture healing. However, undifferentiated MSC may act only through an inductive paracrine effect without direct bone formation. Here, we developed an injectable product constituted of human bone-forming cells derived from bone marrow (BM)-MSC (ALLO-P2) that display more potent bone repair properties not only by stimulating host osteoinduction but also by direct bone formation. In vitro, ALLO-P2 overexpressed markers such as ALP compared to BM-MSC isolated from the same donors, suggesting their engagement into the osteogenic lineage. In vivo, a single dose of ALLO-P2 significantly enhanced bone neoformation 14 days post-administration over the calvaria of NMRI-Nude mice compared to the control excipient. Histological analyses and mouse/human type I collagen double-immunolabelling revealed the presence of mineralized bone nodules of mixed host and donor origins in mice administered with ALLO-P2. Together, these results show that ALLO-P2 is a potential promising clinical candidate to promote bone repair, since it can be produced at high yields, is injectable and boosts ossification mechanisms involved in the physiological repair process.

**S29.6 MACROMOLECULAR CROWDING IN UMBILICAL CORD MESENCHYMAL STEM CELL CULTURE**

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Macromolecular crowding (MMC) is a biophysical phenomenon that accelerates thermodynamic activities and biological processes by several orders of magnitude. Herein, we ventured to identify the optimal crowder and to assess the influence of MMC in umbilical cord mesenchymal stem cell. 7 types of carrageenan (κ\(\lambda\), κ-LV1, κ-LV2, λ-MV, λ-HV, τ-MV, τ-HV) acted as crowder and biophysical properties were assessed respectively. Human umbilical cord mesenchymal stem cells were seeded at 15,000 cells/cm\(^2\) in 24 well plates and allowed to attach for 24 h. Subsequently, the medium was changed to medium with 7 types of carrageenan (10, 50, 100, 500 μg/ml) and 100 μM L-ascorbic acid phosphate (Sigma Aldrich). Medium without carrageenan was used as control. Cell morphology and SDS-PAGE analysis were conducted after 3, 5 and 7 days. Biophysical assessment showed 7 types of carrageenan have increased particle size with concentration, good polydispersity and negative charges. SDS-PAGE and densitometric analyses revealed significant increase \((p < 0.001)\) in collagen deposition in the presence of 10 μg/ml carrageenan \(\lambda\) and \(\tau\) at all the time points. SDS-PAGE and densitometric analysis also showed that the highest collagen deposition was observed in culture at 50 μg/ml carrageenan \(\lambda\). No significant difference was observed in cell morphology between the groups. Collectively, these data primarily illustrate the beneficial effect of carrageenan \(\lambda\) in human umbilical cord mesenchymal stem cell culture.

**S30.1 PATIENT REPORTED OUTCOME MEASURES IN SHOULDER AND ELBOW SURGERY: A GUIDE FOR NON-CLINICIANS**

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Increasingly more emphasis is being placed on Patient Reported Outcome Measures (PROMs). There are many used and reported in clinical studies, but there are no universally accepted or preferred measures. It is important for a researcher with a non-clinical background to understand how these assessments are performed, the type of information provided by each of the measures, and which diseases states are best reported by each measure.

**S30.2 IS OSTEOARTHRITIS A VASCULAR DISEASE?**

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OA pathophysiology has a vascular component consisting of venous stasis resulting in intraosseous hypertension and hypoxia. In response, osteoblasts change their cytokine expression, accelerating bone remodelling and cartilage breakdown consistent with OA. We have characterized circulatory kinetics in OA bone in animal models with dynamic contrast enhanced MRI (DCE-MRI) and \(^{18}\)F PET and have demonstrated venous stasis and reduced perfusion that temporally precede and spatially coincide with OA lesions. Osteoblast uptake of \(^{18}\)F is consistent with abnormal perfusion, bone remodelling, and severity of OA. Circulatory kinetics with DCE-MRI in humans with OA of the knee exhibit similar venous outflow obstruction. Venous stasis is associated with hypoxia in subchondral bone. As an example of the effects of hypoxia on OA osteoblasts, we have described upregulation of fibrinolytic peptides, but a deficiency in the upregulation of PAI-1, leading to the generation of plasmin by human OA osteoblasts exposed to hypoxia in vitro. Plasmin is a serine protease that has been shown to degrade cartilage in OA. Abnormal circulatory kinetics by DCE-MRI may be an imaging biomarker of OA. Pharmacologic modulation of venous stasis would have a salutary effect on the physicochemical microcirculation of subchondral osteoblasts and the pathophysiology of OA.

**S30.3 THE BIOLOGY- BONE GRAFT VERSUS SUBSTITUTES FOR SPINAL FUSION**
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Cervical and lumbar spine fusion procedures are increasing every year. Nonetheless, these procedures are associated with high infection rates, resulting in additional cost burden. The conundrum of achieving efficient spinal fusions with minimum complications requires an ideal bone graft with osteoconductive, osteoinductive, osteogenic and structural characteristics. Synthetic bone graft substitutes with or without autograft, allograft or synthetic bone substitutes have been commonly used for fusion procedures. We carried out a meta-analysis of comparative studies and prospective case series (n = 29) with cervical and lumbar fusion procedures using synthetic bone graft substitutes, autograft or allograft and other biologics. Synthetic bone graft substitutes analysed included HA (Hydroxyapatite), β-TPC (Tri Calcium Phosphate), β-TSC (Tri Calcium Sulfate), PMMA (Polymethylmetacrylate), Surgibone, BOP (Biocompatible Osteoconductive Polymer). The analysis revealed suboptimal evidence for the efficacy and safety of synthetic products used in spinal fusion procedures. Further studies are needed to determine beneficial effects of synthetic substitutes. However, the infection rate could be highly decreased with surface and composition modification of widely used polyether ether ketone (PEEK) implants. Laser modification of surface characteristics and collagen fleeces with micro and nano pore structures can prove to be excellent surface for increased osteoblasts cell proliferation and vitality.

S30.4 FEMORAL SHAFT FRACTURES: BONE BEHAVIOUR UNDER HIGH AND LOW ENERGY TRAUMA IN THE PAEDIATRIC, ADULT AND OLDER POPULATIONS


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Femoral shaft fractures are potentially devastating injuries. Despite this, clinical studies of the biomechanics of this injury are lacking. We aimed to clinically evaluate bone behaviour under high and low energy trauma in paediatric, adult and older patients. Single-centre retrospective study identifying all diaphyseal femoral fractures between Feb 2015-Feb 2017. Peri-prosthetic and pathological fractures were excluded. Patients were subdivided into groups 1 (paediatric, <16yo), 2 (adult, 17-55yo) and 3 (older, >55yo) to reflect immature, peak bone age and osteoporotic bone respectively. Chi-Squared analysis assessed significance of bone age to degree of comminution and fracture pattern. A p-value <0.05 was significant. A total 4130 radiographs were analysed with 206 femoral shaft fractures identified. Forty-three patients were excluded with 163 remaining. Group 1, 2 and 3 included 38, 37 and 88 patients respectively. Mean age 50.8 (SD 32.8) with male-to-female ratio of 1:1.2. Groups 1 and 3 included majority simple fractures (35/38 and 62/88 respectively). Group 2 included more comminuted injuries (33/37). Bone age to degree of comminution proved significant (p<0.05) with a bimodal distribution of simple fractures noted in groups 1 and 3. Energy to fracture was significant in group 2, where a high energy injury was associated with comminution (p<0.05). This study is the first to demonstrate an association between fracture comminution and age. Simple femoral shaft fractures showed a bimodal age distribution in paediatric and older patients regardless of mechanism energy. High energy mechanism trauma was directly related to fracture comminution at peak bone age.

S31.1 BONE ALLOGRAFT WITH MAGNESIUM ENRICHED TISSUE MICROENVIRONMENT PROMOTES LARGE BONE DEFECT HEALING

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Bone allograft is the most widely accepted approach in treating patients suffering from large segmental bone defect regardless of the advancement of synthetic bone substitutes. However, the long-term complications of allograft application in term of delayed union and nonunion were reported due to the stringent sterilization process. Our previous studies demonstrated that the incorporation of magnesium ions (Mg\(^2+\)) into biomaterials could significantly promote the gene up-regulation of osteoblasts and new bone formation in animal model. Hence, our group has proposed to establish an Mg\(^2+\) enriched tissue microenvironment onto bone allograft so as to enhance the bone healing. The decellularization and gamma irradiation process were performed on bovine bone allograft and followed by magnesium plasma treatment. To evaluate the biocompatibility and bioactivity, materials characterizations, *in vitro* and *in vivo* studies were conducted, respectively. Mg composite layer on bone surface ranged from 500nm to ~800nm thick. The cell viability on magnesium enriched allograft was significantly higher than that of the control. The ALP gene expression of hTMSCs in the group of PIII&D treated samples was highly up-regulated. The bone regeneration ability of Mg modified bone allograft implanted in animal model was significantly superior than the control after 2-month post-operation.

**S31.2 TISSUE ENGINEERING OF BONE - AS CLOSE AS WE CAN GET?**

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Bone tissue engineering has the intent to grow bone copies in the laboratory that could be used either for bone regeneration or as model systems to study bone physiology and pathology. Bone marrow- or adipose derived derived mesenchymal stromal cells are commonly used as they have been shown to be capable to differentiate into osteoblasts and depositing a calcium phosphate rich extracellular matrix. However, real bone is more than that: there are commonly three cell types described that are essential contributors to the tissue's native function: osteoblasts, osteocytes and osteoclasts. While all three cell types are being investigated separately, co-cultures of them including their precursors and inactive forms still provide a huge challenge these days, both in terms of culturing and (quantitative) evaluation. In addition, the matrix deposited by the osteoblasts *in vitro* is still far from bone's hierarchical organization *in vivo* that contributes to bone's impressive mechanical properties. Using a large set of microscopic tools (micro-computed tomography, SEM, 3D FIB/SEM, TEM and fluorescence), combined with spectroscopic (FTIR) and molecular tools (qPCR) we show that our 3D model system develops the main features of bone by human stromal cells differentiating first into osteoblasts who further embed themselves to become osteocytes. In their right environment and when stimulated mechanically, the cells are embedded within a collagenous matrix which is mineralized with carbonated hydroxyapatite. While this system still needs the addition of osteoclasts to represent 'real' bone, it allows to study the interaction between osteoblasts and osteocytes and to invest parameters contributing to collagen mineralization in high resolution and cryogenic conditions.

**S31.3 PHOTOPOLYMERIZATION FOR FILLING POROUS CERAMIC MATRIX: IMPROVEMENT OF MECHANICAL PROPERTIES AND DRUG DELIVERING BEHAVIOUR**

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Bone has a remarkable capacity to heal. However, in some instances the amount of bone which is needed to heal exceeds its healing capacity. Due to reported issues with current treatments there is continued research into alternative approaches with a view to producing an off the shelf alternative to the gold standard autologous bone transplants. The current investigated the use of a chitosan/hydroxyapatite scaffold, which was used to covalently bone morphogenetic protein and vascular endothelial growth factor using a UV crosslinking process. Results indicate that the incorporation of hydroxyapatite increased the mechanical properties of the scaffold compared to chitosan alone. Furthermore, crosslinking was confirmed using swelling studies and FTIR analysis. Elisa indicated that physiological doses of BMP were released after 10 days while \textit{in vitro} testing did not indicate a cytotoxic response to the scaffold. \textit{In vivo} testing in a rat femoral defect model indicated the efficacy of the treatment with scaffolds containing BMP and VEGF in combination resulting in more bone in the defect compared to the scaffold alone 8 weeks post-surgery.

**S31.4 CRYOSTRUCTURED SCAFFOLDS FOR OPTIMIZED rhBMP-2 DELIVERY AND BONE REGENERATION**


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In the treatment of bone non-unions an alternative to bone autografts is the use of bone morphogenetic proteins (BMP-2, BMP-7) with powerful osteoinductive and osteogenic properties. In clinical settings, BMPs are applied using absorbable collagen sponges. Supraphysiological doses are needed and major side effects may occur as induce ectopic bone formation, chronic inflammation and excessive bone resorption. In order to increase the efficiency of the delivered for BMPs we designed cryostructured collagen scaffolds functionalized with hydroxyapatite, mimicking the structure of cortical bone (aligned porosity, anisotropic, ANI) or trabecular bone (random distributed porosity, isotropic, ISO). We hypothesize that anisotropic structure would enhance osteoconductive properties of the scaffolds increasing rhBMP-2 regenerative properties. \textit{In vitro}, both scaffolds presented similar mechanical properties, rhBMP-2 retention and delivery capacity. For \textit{in vivo} testing, a rat femoral critical size defect model was created. Four groups were assessed depending on the implant applied to the bone defect: ISO, unloaded isotropic sponge; ISO-BMP, isotropic sponge loaded with 5 μg of hrBMP-2; ANI, unloaded anisotropic sponge; and ANI-BMP, anisotropic sponge loaded with 5 μg of hrBMP-2. Regeneration was allowed for 10 weeks. X-ray, μCT, biomechanical testing and histology were used to evaluate repair. Independently of their structure, sponges loaded with rhBMP-2 demonstrate increased bone volume, and biomechanical properties than their controls (p<0.01 and p<0.05 respectively). Globally, ANI-BMP group demonstrated better bone regeneration outputs with increased defect bridging (p<0.05 when compared ANI-BMP vs ISO-BMP groups). In conclusion, anisotropic cryostructured collagen scaffolds improve the efficiency of rhBMP-2 in bone regeneration.

**S31.5 DETERIORATION OF TRABECULAR MICROARCHITECTURE OCCURS PRIOR TO ALTERATIONS IN MINERAL DISTRIBUTION IN THE TIBIA OF AN ESTROGEN DEFICIENT RAT MODEL**


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Although osteoporosis reduces overall bone mass causing bone fragility, our recent studies have shown that bone tissue composition is altered at the microscopic level, which is undetectable by conventional diagnostic techniques (DEXA) but may contribute to bone fracture. However, the time sequence of changes in bone microarchitecture, mechanical environment and mineral distribution are not yet fully understood. This study quantified the longitudinal effects of estrogen deficiency on the trabecular microarchitecture and mineral distribution in the tibia of Female Wistar rats (6 months) that underwent ovariectomy (OVX, n=10) or sham surgery (SHAM, n=10). Weekly micro-CT scans of the proximal tibia were conducted at 15μm resolution for the first month of estrogen deficiency. Morphometric analysis was conducted to characterise the trabecular bone microarchitecture. The bone mineral composition was characterised with analysis of bone mineral density distributions (BMDD). There was significantly reduced trabecular bone volume fraction at 2 weeks in OVX rats compared to controls (p<0.01). There was no difference in mineral distribution between the OVX and control animals. This study provides the first evidence in uncovering the temporal nature of changes in bone microarchitecture and mineral distribution, showing that structure changes before composition.

S31.6 LONG-TERM OUTCOME OF ACETABULAR REVISION WITH THE BURCH-SCHNEIDER CAGE AND MASSIVE ALLOGRAFTS IN SEVERE BONE DEFICIENCIES

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Introduction. The management of periprosthetic pelvic bone loss is a challenging problem in hip revision surgery. This study evaluates the minimum 10-year clinical and radiographic outcome of major column structural allografts combined with the Burch-Schneider antiprotrusio cage for acetabular reconstruction.

Methods. From January 1992 to August 2005, 106 hips with periprosthetic osteolysis underwent acetabular revision using massive allografts and the Burch-Schneider antiprotrusio cage. Forty-five patients (49 hips) died for unrelated causes without further surgery. Fifty-nine hips in 59 patients underwent clinical and radiographic evaluation at an average follow-up of 15.1 years. There were 17 male and 42 female patients, with age ranging from 29 to 83 years (mean 59). Results. Ten hips required rerevision because of infection (3), aseptic loosening (6), and flange breakage (1). Moreover, 4 cages showed x-ray signs of instability with severe bone resorption. The survivorship of the Burch-Schneider cage at 21.9 years with removal for any reason or radiographic migration and aseptic or radiographic failure as the end points were 76.3 and 81.4, respectively. The average Harris hip score improved from 33.2 points preoperatively to 75.7 points at the latest follow-up (p < 0.001).

Discussion. In hip revision surgery, severe deficiency of pelvic bone stock is a critical concern because of the difficulty in providing a stable and durable fixation of the prosthesis. Although antiprotrusio cages have a limited role in acetabular revision, the use in association with massive allografts in extended bone loss demonstrated highly successful long-term results, enabling bone stock restoration and cup stability.

S32.1 INFLUENCE OF THE MECHANICAL ENVIRONMENT IN HEALING LARGE BONE DEFECTS AND FRACTURES

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The management of bone defects and impaired fracture healing remains one of the most challenging clinical problems. Several treatments exist to aid in the healing of large bone defects, including biologics such as recombinant human bone morphogenetic protein-2 (BMP-2), yet all have met with limited success. Regeneration of bone requires a coordinated network of molecular signals where the local mechanical environment plays a major role in the success of the healing process. The mechanical environment itself is determined by the stiffness of the implant used to stabilize the fracture and weight-bearing, and if fixation is either too flexible or too rigid the healing might fail. The hypothesis is that the healing of large-segmental bone defects and fractures can be accelerated by the imposition of an appropriate mechanical environment. An overview of the progress made in this research area on how the amount of rhBMP-2 could be reduced and its effectiveness increased by providing an optimized mechanical environment to achieve bone union will be presented. Additionally, the latest findings of improved fracture healing through the manipulation of fixation stability introducing a potential clinical strategy to improve the healing outcome of unstable fractures, particularly for non-unions through increased stabilization, will be discussed.

S32.2 INVESTIGATING CHONDROGENESIS UNDER MULTIAXIAL LOAD

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The unique properties of mesenchymal stem cells (MSCs) and their natural presence within the bone marrow make them an attractive source of cells for novel cartilage repair strategies. As mechanics play a critical role in vivo, a more physiological loading regime in vitro would be more appropriate to test novel therapies, and this can be achieved using bioreactors. Using a multiaxial load bioreactor system, we have investigated the effect of mechanical stimulation on human stem cell differentiation in the absence of growth factors, specifically transforming growth factor β (TGFβ). Our bioreactor system allows for the application of shear, compression or a combination of both stimuli to establish the phenotypic changes induced within MSCs. Neither compression alone, nor shear alone induces a change in MSC phenotype with a fibrin-based scaffold. However, we have demonstrated that a combination of compression and shear is able to induce chondrogenic differentiation and this is due to increased endogenous expression and activation of TGFβ. Using this multiaxial load bioreactor system, we can search for novel markers and potential therapeutic targets that only occur under physiological loads. In addition, potential rehabilitation protocols to be used after cell therapy in cartilage repair can also be investigated.

S32.3 CHARACTERISATION OF LEPTIN-RECEPTOR AS A TOOL TO STUDY SKELETAL STEM CELL CONTRIBUTIONS TO BONE MECHANOADAPTATION IN VIVO

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Bone tissue experiences continued remodelling in response to changes in its biochemical and biophysical environment. Given the finite lifespan of osteoblasts, this continued bone formation requires replenishment from a progenitor population. Although this is largely believed to be from a skeletal stem cell population, given the limitation in in-vivo markers for this cell type, progress in demonstrating this mechanism is limited. Therefore, we characterized the LepR-Cre mouse strain and evaluated whether LepR positive cells are the
progenitor population and if they contribute to the osteoblast population over time and in mechanically-induced bone formation in-vivo. Transgenic mouse strains; B6.129(Cg)-Leprtm2(cre)Rck/J to study LepR-expressing cells and B6.Cg-Gt(RosA)26Sortm9(CAG-tdTomato)Hze/J as a reporter strain were obtained from Jackson Laboratories. Characterization studies were performed on LepR:tdTomato mice at embryonic stage (19.5dpc), 8 and 12 weeks old. Mice (12 weeks old) were subjected to compressive tibia loading with a 11N peak load for 40 cycles, every other day for 2 weeks. Histological analysis reveal that LepR is expressed from the embryonic stage in various organs including bones. LepR positive cells are found around blood vessels and on bone surfaces. Flow cytometry analysis show the amount of LepR positive cells negative for CD45 and Ter-119 markers inside the bone marrow increases over time and following tibial loading. Mechanical loading induces an increase in bone mass and bone parameters. This model allows us to track and evaluate the role of LepR positive cells as bone forming cells, and to decipher the role of these cells in mechanically-induced bone formation.

S32.4 DIFFERENTIAL STEM CELL CULTIVATION ON 3D PRINTED PLGA SCAFFOLDS BY MEANS OF DUAL FLUIDIC HIGH THROUGHPUT BIOREACTORS

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After surgical tendon repair, the tendon-to-bone enthesis often doesn’t regenerate, which leads to high numbers of rupture recurrences. To remedy this, tissue engineering techniques are being pursued to strengthen the interface and improve regeneration. In this study, we used biphasic 3D printed PLGA scaffolds with aligned pores at the tendon side and random pores at the bone side to mimic the native enthesis. We seeded these with mesenchymal stem cells and inserted them into dual-flow bioreactors, allowing us to employ tenogenic and chondrogenic differentiation medium in separate flows. MTS assay demonstrated metabolism in dual-flow bioreactors at levels similar to tissue culture plate and rotating bioreactors. After 7, 14 and 21 days, samples were collected and analyzed by histology, RT-PCR and GAG production. H&E staining confirmed a compact cell layer attached to fibers and between porous cavities of scaffolds that increased with time of culture. Interestingly, cultured constructs in dual-flow bioreactors biased towards a chondrogenic fate regardless of which flow they were exposed to, possibly due to high porosity of the scaffold allowing for fluid mixture. Sox9 was upregulated at all timepoints (up to 30x compared to control), and by day 21 Col2A1 was also highly upregulated. Additionally, GAG production in treated constructs (serum-free) was able to match constructs exposed to 10% FBS in controls, demonstrating the functional matrix forming capabilities of this system. Overall, we have validated this dual-flow system as a potential platform to form the enthesis, and future studies will further optimize parameters to achieve distinctly biphasic constructs.

S32.5 MACROMOLECULAR CROWDING AND MECHANICAL STIMULATION FOR CONTROL OF TENOCGETIC PHENOTYPE

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Current cell-based tissue engineering strategies have limited clinical applicability due to the need for large cell numbers and prolonged culture periods that lead to phenotypic drift. In vitro microenvironmental modulators have been proposed to mimic the native tendon. Standard in vitro culture conditions result in delayed extracellular matrix (ECM) deposition, impairing the development of scaffold-free approaches. ECM deposition can be enhanced by macromolecular crowding (MMC), a biophysical phenomenon that governs the milieu of multicellular organisms. We assessed a multifactorial biophysical approach, using MMC and mechanical loading, on different cell sources to determine their suitability for in vitro fabrication of tendon-like tissue. Human dermal fibroblasts (DFs), tenocytes (TCs) and bone marrow mesenchymal stem cells (BMSCs) were cultured with MMC under static and uniaxial strain culture conditions. TCs and DFs exhibited alignment perpendicular to the load, whilst BMSCs did not show preferential alignment. When MMC was used, DFs and BMSCs showed increased deposition of collagen I, the main component in tendon ECM. DFs presented ECM composition similar to TCs with collagen types III, V and VI present. Gene expression analysis revealed upregulation of tenogenic markers by TCs and DFs, such as scleraxis and thrombospondin-4, under both loading and MMC. The combined use of MMC and mechanical stimulation is suitable for TCs phenotype maintenance and can modulate the phenotype of DFs and BMSCs differentially. This study provides insight into response of different cell sources to biophysical cues and contributes to further development of cell therapies for tendon repair and regeneration.

S32.6 SPARC IS A MECHANO-SENSOR THAT REGULATES TENDON DEVELOPMENT AND HOMEOSTASIS


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Mechanical loading plays an essential role in both tendon development and degradation. However, the underlying mechanism of how tendons sense and response to mechanical loading remains largely unknown. SPARC, a multifunctional extracellular matrix glycoprotein, modulates cell extracellular matrix contact, cell-cell interaction, ECM deposition and cell migration. Adult mice with SPARC deficiency exhibited hypoplastic tendons in load-bearing zone. By investigating tendon maturation in different stages, we found that hypoplastic tendons developed at around postnatal 3 weeks when the mice became actively mobile. The in vitro experiments on primary tendon derived stem cells demonstrated that mechanical loading induced SPARC production and AKT/S6K signalling activation, which was disrupted by deleting SPARC causing reduced collagen type I production, suggesting that mechanical loading was harmful to tendon homeostasis without SPARC. In vivo treadmill training further confirmed that increased loading led to reduced Achilles tendon size and eventually caused tendon rupture in SPARC−/− mice, whereas no abnormality was seen in WT mice after training. We then investigate whether paralysing the hindlimb of SPARC−/− mice using BOTOX from postnatal 2 weeks to 5 weeks would delay the hypoplastic tendon development. Increased patellar tendon thickness was shown in SPARC−/− mice by reducing mechanical loading, whereas opposite effect was seen in WT mice. Finally, we identified a higher prevalence of a missense SNP in the SPARC gene in patients who suffered from a rotator cuff tear. In conclusion, SPARC is a mechano-sensor that regulates tendon development and homeostasis.

S33.1 MESENCHYMAL STEM CELL DERIVED EXTRACELLULAR VESICLES TO MODULATE THE TENDON MICROENVIRONMENT IN REPAIR STRATEGIES

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Tendon injuries in both the human and horse represent a challenge due to persistent inflammation combined with inadequate reparative cells and a poorly organised extracellular matrix. The potential of mesenchymal stem cells (MSCs) in regenerating tendon injuries remains to be fully realised. The main mechanism of action by MSCs is considered to be primarily mediated via paracrine mechanisms. This may involve the production and release of extracellular vesicles (EVs) by stem cells with a sub-fraction of these EVs (<100 nm diameter) called exosomes that appear to be the main paracrine effectors. EVs can be readily prepared from MSCs and offer a clinically relevant therapy. However, EVs for tendon repair need to be fully characterised. The horse represents a highly relevant model of tendon and ligament injuries as it shares many features of mechanical loading, function and aetiopathology with the human. We have isolated and characterised EVs from equine MSCs for modulating tendon cell phenotype in an in vitro tendon injury model using IL-1ß. EVs can be isolated from IL-1ß stimulated MSCs although their levels are not significantly increased over controls suggesting that the nature of the stimulated EV cargo may be more important than absolute levels of released EVs.

**S33.2 MAGNETIC ACTUATION IN TISSUE ENGINEERING STRATEGIES TARGETING TENDON REGENERATION**

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Significant challenges remain to accomplishing the development of fully functional tendon tissue substitutes that can lead to clinically effective and successful applications. Scaffolding materials must meet demanding requirements such i) mimic the hierarchical and anisotropically aligned structure of tendon tissues from the nano- up to the macroscale, ii) meet tendon mechanical requirements and non-linear biomechanical behaviour, iii) provide the necessary biophysical/biochemical cues and mechanical responsiveness to induce the tenogenic differentiation of stem cells and potentiating the effects of biochemical supplementation. On the other side, tenogenic differentiation of stem cells is still to be established, as well as the role of such cells (either naïve or pre-differentiated) in promoting tissue regeneration. We have recently found evidences that magnetic actuation can provide means of mechanically stimulating cells in a contact-free manner and, more interestingly, can also modulate inflammatory response, a critical issue for achieving tissue regeneration instead of repair. In summary, synergies of scaffold design and magnetic responsiveness can impact significantly cells behaviour as well as in vivo response and thus widen the therapeutically range of cell-laden tissue engineered constructs in tendon regeneration.

**S33.3 EFFECT OF TGF-β3 AND GDF-5 ON THE EXPRESSION OF TENDON/ LIGAMENT MARKERS IN HUMAN DENTAL PULP STEM CELLS AND PERIODONTAL LIGAMENT CELLS**

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Tendon and ligament injuries represent highly prevalent and unmet clinical challenge that may significantly benefit from tissue engineering therapeutic strategies, once optimal cell source and biomolecules regulating
tendon homeostasis are properly defined. Herein, we aimed to evaluate the expression of tendon/ligament markers in two novel cell populations, namely human dental pulp stem cells (DPSCs) and periodontal ligament cells (PDLCs), in response to supplementation with TGF-β ligands relevant for tendon development and healing, as well as under standard tri-lineage differentiation conditions. DPSCs and PDLCs were isolated from sound human permanent molars removed for orthodontic reasons. Pulp tissue and periodontal ligament were minced and digested with collagenase (3mg/mL) and cells were expanded in α-MEM supplemented with 10% fetal bovine serum (basal medium). To evaluate the susceptibility of DPSCs and PDLCs to tenogenic induction, cells were seeded at density of 1000 cells/cm² and cultured up to 21 days in basal medium or media supplemented with TGF-β3 (10ng/ml), or GDF-5 (50 ng/ml). Cell response was evaluated weakly by analysis of expression of tendon, bone and cartilage markers, employing real time RT-PCR and immunocytochemistry. A significant increase in collagen I and collagen III expression was observed with the culture progression in all conditions, with abundant matrix being deposited by day 14. A significant upregulation of scleraxis expression was demonstrated in response to supplementation with TGF-β3 in both cell populations, when compared to basal medium and medium with GDF-5. It was concluded that TGF-β3 may represent an effective inducer of stem cell tenogenic differentiation.

S33.4 RNA-SEQ AND META-PROFILING FOR A BETTER UNDERSTANDING OF THE MUSCULOSKELETAL SYSTEM BIOLOGY


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RNA-Seq or whole transcriptome shotgun sequencing has been adopted in the last years as a reference technique to determine the presence and the quantity of different species of RNA in determined biological samples, thanks to it allows the identification every single RNA species transcribed from a reference genome. Meta-profiling takes advantage of the public availability of an increasing set of RNA-Seq data produced by different laboratories to summarize the expression levels of the different RNA species of many samples according to their biological context, giving the opportunity to perform comparisons on the gene expression profiles of different tissues by integrating data derived from a high number of studies. By using Genevestigator™, a platform which integrates RNA-Seq data into meta-profiles, we have performed a comparison between the gene expression profiles of bone, cartilage, muscle tendon and skin by means of interrogating its database with different gene sets and families with relevance to the function of the tissues of the musculoskeletal system. The collagen gene family and genes coding for proteoglycans, matrix metalloproteinases and tissue inhibitors of metalloproteinases, mechanotransduction-related proteins and signalling pathways involved in tissue development and differentiation have been analysed. Hierarchical clustering for every gene set was performed for the understanding the differences and similarities between the different tissues included in the analyses. The results of this study will help to improve our understanding of the musculoskeletal system, and will help to identify new biomarkers and signalling pathways of specific relevance for the bone, cartilage, muscle and tendon.

S33.5 QUANTITATIVE ULTRASOUND MEASURES IN THE PATELLAR TENDON ARE ASSOCIATED WITH VISA-P SCORES OF COLLEGIATE BASKETBALL PLAYERS OVER ONE SEASON OF PLAY

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This study of collegiate basketball players evaluated change over time (COT) in ultrasound shear wave (SW) elastography metrics across the basketball season, and correlated to morphologic changes on conventional ultrasound imaging, and VISA-P scores. In eleven male collegiate basketball players (mean age 19, age range 18-21), patella tendon (PT) ultrasound and SW elastography of both knees were performed at pre-season and post-season time points, and players reported their VISA-P scores throughout the season. Patella tendinopathy grade and SW metrics were correlated to VISA-P scores using Spearman correlation coefficients. Paired t-test was used to assess differences in mean SW metrics at pre-and post-season timepoints, accounting for leg dominance. 6 of 11 players (54.5%) had baseline patella tendinopathy on ultrasound progressing in 4 players. The mean change in VISA-P score was 15.18 (+/-8.55). No significant correlation was seen between ultrasound grades of tendinopathy and VISA-P. Pre-season SW velocities did not significantly correlate with baseline VISA-P scores. Post-season SW values and SW COT demonstrated strong correlation with change in VISA-P score in dominant and non-dominant knees. Although not statistically significant, there was a trend towards higher SW velocity for tendinopathy in both dominant and non-dominant knees at both study visits. SW metrics of the PT correlated to change in VISA-P scores in the dominant and non-dominant knees, whereas conventional ultrasound grades of patella tendinopathy did not. There was a trend towards higher SW velocities in patella tendinopathy which may indicate detection of change in intrinsic tissue stiffness.

S33.6 EXTENSOR TENDON ANATOMY: ATTACHMENT OF THE CENTRAL TENDON TO THE DORSUM OF THE PROXIMAL PHALANX SHAFT

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We hypothesized that the finger extensor mechanism has attachments along the dorsal surface of the entire length of the proximal phalanx and that this anatomy has not been clearly defined. The attachment along the dorsal aspect of the proximal phalanx of the index, middle, index and small fingers was dissected in 20 fresh-frozen cadavers. The lateral bands and attachments along the lateral and medial surface were released to appreciate the attachments along the dorsal aspect. We characterized the ligament attachments as very robust, moderately robust, and minimally robust at the distal, middle, and proximal portions. Three orthopaedic surgeons quantified the attachment, finding that 93% of specimens had tendinous attachments and the most robust attachment found at the most proximal and distal aspects adjacent to the articular cartilage. 87% of the specimens had very robust attachments at the proximal portion of the proximal phalanx. The middle portion of the proximal phalanx had moderate to minimally robust attachments. Greatest variability in attachment was found along the most distal portion of proximal phalanx adjacent to the proximal interphalangeal joint (26% of specimens had moderate to minimal robust attachment; 74% had robust attachments). The attachments along the proximal phalanx were attached on the dorsal half of the proximal phalanx, with no fibrous attachments extending past the lateral bands. In summary, we found tendinous attachment along the proximal phalanx that may assist in finger extension and may extend the digit at the metacarpal phalangeal joint without central band contribution.

S34.1 CURRENT CONCEPTS IN THE MANAGEMENT OF TRAUMATIC ELBOW INSTABILITY

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Advancements in treating the unstable elbow. We will review and discuss the kinematics and biomechanics of the forearm, concentrating on the role of soft tissue structures and how they affect forearm and elbow function. During this session, we will review the latest techniques for treating the terrible triad, including solutions to complex injuries of the olecranon, coronoid, and radial head. Techniques presented will address fixation, reconstruction, and salvaging of complex unstable elbow injuries.

S34.2 DISTAL HUMERUS ORIF IN 2018: TIPS AND TRICKS TO OPTIMIZING OUTCOMES

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Advancements in treating distal humerus fractures. We will review and discuss approaches to the elbow to treat different types of fractures. We will discuss the role of soft tissue structures and how they affect elbow function. During this session, we will review the latest techniques for treating the complex articular fractures of the distal humerus to include capitellar and trochlear fractures. Techniques presented will address fixation, reconstruction, and salvaging of complex distal humerus fractures.

S34.3 NON-OPERATIVELY MANAGED PAEDIATRIC SUPRACONDYLAR FRACTURES OF THE HUMERUS: DO THEY ALL NEED “CLOSE” RADIOGRAPHIC FOLLOW-UP?

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Supracondylar fractures of the humerus (SCFH) are the most common type of paediatric elbow fractures. Due to beliefs that non-operatively managed SCFH may displace further from the original position, they are monitored with repeated radiographs and a large number are unnecessarily surgically pinned. Very limited evidence currently exists to support these beliefs. This study aimed to determine the incidence of late “significant” displacement (requiring surgical management) of non-operatively managed paediatric SCFH, and whether they necessitate close radiographic follow-up. Patients aged ≤16, with a SCFH, were included in this retrospective cohort study. All were initially managed non-operatively with at least one follow-up radiograph within six weeks of injury. Data from four consecutive years (2013-2016) was collected using the hospital’s radiology database. Two observers independently analysed patient radiographs and classified fractures by the Gartland and AO systems. The incidence of late displacement was determined using follow-up radiographs and clinic notes. Of the 164 patients included in the study, one patient (Gartland Type II, AO Type III) suffered late displacement at two weeks, requiring surgical fixation. One further patient (AO Type II) had a persistent cubitus varus deformity (Baumann’s angle 90°), with no long-term functional deficit. Incidence of late displacement was 0.6% (n=1). Our findings suggest that stable Gartland Type I/AO Type I and II fractures do not require repeated radiographic follow-up. However, some Gartland Type II/AO Type III fractures require monitoring. This could considerably reduce the financial costs for the healthcare system, and inconvenience to families, associated with repeated follow-ups.

S34.4 SYNTHETIC AUGMENTATION FOR MASSIVE ROTATOR CUFF TEARS

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The treatment of massive chronic tears is problematic. The re-tear rate following surgery for extensive cuff tears remains high, and there is little consensus regarding optimum treatment. To investigate the outcome of a cohort of patients who had open repair of an extensive cuff tear using the Leeds Kuff patch as an augment. A retrospective cohort study of consecutive patients with a massive cuff tear who had surgery in our regional elective orthopaedic centre over a two year period from January 2015 to Dec 2016. All patients followed identical rehabilitation protocols, supervised by physiotherapists with an interest in the shoulder. Outcomes assessment was undertaken at a minimum of 12 months by a registrar or physiotherapist who was not part of the treating team. Pre-op data collection included; range of motion, pain score, Oxford shoulder score (OSS), assessment of muscle atrophy on MRI. Data collection was completed in 15 patients. The mean age was 62 yrs (56 – 75). The mean pre-op OSS was 22, improving to a mean of 43. The range of motion and pain score improved. There were no intra-operative complications. One patient required a second surgery for evacuation of a haematoma at 10 days post op. One patient had an obvious re-tear at 4 months. Open rotator cuff repair with synthetic Kuff patch augmentation for chronic degenerative tears appears worthwhile when assessed at 12 months and they continue to improve even at 18 months. This treatment method may be a useful option for patients > 70 years old.

S34.5 LOSS OF MICRORNA-29A AGGRAVATES SUBACROMIAL BURSA FIBROSIS: A NEW INSIGHT INTO PATHOGENESIS OF ROTATOR CUFF LESION WITH SHOULDER STIFFNESS

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Subacromial bursa fibrosis are linked to rotator cuff lesion with shoulder stiffness; however, the mechanism underlying this shoulder disorder remain elusive. MicroRNA-29s (miR-29s) are emerging fibrosis inhibitor targeting fibrogenic matrices during tissue fibrosis. This study is aimed to investigate clinical relevance and function of miR-29 signalling to subacromial bursa homeostasis in shoulder stiffness. Subacromial bursa in patients with rotator cuff lesion with or without shoulder stiffness who required open acromioplasty were harvested for assessing fibrosis histopathology using Manson’s trichrome staining. Expressions of proinflammatory cytokines, fibrotic matrices, and miR-29s were quantified using RT-PCR and in situ hybridization. Range of motion and pain scores of the stiffness group were higher than those of non-stiffness group. Upregulated proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and fibrotic matrices (collagen 1α1, 3α1, and 4α1) but decreased miR-29a and b expression existed in the stiffness group. Affected tissues exhibited severe fibrotic matrix accumulation, synovial hyperangiogenesis, hyperplasia, and strong miR-29a transcripts. In vitro, IL-1β rather than IL-6 and TNF-α decreased miR-29a expression of subacromial bursa fibroblasts. miR-29a knockdown escalated fibrotic matrix expression, whereas forced miR-29a expression alleviated the IL-1β-induced fibrotic matrix expression. Of interest, miR-29a transgenic mice displayed moderate responses to supraspinatus and infraspinatus tenotomy-induce fibrosis and gait irregularity of affected shoulders. Weak miR-29a signalling causes excessive fibrosis and remodelling in subacromial bursa and ultimately increases the prevalence of shoulder stiffness. This study reveals a new mechanistic underlying shoulder stiffness and highlights that sustained miR-29a potentially ameliorates the severity and function of stiff shoulder.

S34.6 A NOVEL COLLAGEN SCAFFOLD FOR AUGMENTING ROTATOR CUFF REPAIR – AN IN VIVO RAT STUDY

S35.1 ADENOSINE RECEPTORS AS A BIOLOGICAL PATHWAY FOR THE ANTI-INFLAMMATORY AND BENEFICIAL EFFECTS OF LOW FREQUENCY LOW ENERGY PULSED ELECTROMAGNETIC FIELDS.

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The purine nucleoside, adenosine regulates functions in every tissue and organ in the body acting via four G-protein-coupled receptors, A1, A2A, A2B, and A3 adenosine receptors (ARs). Electromagnetic field (EMF) stimulation is an innovative therapeutic technique able to increase cellular anabolic activity and limit the catabolic effects of inflammatory cytokines. The mechanisms of cell reception of EMFs are not well known and much research activity has focused on the interactions between EMFs and membrane receptors. Interestingly, links have been found between ARs and their modulation by such physical agents as pulsed EMFs. EMF exposure mediates a significant upregulation of A2A and A3ARs in chondrocytes, synoviocytes and osteoblasts, leading to the reduction of synthesis and release of pro-inflammatory cytokines. In cultured full-thickness cartilage explants, pulsed EMFs preserve the integrity of the extracellular matrix and antagonize the effect of catabolic cytokines, such as IL-1. Pulsed EMFs, through the increase of ARs, enhance the working efficiency of adenosine without the side effects, desensitization, and receptor down-regulation often related to the use of agonist drugs. Modulation of adenosine receptors by pulsed EMFs could be a mechanism of cell reception of EMFs and an innovative physiologic alternative to the use of adenosine agonists.

S35.2 ELECTROSPINNING APPLICATIONS IN ORTHOPAEDIC PROCEDURES

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Electrospinning of (bio)polymers is a well acknowledged technology used by scientists all over the world to manufacture scaffolds for tissue engineering & 3D cell culture purposes. The ability to control key parameters such as fibre diameter and fibre orientation allow the generation of highly specific scaffolds that closely mimic the native extracellular matrix. Despite the popularity in the R&D lab, the technology itself has only recently seen acceptance as a method for manufacturing clinical-grade medical devices. Subsequently, never before have more electrospun materials obtained market approval (FDA/CE) and are in clinical trials. In this presentation, we share our experience as a manufacturer of clinical-grade medical devices via electrospinning and give insight into the possible applications in orthopaedics.

**S35.3 EFFECTS OF ELECTROMAGNETIC FIELDS ON ARTICULAR CELLS AND OSTEOARTHRITIS**

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Exposure to electromagnetic energy has potent signalling effects upon articular cells including chondrocytes, synoviocytes and osteoblasts. Attention has focused on two actions – the altered synthesis of cytokines and enzymes, and the enhanced synthesis of bone and cartilage extracellular matrix (ECM) molecules. *In vitro* studies with human and bovine articular cartilage have shown increased aggregcan synthesis, glycosaminoglycan content, and biomechanical aggregate modulus with EMF exposure. Osteoarthritic (OA) cartilage responds similarly depending upon the severity of the OA with early OA cartilage responding more robustly. On these bases, two *in vivo* studies have been done with the Dunkin-Hartley guinea pig model of spontaneous OA. Both studies demonstrated preservation of ECM with increased aggrecan synthesis, matrix glycosaminoglycan and type 2 collagen content, and reduced histological-histochemical (Mankin) scores. Suppression of matrix metalloproteases and IL-1, together with increased TGFb were also observed. Responses to various EMF configurations, in terms of amplitude, frequency, and exposure duration have been described, indicating dose responsiveness. These studies suggest the conclusion that exposure to specific EMFs reduce the progression of early OA. A randomized clinical trial is underway. EMFs may be a disease-modifying therapy for OA, resulting in maintenance of ECM and improvement in the cytokine environment of OA joints.

**S35.4 PULSED ELECTROMAGNETIC FIELDS MODULATE METABOLIC ACTIVITY, MYOKINE RELEASE AND DIFFERENTIATION INTO MYOTUBES OF MYOBLASTS GROWN IN VITRO**

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Pulsed Electromagnetic Fields (PEMFs) promote joint tissue anabolic activities, particularly in cartilage and bone. Here we investigated the effect of selected PEMFs (75Hz, 1.5mT, 1.3msec) in a differentiating model of murine myoblasts (C2C12) *in vitro*. C2C12 were seeded at 5X10³ cells/cm² in 4 well plates and left to adhere for 24h. Subsequently, cells were either maintained in growth medium (GM) or induced towards myogenic differentiation in low-serum conditions, with and without PEMF exposure, for 4 days.
Morphological analysis, myotube formation and fusion index (FI) were assessed with fluorescence microscopy techniques. Metabolic activity was determined by MTT; moreover, a multiplex cytokine array (RayBiotech) allowed cell supernatant molecule quantification. Cells exposed to PEMFs in GM acquired a distinctive elongated morphology, with increased bi-nuclear figures (3.2-fold FI increase over PEMF-unexposed cells) and displayed a significantly higher metabolic activity (+31%, p<0.05 over PEMF-unexposed cells). PEMF exposure increased metabolic activity also under myogenic differentiation (+15% over PEMF-unexposed differentiating cells, p<0.05), with the formation of long, thick multinuclear myotubes, suggesting a role of PEMFs in enhancing myogenesis (7.7-fold FI increase over PEMF-unexposed cells). 4-day culture supernatants revealed the presence of several myokines (KC/CXCL1, LIX, MCP-1, TIMP-1). Preliminary analysis showed a 1.16-fold increase (n=2) of LIX and, notably, a 1.91-fold increase (n=2) of TNF-RI, in cell supernatants of PEMF-exposed over PEMF-unexposed cells. Collectively, these results suggest that PEMF may successfully be applied in models of muscle cell trauma to optimise muscle fibre repair, by fine-tuning the release of myokines, promoting myoblast proliferation and myotube formation.

S35.5 MEDIATING HUMAN STEM CELL BEHAVIOUR VIA DEFINED FIBROUS ARCHITECTURES BY MELT ELECTROSPINNING WRITING

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The architecture within which cells reside is key to mediating their specific functions within the body. In this study, we use melt electrospinning writing (MEW), a recently developed 3D printing technology unique in its ability to generate ECM like fibres and control their deposition, to fabricate cell micro-environments with various fibrous architectures to study their effect on human stem cell behaviour. We designed, built and optimised a MEW apparatus and used it to fabricate four different platform designs of 10.4±2μm fibre diameter, with angles between fibres on adjacent layers of 90°, 45°, 10° and R (random). Characterisation was conducted via scanning electron microscopy (SEM) imaging and tensile testing, and human skeletal stem cells (hSSCs) were seeded to scaffolds to study the effect of architecture on cell morphology and mechanosensing. Cell morphology was significantly altered between groups, with cells on 90° scaffolds having a lower aspect ratio, greater spreading, greater cytoskeletal tension and nuclear YAP expression. Long term cell culture studies were then conducted to determine the differentiation potential of scaffolds in terms of alkaline phosphatase activity, collagen and mineral production. Across these studies, an increased cell spreading in 3-dimensions is seen, with decreasing alignment of architecture correlated with enhanced osteogenesis, as seen by significant fold increases in ALP (2.8), collagen (2.5) and calcium (3.6) in the 90° scaffold architecture compared to 10°. This study therefore highlights the critical role of fibrous architecture in regulating stem cell behaviour with implications for tissue engineering and disease progression.

S35.6 A CO-CULTURE MODEL FOR THE STUDY OF OSTEOGENESIS ON SCAFFOLDS FABRICATED USING MELT ELECTROSPINNING WRITING TECHNIQUE

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Bone regeneration using a scaffold-based tissue engineering approach involves a spectrum of overlapping processes, which are driven by cell-to-cell, cell-to-extracellular matrix (ECM) and cell-to-biomaterials interactions. Traditionally, the study of osteogenesis potential of tissue-engineered constructs (TECs) in vitro only considers the osteoblasts- or mesenchymal cells (MSCs)-to-biomaterials interactions. However, this
poorly recapitulates the process of bone regeneration under physiological conditions. In this study, a growth factors free co-culture model, comprising osteoblasts and monocytes was established to allow for the study of the osteogenesis potential of a TEC taking into consideration osteoblasts-to-monocytes and cells-to-biomaterials interactions. Scaffolds made of medical-grade polycaprolactone (mPCL) were fabricated by means of melt electrospinning writing technique. Subsequently, scaffolds were coated with a thin layer of calcium phosphate (CaP) by means of chemical deposition. Scaffolds with CaP coating were seeded with human-derived primary osteoblasts and monocytes and cultured for up to nine weeks. At several time-points, cells were evaluated for alkaline phosphatase and tartrate-resistant acid phosphatase activity. Additionally, cell morphology was observed through fluorescence microscopy and osteoblastic- and osteoclastic-related gene expression was analyzed by quantitative reverse transcription-polymerase chain reaction. The simultaneous presence of osteoblasts and monocytes and CaP accelerated cell matrix formation on scaffolds. Quantitative gene expression profile showed similar findings. Whereby, osteoblastic- and osteoclastic-related gene expression was highest in the PCL/CaP co-culture groups compared to other groups. This indicated synergistic effects of soluble factors secreted by cells and solubilized inorganic components from the scaffolds in promoting matrix deposition.

PS1.1 CANCER, BONE, MUSCLE AND METABOLISM: WHAT’S THE CONNECTION?

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Breast and other cancers commonly metastasize to bone to cause bone destruction, pain, fractures hypercalcemia and muscle weakness. Recently, we described a specific molecular mechanism by which bone-derived transforming growth factor (TGF)-beta, released as a consequence of tumor-induced bone destruction causes muscle dysfunction, before the loss of muscle mass. Circulating TGF-beta induces oxidation of the ryanodine receptor (RYR1) on the sarcoplasmic reticulum of skeletal muscle to induce calcium leak and muscle weakness. Blocking TGF-beta, or its release from bone (with bisphosphonates), preventing oxidation of or stabilizing RyR1 all prevented muscle weakness in mouse models of breast cancer bone metastases. In addition to these effects on skeletal muscle, circulating TGF-beta may act on beta cells of the pancreas to impair insulin secretion and result in glucose intolerance. These and other potential systemic effects of TGF-beta released from the tumor-bone microenvironment or from cancer treatment-induced bone destruction implicate bone as a major source of systemic effects of cancer and cancer treatment. Therapy to block the systemic effects of the bone microenvironment will improve morbidity associated with bone metastases and cancer treatment.

PS1.2 NOVEL TISSUE ENGINEERING AND REGENERATIVE MEDICINE APPROACHES TO HEAL MUSCULOSKELETAL TISSUES AND THEIR RELEVANCE TO ORTHOPAEDICS

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The selection of a proper material to be used as a scaffold or as a hydrogel to support, hold or encapsulate cells is both a critical and a difficult choice that will determine the success of failure of any tissue engineering and regenerative medicine (TERM) strategy. We believe that the use of natural origin polymers, including a wide
range of marine origin materials, is the best option for many different approaches that allow for the regeneration of different tissues. In addition to the selection of appropriate material systems it is of outmost importance the development of processing methodologies that allow for the production of adequate scaffolds/matrices, in many cases incorporating bioactive/differentiation agents in their structures. An adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation, and in many cases the selection of a specific sub-population, methodologies should be developed and optimized. We have been using different human cell sources namely: mesenchymal stem cells from bone marrow, mesenchymal stem cells from human adipose tissue, human cells from amniotic fluids and membranes and cells obtained from human umbilical cords. The development of dynamic ways to culture the cells and of distinct ways to stimulate their differentiation in 3D environments, as well as the use of nano-based systems to induce their differentiation and internalization into cells, is also a key part of some of the strategies that are being developed in our research group. The potential of each combination materials/cells, to be used to develop novel useful regeneration therapies will be discussed. The use of different cells and their interactions with different natural origin degradable scaffolds and smart hydrogels will be described. Several examples of TERM strategies to regenerate different types of musculoskeletal tissues will be presented. Relevance to orthopaedics will be highlighted.
S36.1 KARTOGENIN-BASED INTRA-ARTICULAR THERAPEUTICS TO TREAT OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common arthritis. Early OA is treated with pain-relieving medication while advanced diseases are treated with joint replacement. Intraarticular (IA) injection has been also used as a local therapy for OA. Only corticosteroids and hyaluronic acid has been clinically used for IA injection up to now. While these drugs are effective in alleviating pain relief and mitigating inflammation, they do not regenerate damaged cartilage. We have developed drug delivery system for OA treatment using a new molecule kartogenin which are known to have regenerative effects for cartilage. These systems include kartogenin-conjugated chitosan nano/microparticles, thermoresponsive nanospheres containing kartogenin and diclofenac, hyaluronic acid hydrogel containing PEGylated kartogenin micelles. We have found that injection of these systems arrested the progression of OA as well as inhibiting inflammation in surgically-induced OA model in rats. These data will be introduced in this talk.

S36.2 CALCIUM-CONTAINING CRYSTALS – A POTENTIAL THERAPEUTIC TARGET IN OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common cause of joint disease and associated disability. Despite this, its pathogenesis remains incompletely understood and no specific drug exists to prevent or reverse the structural changes in OA. Basic calcium phosphate (BCP) crystals are extremely common in OA. BCP crystals consist primarily of hydroxyapatite, with smaller amounts of octacalcium phosphate, tricalcium phosphate and magnesium whitlockite. They are present in 100% of joints at the time of knee and hip joint replacement surgery. Their presence strongly correlates with radiographic severity of osteoarthritis. In mice, intra-articular BCP crystals elicit synovial inflammation and cartilage degradation. The potential mechanisms by which calcium-containing crystals may promote articular damage have been studied in the laboratory setting and in vitro properties of BCP crystals have been observed that emphasise their pathogenic potential. BCP crystals interact with articular cells such as fibroblasts and chondrocytes to induce mitogenesis with resultant cellular proliferation likely leading to synovial lining hypertrophy. BCP crystals also upregulate production of cytokines such as tumour necrosis factor alpha (TNF-α), interleukin 1 (IL-1), increase prostaglandin E2 via the cyclooxygenase pathway, stimulate matrix metalloproteinases production and increase nitrous oxide production. Therefore, BCP crystals have potent biologic effects and represent a potential therapeutic target in OA.

S36.3 EPIGENETIC DRUGS AS POTENT MODULATORS IN OSTEOARTHRITIS

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Osteoarthritis (OA) is a leading cause of joint deformity and functional limitation. An imbalance of anabolic and catabolic activity results in destruction of the extracellular matrix of articular cartilage. There is evidence to support the role of DNA methylation in the pathogenesis of OA, but the effect of other epigenetic modifiers is yet to be described. This study looks at the effect of novel epigenetic modulators, PFI-1, a bromodomain inhibitor, and SGC707, a histone methytransferase inhibitor, and their effects on gene expression in the pathogenesis of OA. Chondrocytes were extracted from OA femoral heads (n=6), cultured and incubated. Samples were treated with media alone (control), interleukin 1-beta (IL-1β) plus oncostatin M (OSM) alone, or in combination with increasing concentrations of PFI-1 or SGC707. Levels of expression of \textit{iNOS}, \textit{COX2}, \textit{IL8}, \textit{IL1B}, \textit{matrix metalloproteinase-13 (MMP13)}, \textit{RUNX2} and \textit{COL9A1} were measured using qRT-PCR, and expressed relative to \textit{GAPDH}. PFI-1 (0.5 and 5μM) suppressed expression of catabolic genes in OA chondrocytes, at basal levels and when co-stimulated with IL-1β+OSM. Catabolic gene expression decreased (\textit{iNOS}, \textit{COX2}, \textit{IL-8}, \textit{IL-1β} and \textit{MMP}), and \textit{RUNX2} expression was also suppressed. There was no effect on expression of the anabolic gene \textit{COL9A1}. SGC707 (0.1 and 1μM) did not induce a reduction in expression of all the catabolic genes. This study has demonstrated that PFI-1 has a potent protective effect against cartilage degradation, by modulating the expression of catabolic genes in OA chondrocytes. This further validates the role of epigenetics in OA, with implications for therapeutic interventions in the future.

**S36.4 DETERMINATION OF THE ROLE OF NEURONAL INTERLEUKIN-16 IN THE MECHANISM OF CALCIFICATION THAT OCCURS DURING PROGRESSION OF OSTEOARTHRITIS USING CRISPR**

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Osteoarthritis (OA) is a debilitating joint disease that severely affects elderly populations. At present there are no effective treatments for OA and mechanisms of disease progression are poorly understood. Previous work has identified that neuronal-Interleukin-16 (nIL-16) was significantly up-regulated in cartilage during the later stages of OA. Preliminary investigations identified co-localisation of nIL-16 with the Transient Receptor Potential cation ion-channel sub-Family-V-member-4 (TRPV4) in the primary cilium and the pericellular matrix of human OA chondrocytes. Perturbation of both TRPV4 and cilia are strongly associated with OA. We hypothesised that nIL-16 and TRPV4 work in tandem in a pathway that leads to chondrocyte hypertrophy and calcification that is seen in late OA and contributes to the loss of joint integrity. This makes it a promising target for development of a gene therapy to combat the disease. With the aim of elucidating the mechanism involved, nIL-16 knock-out cell lines generated using the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Cas9 system will be used to knock out nIL-16 PDZ domains to investigate whether this is the mechanism in which nIL-16 functions to anchor TRPV4 to the membrane of chondrocytes at the primary cilium. This work will be carried out using an immortalized hTERT mesenchymal stromal cell (MSC) cell line and effects on terminal MSC chondrogenesis, where hypertrophy mimics the process of calcification seen in OA, will be used to define functional effects of the knockout. Cell lines will be made using the RALA peptide (Phion Therapeutics), a bioinspired nanoparticle, for delivery the CRISPR/Cas9 system.

**S36.5 THE INFLUENCE OF BODY MASS INDEX ON MICROSTRUCTURAL AND PATHOLOGICAL CHANGES IN OSTEOCHONDRAL UNIT OF OSTEOARTHRITIC TIBIA PLATEAUS**

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Osteoarthritis (OA) is traditionally believed to affect the osteochondral unit by wear-and-tear from the superficial zone to the deep zone of cartilage and extended to subchondral plate. Obesity is commonly considered as a risk of OA development and hence total knee replacement (TKR), but the mechanism remains unclear. We hypothesized that obesity accelerated OA development by deteriorating tidemarks and increasing bone remodelling. 616,495 cases of TKR for OA from Australia and British joint replacement registries were collected, and data indicated that patients with higher BMI had TKR at earlier age. Specifically, patients with BMI $\leq$25kg/m$^2$ showed 8 years younger than patients with BMI $\geq$40kg/m$^2$ ($P<0.0001$) when they received TKR. We next examined tibia plateaus of 88 knee OA patients by micro-CT and histomorphometry. Linear regression showed that less cartilage degradation was associated with increased BMI in the load-bear compartment ($p<0.05$), while 58.3% of patients with BMI $\geq$40kg/m$^2$ demonstrated a clear anatomical separation close to tidemarks filled with fibrosis, erythrocytes and bone fragments (compared to BMI $\leq$25kg/m$^2$ group: 7.7%, $p<0.01$). In subchondral bone, elevated bone formation was associated with increased BMI, as higher thickness of osteoid ($p<0.01$), percent osteoid volume ($p<0.01$), percent osteoid surface ($p<0.01$) were found in obese patients. However, no alteration of bone resorption and microstructural parameters was found to be associated with BMI. We suspected that the abnormal loading in knee joint due to high BMI led to the direct deterioration of binding site of osteochondral unit, which might be the mechanism of the rapid progression in obesity-related OA.

S36.6 A RANDOMISED CONTROL TRIAL OF INTRA ARTICULAR INJECTATES IN KNEE OSTEOARTHRITIS: RESULTS OF CORTICOSTEROID VERSUS NSAID INJECTIONS

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Knee osteoarthritis is a common, debilitating condition. Intra articular corticosteroid injections are a commonly used non-operative treatment strategy. Intra articular hip injection with Ketorolac (an NSAID) has proven to be as efficacious as corticosteroids. No prior study compares the efficacy of Ketorolac relative to corticosteroids for relief of discomfort in knee osteoarthritis. The study design was a single centre double blinded RCT. Severity of osteoarthritic changes were graded on plain film weightbearing radiographs using the Kellgren and Lawrence system. Injection was with either 30mg Ketorolac or 40mg Methylprednisolone, given by intra-articular injection, in a syringe with 5mls 0.5% Marcaine. Pre-injection clinical outcomes were assessed using the Numerical Pain Score (NPS), WOMAC, and Oxford knee scores. Patients’ NPS scores were assessed at Day 1 and Day 14 post-injection. An assessment of all clinical outcomes took place in clinic at six weeks. There were 72 participants (83 knees) in the study. No patients were lost to follow-up. Mean age was 62.66 years (Range 29-85). 42 knees received a corticosteroid injection, 41 a NSAID injection. Mean Kellgren and Lawrence score was 3.1. There was no significant difference in pre-injection clinical scores in either group. There was a significant improvement of NPS on Day 1 and 14 in both injection groups($p<0.05$). These improved pain scores were sustained at 6 weeks in both groups. WOMAC and Oxford Knee Scores showed a statistically significant improvement in the corticosteroid group. WOMAC scores showed significant improvement in the NSAID group, however these improvements didn’t achieve statistical significance using the Oxford Knee Score. Corticosteroid or NSAID injectate are a safe and effective non-operative treatment strategy in the patient with knee osteoarthritis. Ketorolac appears to provide effective medium-term improvement of pain and clinical scores. Further follow-up is recommended to investigate if this trend in sustained.
S37.1 ADVANCED BIOMECHANICAL TESTING – EXAMPLES FROM THE FIELD OF ORTHOPAEDIC DEVICE MODULARITIES & CEMENTED IMPLANT FIXATION IN KNEE ARTHROPLASTY

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Modular hip prostheses were introduced to optimize the intra-surgical adaptation of the implant design to the native anatomy and biomechanics of the hip. The downside of a modular implant design with an additional modular interface is the potential susceptibility to fretting, crevice corrosion and wear. For testing hip implants with proximal femoral modularity according to ISO & ASTM, sodium chloride solutions are frequently used to determine the fatigue strength and durability of the stem-neck connection. The present study illustrate that the expansion of standard requirements of biomechanical testing is necessary to simulate metal ion release as well as fretting and crevice corrosion by using alternative test fluids. To assess the primary stability of tibial plateaus in vitro, different approaches had been undergone: cement penetration depth analysis, static tension or compression loading until interface failure. However, these test conditions do not reflect the in vivo physiologic loading modes, where the tibial plateau is predominantly subjected to combined compression and shear forces. The objectives were to evaluate the impact of the tibial keel & stem length on the primary stability of a posterior-stabilised tibial plateau under dynamic compression-shear loading conditions in human tibiae.

S37.2 IS SYNOVIAL FLUID A MIRROR OF EQUINE JOINT PATHOPHYSIOLOGY? REVIEW OF THE TRIBOLOGICAL IDENTITY OF EQUINE SYNOVIAL FLUID

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The paramount importance of synovial fluid in lubrication and protection of articular joints has long been recognized. Synovial fluid, a dialysate of plasma, forms an interface with both the synovium and cartilage and plays a crucial role in joint lubrication and bearing functions. In an osteoarthritic joint, damage to the articular cartilage causes modifications in the rheological properties of synovial fluid and, reducing the viscoelasticity and increasing the friction between articular surfaces. Viscosupplementation is a treatment for osteoarthritis that uses hyaluronic acid as a (visco)supplement to the diseased joint. The aim of this treatment is to restore the rheological properties of synovial fluid. Osteoarthritis is the most common disease affecting the joints in human population and among the most important causes of pain, disability and economic loss. Therefore, innovative methods are needed to more effectively treat osteoarthritis, directly addressing the disease process. Among various locomotor mechanisms that could serve to illustrate the integrated nature of functional morphology, perhaps none is more complex than the equine locomotor system. Confronting the need for evaluating the current methods to control joint disease, the horse provides an excellent animal model. As it suffers similar clinical manifestations to those seen in human, it may provide tentative biomedical extrapolations.

S37.3 CARTILAGE CONTACT PRESSURE IN THE KNEE IN THE PRESENCE OF A FOCAL DEFECT

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In knee osteoarthritis (OA) patients, a focal cartilage defect is commonly found, especially in the medial compartment. In addition, cartilage softening is often observed at the defect rim. Both factors may alter the loading distribution and thereby the contact pressures, previously related to cartilage degeneration. To determine contact pressure \textit{in-vivo} during motion, computational modelling can be used. The aim of this study was to analyse knee cartilage pressures during walking in healthy and damaged cartilage using a multi-scale modelling approach. Using 3D motion capture and musculoskeletal models, multi-body simulations of the stance phase of gait calculated knee kinematics and muscle, ligament and contact forces. These were subsequently imposed to a finite element (FE) model including tibial and femoral bones and cartilage. FE analyses were performed using intact cartilage as well as including a medial tibial cartilage defect, with and without softening of the defect rim. Specifically during loading response, a medial cartilage defect reduced the contact surface (-28%) and thereby increased the contact pressure (+33%) compared to intact cartilage, particularly on the medial compartment (+75% in contact pressure). Including softening of the cartilage rim increased the contact area (+22%) and decreased contact pressures (-9%) compared to the defect. This indicates that a focal defect increases the cartilage loading. This is partially compensated by softening of the cartilage rim. Therefore, the role of focal defects in altered cartilage loading and consequent OA development always needs to be discussed acknowledging the cartilage status at the defect rim.

\textbf{S37.4 IN VIVO KINEMATIC ANALYSIS AFTER TOTAL KNEE ARTHROPLASTY; COMPARISON BETWEEN INTRA- AND POST-OPERATIVE MEASUREMENTS}


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Our aim was to investigate whether it is possible to predict post-operative kinematics (Post-Ope) from intra-operative kinematics (Intra-Ope) after total knee arthroplasty. Our study were performed for 11 patients (14 knees) who underwent primary PS TKA using CT-based navigation system between Sept.2012 and Sept.2014. The mean subject age was 71.5 ± 5.5 years at the time of surgery. Intra-Ope was measured using the navigation system after implantation during passive full extension and flexion imposed by the surgeon. Under fluoroscopic surveillance, each patient was asked to perform sequential deep knee flexion under both non-weight bearing (NWB) and weight bearing (WB) conditions from full extension to maximum flexion. To estimate the spatial position and orientation, we used a 2- to 3- dimensional (2D3D) registration technique. Intra-Ope and Post-Ope had a common coordinate axis for bones. Evaluations were range of motion (ROM), external rotation angles (ER). The level of statistical significant difference was set at 0.05. Mean ROM in Intra-Ope(130°± 7.9°) was statistically larger than both NWB(121.1°±10.5°) and WB(124.0°±14.7°). No Statistically significant difference was found in the mean ER from 10° to 120° among Intra-Ope (11.2°± 8.5°) and NWB(7.1°±6.0°) and WB(5.3°±3.2°). It is suggested that we could predict Post-Ope from Intra-Ope by considering the increase of the range of motion due to the muscle relaxation condition and the amount of change in the ER.

\textbf{S37.5 VARIATION IN EARLY FUNCTIONAL OUTCOME MEASUREMENTS FOLLOWING TOTAL KNEE ARTHROPLASTY}

M.T.A. Griffin, A.H.R.W. Simpson, D.F. Hamilton
The first three months following Total Knee Arthroplasty (TKA) provide an early window into a patient’s functional outcomes, with the change of function in this time yielding valuable insight. Twenty patients due to undergo primary TKA were recruited to the study. Data were recorded at three time points; pre-assessment clinic (PAC) before the operation, 6-weeks-post-operation (6WKs), at 12-weeks-post-operation (12WKs). Functional activity levels were monitored during early post-operative recovery for changes in early functional outcome, and allowed a comparison of metrics at each time point. This included direct functional testing of power output, timed functional performance in clinic, patient reported outcome measures, and multiday activity monitoring devices. Maximal power output symmetry (Power) was similar at 6WKs vs PAC (p = 0.37). At 12WKs, it had increased (p < 0.05). Timed functional performance (Performance) remained similar across all three time points (p = 0.27). Patient reported activities of daily living (ADL) performance significantly increased at 6WKs vs PAC (p < 0.05). At 12WKs, it remained similar (p = 0.10). Patient daily step count significantly decreased at 6WKs vs PAC (p < 0.05). By 12WKs, this had increased to similar levels to PAC (p = 0.30).

### S37.6 POOR ACCURACY IN DIAGNOSIS OF COMMON KNEE CONDITIONS, HIGHLIGHTING THE INHERENT WEAKNESS OF THE MEDICAL MODEL AND ALGORITHMS

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The medical model of history, examination and investigation forms the bedrock of diagnosis and management of all patients. The essence is the recognition of patterns of symptoms and signs. In the modern era there are an increasing number of non-medical resources ranging from web-based information, computer diagnostic aids and non-specialist healthcare professionals to provide a diagnosis and commence management of a wide range of conditions, including knee problems. We analysed the quality and patterns of clinical presentation in order to answer the question how closely clinical symptoms and examination findings correlate to diagnosis based on MRI scan and/or arthroscopic findings. The analysis was a dataset of a consecutive series of patients, aged 18 to 45, with no past history of knee problems or end stage arthritis, presenting to a single specialist triage physiotherapist, working within an integrated knee service, who fully completed a standardised knee assessment proforma of presenting symptoms and signs at a large district general hospital. The study comprises 86 patients and 98 knees. We analysed this data based on diagnostic findings of MRI scan or arthroscopy to provide definitive intra-articular diagnosis. Based on standard textbook descriptions of common presentations, we went on to define the patients’ presentation history and examination as typical or atypical, with typical meaning the symptoms and signs correlated with the diagnosis. The null hypothesis is that patients have a high chance of typical presentations for common knee conditions. In the 75% of patients with a significant intra-articular pathology we found the majority had chondral rather than meniscal tears 1.7 to 1. Forty four percent of patients had atypical symptoms and 71% had atypical clinical signs, 30% and only 26% of the cohort had both typical symptoms and signs together, reflecting a surprisingly low positive predictive probability of symptoms and signs in this group of patients, particularly those with chondral lesions which was 44%. In this cohort, 57% of the cohort has 3 or more multiple diagnoses. In the diagnostically normal group, 43% had symptoms and signs typical for a meniscal tear. We conclude that clinical symptoms and signs surprisingly inaccurate in guiding intra-articular pathology within the knee, even in a sub-set considered the easy and accurate to assess. The number of multiple diagnoses and the incidence of false positive results also means
that simplistic interpretations of non-definitive diagnoses and linear causation of pain pathways should be treated cautiously.

S38.1 BEING A GOOD GUEST: THE HOST IMPLANT PARADIGM

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Biomaterials are no longer considered innate structures and using functionalisation strategies to modulate a desired response whether it is a host or implant is currently an important focus in current research paradigms. Fundamentally, a thorough understanding the host response will enable us to design proper functionalisation strategies. The input from the host response need to be weighed in depending on the host disease condition. In addition, biomaterials themselves provide immense therapeutic benefits which needs to be accounted for when using functionalisation strategies. Using strategies such as enzymatic and hyperbranched linking systems, we have been able to link biomolecules to different structural moieties. Our recent design efforts have harnessed the therapeutic effects of biomaterials and mapped the molecular fingerprint of this specific host response in a disease target. This approach allows us to rethink functionalisation strategies currently employed in the field. This talk will elucidate some of these ongoing strategies that have applications in the development of the next generation of orthopaedics devices.

S38.2 BIOMETALS FOR REGENERATIVE AND TRANSLATIONAL MEDICINE

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Biometals like Magnesium (Mg) and Zinc (Zn) are essential for life. Mg/Zn-deficiency has been linked to numerous diseases including cardiovascular, bone, diabetics, neurological and neurodegenerative disorders. Moreover, Mg/Zn-based biomaterials have recently emerged as innovative degradable medical implants, typically for cardiovascular and orthopedic application. We study the pathophysiological role of Mg²⁺/Zn²⁺ ion in vascular and bone diseases, as well as metallic Mg/Zn alloys for stent and bone implant applications. We demonstrated some interesting role and mechanism of Mg²⁺/Zn²⁺ ion in controlling cellular functions. Also, metallic Mg/Zn-based medical implants exhibited strong potential as stent and bone fixation device. They have sufficient mechanical strength, promotes tissue regeneration, and are fully bioresorbable with minimal toxicity. The beneficial or therapeutic role of biometals Mg/Zn in medicine and biomaterial applications is still not fully explored, our research aims to answer some fundamental questions and to inspire more future studies related to biometals in health.

S38.3 EFFECTS OF PROCESSING CONDITIONS ON HIERARCHICAL FEATURES OF COLLAGEN-BASED SUBSTRATES FOR MEDICAL DEVICES


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The aim of this work was the structural investigation of different type I collagen isoforms at atomic and nanoscale, as well as the evaluation of the impact of different fabrication treatments on the structural, mechanical and biological properties of collagen-based films. Raw type-I collagens from bovine hide (Typ-BH, CS, SYM) and equine tendon (TypE, TypCH and OPO) were analyzed. Materials were then used for fabricating air-dried films, obtained by: 1) dissolution in distilled water (HH); 2) dissolution in acidic medium (AA); 3) homogenization of acid solubilized fibers (HOM). Crosslinking treatments (DHT, DHT+EDC) were also adopted and studied. Analysis by Wide Angle (WAXS) and Small Angle (SAXS) X-ray Scattering was carried out at the XMI L@b (CNR-IC-Bari); Fourier Transform-IR and biological analysis was performed at UniSalento. WAXS and SAXS data on raw materials demonstrated the preferential orientation of collagen molecules and the preservation of hierarchical nanoscale architecture in equine tendon-derived collagens, in particular in chemically extracted, while randomly oriented molecules were found in bovine dermis collagens, together with a certain degree of salt contamination. Concerning equine collagen, we found that TypCH structure is influenced by crosslinking procedures at atomic scale, whereas both processing conditions and crosslinking treatments affect TypE collagen structure at atomic and nanoscale. WAXS, SAXS and FT-IR analyses showed that the HOM processing was the one which ensures a high content of structural super-organization of collagen into triple helices and a high crystalline domain of the final material. Crosslinking of the films by DHT/EDC combined treatment was shown to affect their mechanical stiffness, the latter depending on the collagen source and the specific processing conditions.

S38.4 CONTROLLED RELEASE OF BIOLOGICAL FACTORS FOR PROGENITOR CELL-MEDIATED ENDOGENOUS REPAIR OF INTERVERTEBRAL DISCS

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The recent description of progenitor/stem cells in degenerated intervertebral discs (IVDs) raised the possibility of harnessing their regenerative capacity for endogenous repair. The aim of this work is to develop an intradiscal polysaccharide microbead-based delivery system for the sequential release of chemokines and nucleopulpogenic factors. This delivery system would sequentially contribute to 1) the recruitment of resident progenitors (CXCL12 or CCL5), 2) the differentiation of the mobilized progenitors (TGF-β1 and GDF5), and 3) the subsequent regeneration of NP. To determine the effects of chemokines on in vitro cell recruitment, human mesenchymal stem cells (MSC) were cultured in Transwells for 4h, with or without CXCL12 or CCL5. In parallel, pullulan microbeads (PMBs) (100μm) were prepared by a simultaneous crosslinking protocol coupled to a water-in-oil emulsification process. Freeze-dried PMBs were loaded with biological factors then release assays were performed at 37°C for 21 days and supernatant concentrations were measured by ELISA. As compared to untreated MSC, MSC migration was improved with a 3.9 (CXCL12) and 7.5 (CCL5) fold increase, respectively. All factors were successfully adsorbed on PMBs and a burst release within the 1st day was observed. At day 7, 27.5% and 83% of CXCL12 and CCL5 were released, respectively and at day 21, 20% and 100% of TGF-β1 and GDF5 were released, respectively. Currently, released cytokine bioactivity is being analysed and an ex vivo ovine IVD model is developed to determine the repair potential of this controlled release approach.

S38.5 DEVELOPMENT OF MULTICOMPARTMENT COLLAGEN DEVICES FOR CONTROLLED AND SYNERGISTIC DUAL DELIVERY

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Complex pathophysiology involves different signalling mechanisms, with a multitude of often interconnected potential therapeutic targets. Therefore, there is a need for the development of multi-compartment delivery vehicles for combinatorial and synergistic therapeutic approaches. In this study, it was hypothesized that multi-compartment crosslinked collagen type I systems can deliver multiple bioactive agents in a controlled manner in an \textit{in vitro} model condition of skin fibrosis. Multi-compartment collagen-based systems were made using solutions of dialyzed type I collagen mixed with 10x PBS, after which they were neutralized and crosslinked with 1 and 2.0 mM 4-arm-succinimidyl glutarate ester PEG (4-arm-PEG-SG), respectively, followed by incubation at 37°C. The systems were characterised through swelling assessment, collagenase degradation assay and compression tests. The release of encapsulated drugs from the hydrogels was studied by ELISA and the effect of the delivered bioactive agents was assessed through imaging and quantification for fibrotic markers in an \textit{in vitro} model. A pilot study using FITC-dextran proved that the inner compartment was capable of promoting sustained release over a long period of time (7 days), which was further confirmed with drug release assays using a TNF-α antagonist and recombinant decorin, fitting the intended therapeutic release profile. Protein expression studies showed a decrease of endogenous collagen type I and α-smooth muscle actin expression (p<0.05) indicating amelioration of fibrosis. In summary, this indicates that this system is suitable for dual delivery of multiple bioactive agents, resulting in a controlled release \textit{in vitro} and illustrating its potential in therapy.

\textbf{S38.6 HIGH RESOLUTION 3D PRINTING OF COLLAGEN}

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Collagen is a key component of the extracellular matrix in a variety of tissues and hence is widely used in tissue engineering research, yet collagen has had limited uptake in the field of 3D printing. In this study, we successfully adapted an existing electronic printing method, aerosol jet printing (AJP), to print high resolution 3D constructs of recombinant collagen type III (RHCIII). Circular samples with a diameter of 4.5mm and 288 layers thick, or a diameter of 6.5mm and 400 layers thick were printed on glass cover slips with print lines of 60μm. Attenuated Total Reflectance Fourier-Transform Infrarred (ATR-FTIR) spectroscopy performed on 4 of the printed samples and dried non-printed RHCIII samples showed that no denaturation had occurred due to the printing process. Printed samples were crosslinked using EDC [N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride, Sigma Aldrich] to improve their stability and mechanical strength. Differential scanning calorimetry (DSC) performed showed a marked difference in the denaturation temperature between crosslinked printed samples and fibrillar non-printed samples and nano-indentation showed that the construct was relatively stiff. Previous results with similar samples have shown that mesenchymal stem cells (MSCs) align with and travel parallel to print direction. Results obtained from these samples show signs that they might be applied in other areas such as bone tissue engineering.

\textbf{S39.1 OSTEOCYTES AND PERIPROSTHETIC JOINT INFECTION}

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Periprosthetic joint infections (PJI) are increasing in prevalence and are recognised as one of the most common modes of failure of joint replacements. Osteomyelitis arising from PJI is challenging to treat, difficult to cure and increases patient mortality 5-fold. PJI can have subtle symptoms and lie dormant or go undiagnosed for many years, suggesting persistent bacterial infection. *Staphylococcus aureus* is the most common pathogen causing PJI. Osteocytes are the most numerous and long-lived cell type in hard bone tissue. Our recent work has shown that *S. aureus* can infect and reside in human osteocytes without causing cell death, both experimentally and in bone samples from patients with PJI. Osteocytes respond to infection by the differential regulation of a large number of genes, suggesting previously unknown immune functions of this important cell type. *S. aureus* adapts during intracellular infection of osteocytes by adopting a quasi-dormant, small colony variant (SCV) phenotype, a property of several bacterial species known to cause PJI, which could contribute to persistent or silent infection. These findings shed new light on the aetiology of PJI and osteomyelitis in general. Further elucidation of the role of osteocytes in bone infection will hopefully lead to improved disease detection and management.

**S39.2 DO BACTERIA CONTRIBUTE TO ASEPTIC LOOSENING OF ORTHOPAEDIC IMPLANTS?**

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Considerable evidence exists that aseptic loosening is initiated by wear particles that recruit macrophages and stimulate their production of pro-inflammatory cytokines. The cytokines primarily act indirectly by inducing production of RANKL, which stimulates osteoclast differentiation, osteolysis, and inflammatory bone loss. There is also considerable evidence that activation of macrophage Toll-like Receptors (TLRs) contributes to this cascade of events. It is however controversial whether bacterially-derived immunostimulatory molecules known as Pathogen-Associated Molecular Patterns (PAMPs) can contribute to aseptic loosening by stimulating their cognate TLRs on macrophages. Priming and subsequent activation of the NLRP3 inflammasome is essential for macrophage production of mature, active IL-1β in response to wear particles. We recently confirmed that wear particles can activate pre primed NLRP3 inflammasomes in the absence of PAMPs. Thus, activation of the NLRP3 inflammasome is the only macrophage-based event in the aseptic loosening cascade that we have found to date is independent of PAMPs. In contrast, priming of the NLRP3 inflammasome by wear particles requires PAMPs as well as their cognate TLRs. These results add to the growing body of evidence that bacterially-derived PAMPs can contribute to aseptic loosening.

**S39.3 COLLAGEN SCAFFOLDS FUNCTIONALISED WITH COPPER-ELUTING BIOACTIVE GLASS FOR THE TREATMENT OF INFECTION AND REGENERATION OF VASCULARISED BONE**


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The bone infection osteomyelitis (typically *Staphylococcus aureus*) requires a multistep treatment process including: surgical debridement, long-term systemic high-dose antibiotics, and often bone grafting. With antibiotic resistance becoming increasingly concerning, alternative approaches are urgently needed. Herein, we develop a one-step treatment for osteomyelitis that combines local, controlled release of non-antibiotic...
antibacterials (copper) within a proven regenerative scaffold. To maximise efficacy we utilised bioactive glass – an established material with immense osteogenic capacity – as a copper ion delivery reservoir. Copper ions have also been shown to stimulate angiogenesis and induce MSC differentiation down an osteogenic lineage. To eliminate grafting requirements, the copper-doped BG was incorporated into our previously developed collagen scaffolds to produce multifunctional antibacterial, osteogenic, and angiogenic scaffolds. Scaffolds were fabricated by freeze-drying a co-suspension of collagen and bioactive glass particles (± copper doping, referred to as CuBG and BG, respectively) at a range of different concentrations (0-300% w/w bioactive glass/collagen). Scaffolds demonstrated a 2.7-fold increase in compressive modulus (300% CuBG vs. 0%; p≤0.01), whilst maintaining >98% porosity. The 300% CuBG scaffolds showed significant antibacterial activity against *Staphylococcus aureus* (p≤0.001). In terms of osteogenesis, both 100% and 300% CuBG scaffolds increased cell-mediated calcium deposition on the scaffolds at day 14 and 28 (p≤0.05 and p≤0.001), as confirmed by alizarin red staining. 100% CuBG scaffolds significantly enhanced angiogenesis by increased tubule formation (p≤0.01) and VEGF protein production (p≤0.001) (all ≥ n=3). In summary, this single-stage, off-the-shelf treatment for osteomyelitis shows potential to minimise bone grafting and antibiotic dependence, while reducing hospital stays and costs.

**S39.4 INDUCTION HEATING FOR DECREASING BACTERIAL LOAD OF *STAPHYLOCOCCUS EPIDERMIDIS* FROM BIOFILM: IN VITRO RESULTS**

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The main problem of infected orthopaedic implants is that the presence of microorganisms in an organized biofilm making them difficult accessible for antibiotics. This biofilm consists of a complex community of microorganisms embedded in an extracellular matrix that forms on surfaces such as an implant. Non-contact induction heating uses pulsed electromagnetic fields to induce so-called ‘eddy currents’ within metal objects which causes them to heat up. This heat causes thermal damage to the bacterial biofilm hence killing the bacteria on the metal implant. The purpose of this study is to determine the effectiveness of induction heating on killing *Staphylococcus epidermidis* in a biofilm. *S. epidermidis* biofilms were grown on Titanium alloy (Ti6Al4V) coupons and subsequently were heated with a custom-built induction heater to temperatures of 60°C, 70°C, 80°C and 90°C for 3.5 minutes. Temperature was controlled with an infra-red thermal sensor and micro-controller. We also included two control conditions without induction heating: C1 without induction heating and C2 with chlorhexidine 0.5% in 70% alcohol without induction heating. Experiments were repeated 5 times. In the C1 group (no induction heating), 1.3 * 10(7) colony forming units (CFU)/cm(-2) of *S. epidermidis* were observed. For 60 °C, 70C, 80 C and 90°C a 3.9-log reduction, 5.3-log reduction, 5.5-log reduction and 6.1-log reduction in CFU/cm(-2) were observed, respectively. For the C2 (chlorhexidine) there was a 6.7-log reduction CFU/cm(-2). We concluded that induction heating of Titanium coupons is effective in reducing bacterial load *in vitro* for *S. epidermidis* biofilms.

**S39.5 CUTIBACTERIUM ACNES ISOLATED FROM BONE PROSTHESIS VS SKIN: DIFFERENCE IN VIRULENT BEHAVIOR**


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Prosthetic Joint Infections (PJs) are increasing with the use of orthopedic devices on an ageing population. *Cutibacterium acnes* is a commensal organism that plays an important role in the ecosystem healthy human skin, yet this species is also recognized as a pathogen in foreign body infection: endocarditis, prostatitis and specifically in PJs. *C. acnes* is able to escape the immune system. This phenomenon could reflect two bacterial behaviour: the bacterial internalization by host cells and the biofilm formation. In this study, we studied different clinical strains of *C. acnes*. We noticed that *C. acnes* isolated from PJs form 2 fold-more biofilm than the strains isolated from a normal skin in two models (Crystal violet staining and fluorescent microscopy (*p*=0.04 and *p*=0.02, respectively, Mann-Whitney test). We did not observe any difference in the internalization rate of those strains by osteoblasts. However, the quantity of biofilm formed by *C. acnes* before and after the internalization was compared. A significant increase in biofilm formation was observed for the strains isolated from the skin (x2.3±0.07; *p*=0.008, Mann-Whitney test). However, the hydrophobicity of the skin strains is significantly less important than for the PJs strains (24.8±13% vs 56.6±12% respectively; *p*=0.003, Mann-Whitney test) but this did not change after internalization suggesting that there is no cell wall evolution. In conclusion, we studied for the first time the impact of bacterial internalization by osteoblasts on the virulent behaviour of *C. acnes*, which could explain the hidden pathogenicity of this commensal bacterium.

**S39.6 COMPARISON OF DIFFERENT ANTIBIOTIC PROPHYLAXIS REGIMENS IN THE RISK OF REVISION FOR INFECTION FOLLOWING PRIMARY JOINT ARTHROPLASTY OF THE HIP AND KNEE IN THE NETHERLANDS**


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Administration of perioperative antibiotic prophylaxis (AP) reduces the risk of prosthetic joint infection (PJI) following primary total hip (THA) and knee (TKA) arthroplasty. The optimal type of antibiotic used, and duration of prophylaxis are subject to debate. We compared the risk of revision surgery for PJI in the first year following THA and TKA by AP regimen. A national survey collecting information on hospital-level AP regimen policy was conducted across the Netherlands and linked to data from the LROI arthroplasty registry for 2011-2015. PJI status was defined using the surgical indication reported at revision by surgeons in the registry form. Restricted cubic splines Poisson model adjusted for hospital clustering were used to conduct the comparisons on 130,712 THAs and 111,467 TKAs performed across 99 institutions. These included 399 THAs and 303 TKAs revised for an indication of PJI. Multiple shot of Cefazolin (MCZ), of cefuroxime (MCX) and single shot of Cefazolin (SCZ) were respectively administrated to 87%, 4% and 9% of patients. For THA, the rates of revision for PJI were respectively 31/10,000 person-years 95%CI[28, 35], 39[25, 59] and 23[15, 34] in the groups which received MCZ, MCX and SCZ; respectively, the rates for TKA were 27[24, 31], 40[24, 62] and 24[16, 36] . No evidence of difference between AP regimens was found in the unadjusted and adjusted model (age, gender, BMI and ASA grade). Further work is advocated to confirm whether there is an association between AP regimen collected at patient-level and the risk of subsequent revision for PJI.

**S40.1 CLINICAL TRIALS ON BEARING SURFACES IN TOTAL HIP REPLACEMENT**

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We have undertaken a series of clinical trials over the last 20 years to look at different bearing surface combinations in young adults. We continue to follow these patients well beyond the planned duration of the trials and new information is constantly becoming available. The first trial compared ceramic-on-ceramic with ceramic-on-standard-polyethylene. These patients have now been followed for 20 years with significant wear in the polyethylene group but virtually identical revision rates. The second trial ceramic-on-ceramic, cobalt-chrome-on-standard-polyethylene and cobalt-chrome-on-cross-linked-polyethylene. In this group the ceramic-on-ceramic patients have the lowest revision rate; the ceramic-on-polyethylene group demonstrates a lower wear rate than cobalt-chrome-on-polyethylene. The third trial looks at cobalt-chrome versus zirconium on either cross-linked polyethylene or conventional polyethylene. At 10 years there remains no evidence of improved performance from the zirconium surface as compared to cobalt-chrome. The cross-linked polyethylene group is clearly outperforming the conventional polyethylene in terms of wear rate but at 10 years the revision rates remain the same in all groups. Cross linked polyethylene appears to be the major determining factor in prosthetic longevity and appears to be more important than the counter face material.

S40.2 METAL-ON-METAL HIP FAILURES: WHY DID THIS HAPPEN

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Metal on metal hip replacements have been one of worst failures in recent years in terms of orthopaedic implants. Some of these devices have had catastrophic failure rates, with reports of 48% failure at 6 years. The failure of these devices has led to considerable suffering, pain and reduction in quality of life; consequently, they have given rise to high costs and multi-million-dollar legal cases. This talk will describe the history of the current metal on metal failure and discuss some of the reasons why might have occurred. It will also consider the reasons that wear debris arising from the trunnion is worse in terms of biological activity then that arising from the bearing surfaces.

S40.3 EFFICACY AND PROCEDURE SURVIVAL OF METAL-ON-METAL HIP RESURFACING IN PATIENTS AGED LESS THAN 50 YEARS: A PROSPECTIVE OBSERVATIONAL COHORT STUDY WITH MINIMUM TEN-YEAR FOLLOW-UP

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The aim of this study was to report the procedure survival and patient-reported outcomes in a consecutive series of patients <50yrs at the time of hip arthroplasty with a metal-on-metal hip resurfacing system who have progressed to a minimum of 10yrs follow-up. Patients presenting for treatment of degenerative conditions of the hip electing to undergo hip resurfacing were included in a clinical registry (N=226 patients; 238 procedures). Procedure survival was confirmed by crosschecking to the Australian Orthopaedic Association National Joint Replacement Registry and comparing to all procedures by other surgeons nationwide. Kaplan-meier survival curves with 95% confidence intervals were constructed, while patient-reported outcome measures were compared with t-tests and postoperative scores assessed with anchor analysis to age and gender-matched normative data. At mean follow up of 12 years, six cases were revised with a cumulative survival rate of 96.8% (95%CI 94.2-99.4) at 15 years. Majority of revisions were early (<3yrs) and occurred in females (N=4). Patient-reported general health, disease state, hip function and activity level maintained large improvements beyond 10 years post-implantation and were equal to or exceeded age and gender-matched normative data. Metal-on-metal hip resurfacing in males and females aged <50 years at time of surgery
demonstrated a high rate of cumulative survival beyond 10 years follow up. The results demonstrate excellent outcomes in this age group.

**S40.4 HIP JOINT SYNOVIAL FLUID BONE RESORPTION MARKERS IN SUBCHONDRAL INSUFFICIENCY FRACTURE OF THE FEMORAL HEAD**


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Similar to the radiological findings in rapidly destructive arthrosis of the hip joint (RDA), subchondral insufficiency fracture of the femoral head (SIF) can result in progressive femoral head collapse of unknown etiology. We thus examined the osteoclast activity in hip joint fluid in SIF with progressive collapse in comparison to that in RDA. Twenty-nine hip joint fluid samples were obtained intraoperatively with whole femoral heads from 12 SIF patients and 17 RDA patients. SIF cases were classified into subgroups based on the presence of ≥2mm collapse on preoperative radiographs: SIF with progressive collapse (n=5) and SIF without progressive collapse (n=7). The levels of tartrate-resistant acid phosphatase (TRACP)-5b, interleukin-8, vascular endothelial growth factor (VEGF), and matrix metalloproteinase (MMP)-9 were measured. Numbers of multinuclear giant cells at the subchondral region were assessed histopathologically using mid-coronal slices of each femoral head specimen. Median levels of all markers and median numbers of multinuclear giant cells in SIF with progressive collapse were significantly higher than those in SIF without progressive collapse, while there were no significant differences in SIF with progressive collapse versus RDA. Regression analysis showed that the number of multinuclear giant cells correlated positively with the level of TRACP-5b in joint fluid. This study suggests an association of increased osteoclast activity with the existing condition of progressive collapse in SIF, which was quite similar to the findings in RDA. Therefore, high activation of osteoclast cell may reflect the condition of progressive collapse in SIF as well as RDA.

**S40.5 IMPROVING THE WHO FIVE STEPS TO SAFER SURGERY BUNDLE: ENHANCING QUALITY FOR BETTER OUTCOMES**


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The World Health Organisation (WHO) Surgical Safety checklist is an evidence-based tool shown to reduce surgery-related morbidity and mortality. Despite audits showing 96% checklist compliance, our hospital had 3 surgical never events in 10 months, 2 of which were in orthopaedics. By March 2018, the authors aimed to achieve 100% compliance with all 5 sections of the WHO Five Steps to Safer Surgery bundle for all surgical patients. Additionally, the authors aimed to assess the impact of the quality of bundle delivery on preventable errors related to human factors. Quantitative assessment involved direct observations of compliance in theatres. Qualitative data in the form of rich, descriptive observations of events and discussions held during checklist delivery was analysed thematically. Interventions included trust-wide policy changes, awareness sessions, introduction of briefing and debrief pro formas and documented prosthesis checks. For elective surgeries, checklist compliance increased to 100% in 4 of 5 sections of the bundle. The incidence of reported preventable critical incidents decreased from 6.7% to 2.4%. A chi-squared test of independence demonstrated a significant relationship between the implementation of changes and completion of the checklist, X² (1, N = 1019) = 25.69, p < 0.0001. Thematic analysis identified leadership, accountability, engagement, empowerment, communication, and teamwork as factors promoting effective checklist use. Our
findings highlight the benefits of a qualitative approach to auditing checklists. Exploring the role of human factors and promoting staff awareness and engagement improves checklist compliance and enhances its effectiveness in reducing surgery-related adverse outcomes.

**S40.6 PRIMARY TOTAL HIP REPLACEMENT: IRISH REGISTRY DATA FOR FIXATION METHODS AND BEARING OPTIONS AT A MINIMUM OF 10 YEARS**

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This was a retrospective study of registry data from a National Orthopaedic Hospital for all THRs with 10-year follow-up data. Inclusion criteria were all THRs with a minimum of 10-year follow-up data. All metal-on-metal (MoM) THRs and MoM resurfacings were excluded from the analysis due to the high rate of revision associated with these bearings. Univariate and multivariate analyses controlling for confounding variables were performed to compare outcomes. A total of 1,697 THRs were performed in 1,553 patients. The four significant predictors for revision were fixation type \( (p<0.01) \), surface bearing type \( (p<0.01) \), age \( (p<0.05) \) and head size \( (p<0.05) \). Gender, BMI and approach had no effect on revision rates. The lowest 10-year all-cause revision rates were seen in cemented THRs at 1.7%. Ceramic-on-poly bearings had the lowest revision rate at only 1.2%. Metal-on-poly bearings had a 1.7% revision rate. Ceramic on ceramic bearings had a 7.1% revision rate with 1 revision for squeak and 1 revision for ceramic head fracture. The causes for revision in order of decreasing frequency were as follows: Infection \( (n=13, 0.7\%) \), dislocation \( (n=7, 0.4\%) \), periprosthetic fracture \( (n=3, 0.2\%) \) and aseptic loosening \( (n=2, 0.1\%) \). There were 2 re-revisions at 10 years in total. The smaller 22.225mm head sizes had a significantly lower revision rate than other head sizes \( (p<0.05) \). Ceramic-on-poly bearings, cemented fixation and smaller head sizes perform better in the experience of this registry. However, with multivariate analysis, these differences were shown to be insignificant.

**S41.1 CLINICAL STATE OF THE ARTICULAR CARTILAGE REPAIR**

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Articular cartilage injury has a high prevalence in elite and recreational athletes. Articular cartilage repair remains a challenge due to cost effectiveness and clinical effectiveness issues. There are now several effective technologies and it is possible to return to competitive sports following many of the procedures available. The durability of repair tissue is variable and there remains extensive growth in the Scientific world. Evolving cartilage restoration technologies focus on increasing cartilage quality and quantity, while optimising surgery and rehabilitation. In UK ACI has undergone extensive cost effectiveness analysis and the in-depth review has shown that ACI is cost effective compared to microfracture. ACI is indicated for lesions \( >2 \text{cm sq} \) but NICE has considered that it is not indicated for problems after microfracture. This presentation details the various options available to surgeons and examines the cost effectiveness.

**S41.2 CELL STRATEGIES FOR CARTILAGE REPAIR**

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Osteoarthritis (OA) of the spine and diarthrodial joints is by far the most common cause of chronic disability in people over 50 years of age. The disease has a striking impact on quality of life and represents an enormous societal and economic cost, a burden that will increase greatly as populations age. OA is a complex condition with broad pathology. Damage to the articular cartilage is a consistent feature, accompanied by changes to the subchondral bone and synovium. Progression of the disease involves further degeneration of the articular cartilage, damage to the underlying bone and morphological changes that include subchondral bone thickening, development of cysts, osteophytes and inflammation of the synovium. Enhanced production of proinflammatory cytokines and matrix metalloproteinases accelerates degradation of the articular cartilage. It is striking that no approved pharmacological intervention, biological therapy or procedure prevents the progressive destruction of the OA joint. All current treatments, without exception, produce symptomatic rather than regenerative results. While there have been some exciting developments in the search for OA treatments in the last decade, including matrix metalloproteinase inhibitors, anti-TNF and anti-IL1 drugs for example, none of these has to date emerged as an effective medicinal product. There is thus an urgent and compelling need to identify, validate and test new biological therapeutics. Stromal cell therapy represents one such compelling approach. The results from several early clinical studies have indicated that this approach holds a great deal of promise for the treatment of OA. Most studies have involved direct intraarticular injection of a suspension of mesenchymal stromal cells (MSCs) for treatment of knee OA. Results from a number of controlled patient studies have suggested that this treatment results in an effective repair response. Although data regarding mechanism of action are limited, it appears that the cells have an anti-inflammatory effect, possibly targeting cells within the synovium, rather than a direct cartilage repair effect. Several recent reports have highlighted a dramatic and sustained response in patients receiving MSC treatment. For example, allogeneic expanded adipose-derived MSCs have been shown to be safe and effective in the treatment of complex perianal fistulas in Crohn's disease. Also, allogeneic bone marrow-derived MSCs has a been shown to have a positive effect in pediatric acute graft versus host disease. These observations point to a mechanism of action that involves host immunomodulation, but this needs further examination. Within the field of musculoskeletal disease effective translation of MSC technology has been hindered by a lack of randomized controlled patient studies, severe inconsistencies regarding the preparation and characterization of the cell product, and an incomplete understanding of the therapeutic mechanism. Direct to consumer clinics have flourished in some countries, providing cell treatments to OA patients. Most or all of these utilize unexpanded cell fractions from marrow or fat without even rudimentary product characterization and may report an exaggerated clinical outcome. Data from these clinics is not likely to yield information that will be useful. In fact, a recent systemic review of clinical trials involving MSC treatment in OA indicated that only a limited number of studies provided high quality evidence and long term follow up. Many suffered from a lack of consistency, including a diversity of methods for MSC preparation, and thus did not contribute to a supporting evidence base. There is a compelling need to provide clear and unambiguous clinical proof of concept relating to MSC treatment for OA. The ADIPOA2 study, currently active in Europe, will go some way towards achieving this. This is a 150 patient, phase 2b study designed to to assess the efficacy of a single injection of autologous adipose-derived MSCs in the treatment of mild to moderate OA of the knee, active and unresponsive to conservative therapy for at least 12 months.

S41.3 CARTILAGE AS BIOMATERIAL FOR TISSUE ENGINEERING - SOLUTIONS FOR REPOPULATION OF DENSE MATRIXES

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Organ and tissue decellularisation are promising approaches for the generation of scaffolds for tissue regeneration since these materials provides the accurate composition and architecture for the specific tissues.
Repopulation of the devitalized matrixes is the most critical step and a challenge, especially in dense tissues such as cartilage. To overcome this difficulty, several chemical and mechanical strategies have been developed. Chemical extraction targeting specific matrix components such as elastin, makes auricular cartilage accessible for cells via channels originating from the elastic fiber network. However, chemical treatment for glycosaminoglycan removal is not sufficient to allow cell ingrowth in articular cartilage. As alternative, laser perforation has been developed allowing to engrave fine structures with controlled size, distance and depth, with reproducibility and high throughput. Two of the most commonly used laser technologies used in the medical field, the CO₂ and femtosecond laser, were applied to hyaline cartilage with very different structural effect. Within this talk, the structuralizing possibilities of laser and enzymatic treatments, the effect on the matrix and the general advantages and disadvantages for tissue engineering are discussed. We believe that the optimal combination of chemical and laser treatment has high potential for a new generation of biomaterials for tissue engineering.

**S41.4 EXTRUDED PERFUSION BIOREACTOR: A VERSATILE CUSTOM-MADE PLATFORM TO STUDY SHEAR STRESS IN CARTILAGE TISSUE ENGINEERED CONSTRUCTS**

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Bioreactors have been used in articular cartilage tissue engineering (AC-TE) to apply different mechanical stimuli in an attempt to better mimic the native AC microenvironment. However, these systems are often highly complex, costly and not very versatile. In this work, we propose a simple and customizable perfusion bioreactor fabricated by 3D-extrusion to study the effect of shear stress in human bone-marrow mesenchymal stem cells (hBMSC) cultured in 3D porous polycaprolactone (PCL) scaffolds. Prototype models were designed in a CAD-software to perfectly fit the scaffolds and computational fluid dynamics analysis was used to predict the fluid velocities and shear stress forces inside the bioreactor. For the culture studies, hBMSC-PCL constructs were cultured under static expansion for 2 weeks and then transferred to the ABS-extruded bioreactors for continuous perfusion culture (0.2mL/min) under chondrogenic induction for additional 3 weeks. Perfused constructs showed similar cell proliferation and higher sGAG production in comparison to the static counterparts (bioreactor without perfusion). Constructs exposed to shear stress stimuli presented higher expressions of chondrogenic genes \((COLII/Sox9/Aggrecan)\) and reduced expressions of \(COLI\) and \(Runx2\) (osteogenic) than static group. However, the higher expression of \(COLX\) in the perfused constructs suggests a shear stress role in AC hypertrophy. Both conditions (perfused/static) stained positively for GAG deposition and for the presence of collagen II and aggrecan. Overall, the results provide a proof-of-concept of our customizable extruded bioreactor envisaging applications as a platform for AC-TE repair strategies and in the development of more reliable *in vitro* models for disease modelling and drug screening.

**S41.5 REINFORCED COLLAGEN-GAG SCAFFOLDS FOR CARTILAGE REPAIR USING 3D PRINTED POLYMERS**

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Damage to articular cartilage is difficult to treat, as it has a low capacity to regenerate. Biomimetic natural polymer scaffolds can potentially be used to regenerate cartilage. Collagen hyaluronic acid (CHyA) scaffolds have been developed in our laboratory to promote cell infiltration and repair of articular cartilage. However, the low mechanical properties of such scaffolds potentially limit their use to the treatment of small cartilage defects. 3D-printed polymers can provide a reinforcing framework in these scaffolds, thus allowing their application in the treatment of larger defects. The aim of this study was to create mechanically functional biomaterial scaffolds by incorporating a CHyA matrix into 3D-printed polymer meshes resulting in an integrated porous material composite with improved mechanical properties for repair of large cartilage defects.

3D-printed meshes were developed to facilitate an architecture suitable for nutrient flow, cell infiltration, and even CHyA incorporation. And the meshes were freeze dried in custom made moulds to create a pore structure suitable for chondrogenesis. Uniaxial compressive testing of the scaffolds revealed improved mechanical properties following reinforcement with printed meshes, with the compressive modulus increasing from 0.8kPa (alone) to 0.5MPa (reinforced structure). The reinforced scaffolds maintained interconnected pores with the mean pore diameter increasing from 130 to 175μm. The reinforcement had no negative impact on MSC viability, with 90.1% viability in reinforced scaffolds at day 7. The compressive modulus of the reinforced CHyA scaffold is close to native articular cartilage, suggesting that this approach can be used for treatment of large cartilage defects.

S41.6 CHONDROCYTES FROM PATIENTS WITH FAMILIAL OSTEOCHONDritis DISSEcANS EXHIBIT AN ER STRESS RESPONSE AND DEFECTIVE MATRIX ASSEMBLY

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Familial osteochondritis dissecans (FOCD) is an inherited defect of cartilage and bone characterized by development of large cartilage lesions in multiple joints, short stature and early onset osteoarthritis. We have studied a family from Northern Sweden with FOCD over five generations. All affected family members have a heterozygous missense mutation on exon 17 of the aggrecan gene, resulting in a Val-Met amino acid replacement in the G3 aggrecan C-type lectin domain (CLD). Aggrecan, a major proteoglycan of articular cartilage produced by chondrocytes, has a large protein core richly substituted with sulfated glycosaminoglycan chains. The unique structure, its high concentration within the cartilage extracellular matrix and its ability to form a supermolecular complex with hyaluronan and bind to other matrix proteins all profoundly influence the biomechanical properties of the tissue. Deletion of CLD in a chick aggrecan construct was found to influence its secretion from chondrocytes and human aggrecan constructs carrying the V2303M mutation showed diminished interactions with the ECM proteins tenascin-R, fibulin-1 and fibulin-2. To investigate the pathogenesis of FOCD, we studied chondrogenic differentiation of patient bone marrow mesenchymal stem cells and induced pluripotent stem cells. We demonstrated that the mutation results in accumulation of unfolded or misfolded aggrecan within the lumen of the chondrocyte endoplasmic reticulum. Associated with this is the failure to assemble a normal extracellular matrix. This explains the susceptibility of these patients to cartilage injury and the degenerative changes that lead to early onset osteoarthritis.

S41.7 A FIVE-YEAR REVIEW OF THE OUTCOMES OF TALAR OSTEOCHONDRAL LESIONS TREATED WITH MATRIX ASSOCIATED STEM CELL TRANSPLANTATION

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Osteochondral lesions (OCLs) of the talus are a challenging and increasingly recognized problem in chronic ankle pain. Many novel techniques exist to attempt to treat this challenging entity. Difficulties associated with treating OCLs include lesion location, size, chronicity and problems associated with potential graft harvest sites. Matrix associated stem cell transplantation (MAST) is one such treatment described for larger lesions >15mm² or failed alternative therapies. This cohort study describes a 5 year review of the outcomes of talar lesions treated with MAST. A review of all patients treated with MAST by a single surgeon was conducted. Pre-operative radiographs, MRIs and FAOS outcome questionnaire scores were conducted. Intraoperative classification was conducted to correlate with imaging. Post-operative outcomes included FAOS scores, return to sport, revision surgery/failure of treatment and progression to arthritis/fusion surgery. 32 patients were identified in this cohort. There were 10 females, 22 males, with an average age of 35. 01. 73% had returned and continued playing active sport. 23 patients underwent MAST in the setting of a failed previous operative attempt, with just 9 having MAST as a first option. 9 patients out of 32 had a further procedure. Two patients had a further treatment directed at their OCL. Two patients had a fusion, 2 had a cheilectomy at > 4 years for impingement, one had a debridement of their anterolateral gutter, one had debridement for arthrofibrosis, one patient had a realignment calcaneal osteotomy with debridement of their posterior tibial tendon. MAST has demonstrated positive results in lesions which prove challenging to treat, even in a “failed microfracture” cohort.

S42.1 THE EFFECT OF SURGICAL APPROACH AND IMPLANT DESIGN ON GAIT ANALYSIS AFTER TOTAL HIP REPLACEMENT

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Total hip arthroplasty (THA) is one of the most successful surgery. However, patients’ expectations have increased over the last two decades in regards to hip function after joint replacement, the patients assume to return their daily and sport activities without major limitations. This presentation will examine the effect of surgical approaches and implant designs as well as rehabilitation protocol on the clinical and biomechanical outcomes after THA. The new implant designs for THA aim to improve joint function whereas the surgical approaches intend to reduce muscle damage to regain muscle strength. One important determinant measured from gait analysis is the hip abduction moment as the abductors play a key role in stabilizing the pelvis in the frontal plane, particularly in phases of transition, such as the single leg stance in walking or stair climbing. This showed that muscle strength needs to be preserved. To minimize the risk of hip joint instability, a strong focus of implant development has been carried out. To illustrate this important concept within the context of gait analysis, I will present two studies that examine the influence of surgical approach and biomechanical reconstruction; and the second, is a prospective RCT comparing a dual mobility implant to a standard total hip replacement.

S42.2 FREE-ENERGY ANALYSIS OF CELL SPREADING ON LIGAND-COATED ELASTIC SUBSTRATES

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Several experimental studies demonstrate that controlled substrate micro-patterning has a significant impact on cell behaviour. Several experiments reveal cell spread area is dependent on both substrate rigidity and ligand density. The biomechanisms underlying such observations are not fully understood. We demonstrate that a thermodynamically consistent statistical mechanics model explains several of the key phenomena observed experimentally. We implement a steady-state thermodynamically consistent framework for stress-fibre formation and focal adhesion assembly. A Markov chain Monte-Carlo (MCMC) methodology is used to compute the distribution of cell spread states for a given substrate ligand density and stiffness. Several million spread states are considered by imposing a sequence of random trial moves on the cell. For each spread state, we compute quantities such as the cytoskeletal protein distribution, SF orientation, and FA distribution via a mixed finite element/boundary element method scheme. The free energy of all accepted states averaged equates to the homeostatic free energy. Following completion of the MCMC scheme we can construct the probability distribution for an observable of interest. For cells on a rigid substrate both the mean spread area and SD increase as the collagen density increases. A peak spread area is observed at a collagen density of 300 ng.cm$^{-2}$, with an area $A/A_0 \approx 2.7$. Further increases in collagen density lead to a reduction in cell area, motivated by focal adhesion free energy. On a compliant elastic substrate, lower spread areas are observed (peak $A/A_0 \approx 1.8$). Our computed dependence of spread area on substrate stiffness and ligand density has been observed experimentally.

**S42.3 HIP MUSCLE AND CONTACT FORCES IN POST-SURGICAL CAM FAI DURING GAIT**

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Cam-type femoroacetabular impingement (FAI) is a common cause for athletic hip injury and early hip osteoarthritis. Although corrective cam FAI surgery can improve patient reported outcome measures (PROMs), it is not clear how surgery affects muscle forces and hip joint loading. Surgery for FAI may redistribute muscle forces and contact forces at the hip joint during routine activities. The purpose of this study was to examine the muscle contributions and hip contact forces during gait in patients prior and after two years of undergoing surgery for cam FAI. Kinematics and kinetics were recorded in 11 patients with symptomatic cam FAI as they completed a gait task. Muscle and hip contact forces during the stance phase were estimated using musculoskeletal modelling and static optimization in OpenSim. All patients reported improvements in PROMs. Post-operatively, patients showed reduced forces in the long head of the biceps femoris at ipsilateral foot-strike and in the rectus femoris at the contralateral foot-strike. The reduced muscle forces decreased sagittal hip moment but did not change hip contact forces. This was the first study to evaluate hip muscle and contact forces in FAI patients post-operatively. Although hip contact forces are not altered following surgery, muscle forces are decreased even after two years. These findings can provide guidance in optimizing recovery protocols after FAI surgery to improve hip flexor and extensor muscle forces.

**S42.4 DISCERNING THE EFFECT OF SUBSTRATE DIRECTED DIFFERENTIATION ON MESENCHYMAL STROMAL CELL METABOLIC ACTIVITY USING FLIM**

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Recapitulating tissue elasticity can direct mesenchymal stromal cell (MSC) differentiation; however, it is unclear how substrate elasticity affects MSC metabolism. It is hypothesized MSCs subjected to stiffnesses,
atypical of standard tissue culture plastic, display altered metabolic phenotypes during differentiation. In this study, such alterations in MSC metabolic profiles, based on the fluorescence lifetime of NAD(P)H, a critical co-factor in energy production, were monitored using Fluorescence lifetime imaging microscopy (FLIM) as an evaluation tool. Polyacrylamide substrates with varying stiffnesses were fabricated to model the native elasticity of cartilage and bone. MSCs cultured on these substrates exhibited potent alterations in their metabolic status over a 14-day period that were detectable as early as day 3 using FLIM. Overall, soft substrates induced a more glycolytic response after 10 days of culture that persisted at day 14 (as measured by protein-bound NAD(P)H contributions to the lifetime decay). Similarly, by day 10; MSCs on intermediate-stiffness substrates favoured glycolysis. MSCs on stiffer substrates initially displayed a glycolytic phenotype followed by a transition to oxidative phosphorylation by day 10. Staining for mineralised nodules and glycosaminoglycans verified MSCs on stiffer substrates differentiating towards an osteogenic lineage, while MSCs on intermediate substrates showed similarities with differentiated chondrocytes. Overall, it can be concluded that matrix stiffness can induce metabolic perturbations in MSCs for up to 14 days. From this research, ideal culture conditions in which the metabolics of MSCs could be manipulated to promote maximum potency could potentially be defined in the future.

**S42.5 COMPARISON OF THE BIOMECHANICAL STABILITY AMONG INTRAMEDULLARY NAIL, COMPRESSION PLATE, AND EXTERNAL FIXATOR USED FOR ATROPHIC NON-UNION MODEL**

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There is a growing trend towards using pre-clinical models of atrophic non-union. This study investigated different fixation devices, by comparing the mechanical stability at the fracture site of tibia bone fixed by either intramedullary nail, compression plate or external fixator. 40 tibias from adult male Wistar rats’ cadavers were osteotomised at the mid-shaft and a gap of 1 mm was created and maintained at the fracture site to simulate criteria of atrophic non-union model. These were divided into five groups (n=8 in each): the first group was fixed with 20G intramedullary nail, the second group with 18G nail, the third group with 4-hole plate, the fourth group with 6-hole plate, and the fifth group with external fixator. Tibia was harvested by leg disarticulation from the knee and ankle joints, the soft tissues were carefully removed from the leg, and tibias were kept hydrated throughout the experiment. Each group was then subdivided into two subgroups for mechanical testing: one for axial loading (n=4) and one for 4-point bending (n=4). Statistical analysis was carried out by ANOVA with a fisher post-hoc comparison between groups. A p-value less than 0.05 was considered statistically significant. Axial load to failure data and stiffness data revealed that intramedullary nails are significantly stronger and stiffer than other devices, however there was no statistically significant difference axially between the nail thicknesses. In bending, load to failure revealed that 18G nails are significantly stronger than 20G. We concluded that 18G nail is superior to the other fixation devices, therefore it has been used for in-vivo experiments to create a novel model of atrophic non-union with stable fixation.

**S42.6 VISCOELASTIC MICRO-MECHANICAL CHARACTERIZATION OF (BIO)MATERIALS: AN APPLICATION TO THE OSTEOCHONDRAL INTERFACE**

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The micro-mechanical properties of complex biomaterials play an important role in tissue engineering and regenerative medicine, by regulating cellular processes and signalling. Local characterization of complex tissues while immersed in liquids proves to be very difficult to perform. We therefore present a method to derive viscoelastic micro-mechanical properties via non-destructive nano-indentation measurements in liquid. This technique is featured with a fiber-optical ferrule-top micro-machined force transducer, enabling a wide range of mechanical tests: from quasi-static experiments to derive elastic moduli, to step-response tests (e.g. creep, stress-relaxation), dynamic mechanical analysis (DMA) and constant strain rate tests to characterize sample viscoelastic behaviour. As a complex application we here present the osteochondral (OC) interface, which gradually ranges from hard and stiff bone regions towards softer and viscoelastic articular cartilage covering joint surface. The osteochondral plugs were collected from medial femoral condyle of cadaveric knees and measured at 37°C to mimic in-vivo physiological-like conditions. The stiffness of articular cartilage was 1.58±0.06 MPa, whereas subchondral bone plate could be categorized in “softer” region with 68.24±37.43 MPa, and a “stiffer” region with 683.68±622.88 MPa. The high stiffness in the “hard” region could be attributed to the mineralized matrix in the contact area, whereas the contribution of gel-like material, containing cell processes, along with osteocytes was larger in the “soft” region of the subchondral bone plate, leading to lower stiffness. These results might correlate with differences in extracellular matrix (ECM) composition and micro-architecture and are essential for engineering functional gradient scaffolds to better understand cell-ECM interactions.

S42.7 MECHANOBIOLOGICAL RESPONSES OF OSTEOBLASTS IN 3D HYDROGEL UNDER ESTROGEN WITHDRAWAL AND MECHANICAL STIMULATION

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3D cell culture studies more accurately represent the complex in vivo mechanical environment of human bone and are, thus, superior to 2D studies when testing the efficacy of osteoporosis therapies. As such, the objective of this study was to use a 3D model to investigate the effect of sclerostin antibodies. Sclerostin is a protein, which inhibits osteoblasts and is downregulated under mechanical stimulation. It is not yet known how expression of sclerostin mediates the site-specific and temporal changes in mineralisation. To address this, we developed a 3D cellular niche of MC3T3 osteoblasts encapsulated within gelatin and applied mechanical loading to the constructs using a custom-designed compression bioreactor system (0.5% strain at 0.5 Hz, 1 hr/day) (VizStim) under continuous perfusion of cell culture media. Osteoblasts were pretreated with estrogen for 14 days, followed by estrogen withdrawal (EW) to simulate postmenopausal conditions. 3D constructs were successfully fabricated and actin staining revealed the formation of dendritic cells under both static and stimulated conditions indicative of osteocyte-like cells. Under static conditions, estrogen treatment enhanced production of calcium by osteoblasts when compared to the same cells cultured under estrogen deficient conditions. Overall, preliminary results propose a link between mechanical stimulation, estrogen deficiency and mineral production by osteoblasts. Ongoing studies are comparing the static and stimulated groups after a longer culture period of 21 days using sclerostin antibodies. This research aims to deliver further understanding of the mechanical regulation of bone formation, and will inform novel approaches for regeneration of bone tissue and treatment of osteoporosis.

S42.8 MINERAL HETEROGENEITY IS ALTERED IN THE FEMORAL HEADS OF OSTEOPOROTIC AND DIABETIC HUMAN PATIENTS

Recent studies have shown that bone mineral distribution is more heterogeneous in bone tissue from an animal model of osteoporosis and osteoporotic human vertebral trabeculae. These tissue alterations may play a role in bone fragility seen in osteoporosis, albeit that they are not detectable by current diagnostic techniques (dual-energy X-ray absorptiometry, DXA). Type II Diabetes Mellitus (T2DM) also increases a patient’s fracture risk beyond what can be explained or diagnosed by DXA, and is associated with impaired bone cell function, compromised collagen structure and reduced mechanical properties. However, it is not currently known whether osteoporosis or T2DM leads to an increased mineral heterogeneity in the femoral head of humans, a common osteoporotic fracture site. In this study, we examine bone microarchitecture, mineralisation and mechanical properties of trabecular bone from osteoarthritic, diabetic and osteoporotic patients. We report that while osteoporotic trabecular bone has significantly deteriorated mechanical properties and microarchitecture compared to the other groups, there is also a significant increase in mean mineral content. Moreover, the heterogeneity of the mineral content in osteoporotic bone is significantly higher than osteoarthritic (+35%) and diabetic (+13%) groups. We propose that the compromised architecture following bone loss at the onset of osteoporosis alters the mechanical environment, which initiates compensatory changes in mineral content. We show for the first time that trabecular bone mineralisation is significantly more heterogeneous (+20%) in T2DM compared to osteoarthritic controls. Interestingly, bone microarchitecture and mechanical properties are not significantly different between diabetic and osteoarthritic groups despite this increase in mineral heterogeneity.

**S42.9 PULL OUT STRENGTH OF SUTURE ANCHOR FIXATION IN DIFFERENT ANCHOR INTERVALS**

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Suture anchor have been used in surgical procedure of tendon or ligament repair. Recently, there has been developed an all suture anchor (soft anchor) which can be used even when the insertion area is narrow. But, the stability of soft anchors due to narrow zone has not been elucidated. This purpose of this study was to investigate stability of soft anchors with respect to their fixation intervals. Polyurethane foams with two different bone densities (10 pcf; 0.16g / cm³, 20 pcf; 0.32g / cm³) were used. All suture anchors and conventional suture anchors were fixed at 10mm, 5mm, and 2.5mm intervals. The failure load was measured using a mechanical testing machine. The average load to failure of conventional suture anchor were 200.4N, 200.2N, 184.7N in the 10mm, 5mm and 2.5mm interval with 10pcf foam bones and 200.4 N, 200.2 N and 184.7 N with the 20 pcf foam bone respectively. Average load to failure load of soft anchor was 97.3N, 93.9N and 76.9N with 10pcf foam bones and 200.4 N, 200.2 N and 184.7 N with 20 pcf foam bone. Suture screw spacing and bone density are important factors in anchor pullout strength. In osteoporotic bone density, insertion of the suture screw interval of 5 mm might be necessary.

**S43.1 CELLULAR ACTIVATION OF TENDON REPAIR BY COLLAGEN MATRIX DAMAGE**

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Tendon tissue equilibrium very heavily depends on appropriate mechanical loading within a narrow, and still poorly defined, physiological range. We will present an overview of our recent work on the tendon cell-matrix interactions that drive tissue homeostasis, matrix remodelling and eventual tissue degeneration, and discuss a roadmap for unravelling these mechanically regulated signalling pathways for the development of effective treatment strategies. Our data suggest that tissue damage accumulates in the tendon until “intrinsic repair mechanisms” are overwhelmed. At this point, the metabolic cost of extracellular matrix remodeling exceeds the locally available nutrient supply. We hypothesize that upon reaching this “Metabolic Tipping Point”, the vascular system is recruited along with accompanying nerve supply (and pain) and the tissue enters into a chronic disease state characterized by high matrix turnover and increasingly poor tissue quality. In this paradigm, a delicate mechanically regulated balance exists between recruitment and suppression of the extrinsic vascular system by the resident tendon core cells. Upon injury or damage, this regulation in turn steers the tissue towards either functional remodeling or chronic tendon disease.

**S43.2 ENGINEERED TENDON COMPLEX FOR ROTATOR CUFF REPAIR AND REGENERATION**

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A rotator cuff tear is one of the most common traumatic and degenerative tendon injuries resulting in over 4.5 million physician visits in the US alone. Functional restoration of rotator cuff defects usually requires surgical repair, estimated at 300,000 cases in the US annually. However, postoperative retear of repaired tendons ranges from 20% in small to medium tears to over 90% in large and massive tears. Recently, augmentation with grafting materials to strengthen a reparable tear or to bridge an unrepairable defect has become a common and attractive strategy to reduce the retear rate, especially for large or massive tears. Current graft materials, however, have encountered great challenges in achieving these goals. To meet these challenges, we have developed an engineered tendon with layered tendon-fibrocartilage-bone composite (TFBC) from patellar-tibia unit revitalized by seeding bone marrow derived stem cells (BMDSCs) within the slices, and then reassembled to an engineered tendon. Both *in vitro* and *in vivo* results have shown that engineered TFBC enhance the biomechanical strength and biological healing using canine model.

**S43.3 KNOCKOUT MOUSE MODELS: TOOLS FOR DECIPHERING TENDON BIOLOGY**

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The establishment of a proper musculoskeletal system depends on the well-organized and synchronized development of muscle, tendon and cartilage/bone. In tendon biology, a great progress in identifying tendon-specific genes (Scleraxis, Mohawk, Tenomodulin) had been made in the last decade. However, there are many open questions regarding the exact function of genes in tendon development and homeostasis. The purpose of this study was to perform a systematic review of publications describing tendon-related genes, which were studied in-depth and characterized by using knockout technologies and the respectively generated transgenic mouse. Method: Literature search was carried out in Pubmed using “tendon” and “mouse knockout” and “phenotype” and was not limited to year. Results: We report in a tabular manner, that from a total of 25 tendon-related genes, in 23 of the respective knockout mouse models phenotypic changes were detected. Additionally, in some of the models it was described at which developmental stages these changes appeared and progressed.
Interestingly, so far only loss of Scleraxis and TGFbeta signaling led to severe tendon developmental phenotypes, while mice deficient for various proteoglycans, Mohawk, EGR1 and 2, and Tenomodulin exhibited mild phenotypes. This suggests that in general the tendon developmental program is well backed up and specifically that among the members of the proteoglycan family there are clear compensatory effects. In future, it will be of great importance to discover additional master tendon transcription factors as well as genes that play indispensable roles in tendon development.

S43.4 SCAFFOLD-FREE APPROACH IN TENDON ENGINEERING

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Tendons are dense connective tissues and critical components of the musculoskeletal system with known long repair process. Tissue engineering is a promising approach for achieving complete recovery of ruptured tendons. The most studies have focused on the combination of cells with various carriers; however, frequent times the biomaterials do not match the tissue organization and strength. For this reason, we first reviewed the literature for an alternative scaffold-free strategy for tendon engineering and second, we compared the cell sheet formation of two different cell types: bone marrow-derived mesenchymal stem cells (BM-MSCs) and tendon stem/progenitor cells (TSPCs). Methods: Literature search was performed in Pubmed using “tendon tissue engineering” and “scaffold-free” keywords and was limited to the last ten years. By using a 3-step protocol, BM-MSCs and TSPCs were induced to form cell sheets in 5 weeks. The sheets were compared by analysis for weight, diameter, cell density, tissue morphology (H&E and scoring) and cartilaginous matrix (DMMB and S.O. staining). Results: Scaffold-free models (cell sheets and pellet cultures) are available; however, further optimization is needed. Comparison between the two cell types clearly demonstrated that TSPCs form more mature cell sheet, while BMSCs form larger but less organized and differentiated sheet as judged by higher cell density and lower scoring outcome. Future efforts will focus on identifying mechanisms to speed BM-MSC sheet formation and maturation, which can in turn lead to development of new methodology for scaffold-free tendon tissue engineering.

S43.5 TENOGenic DIFFERENTIATION PROTOCOL IN XENOGENIC-FREE MEDIA ENHANCES TENDON-RELATED MARKER EXPRESSION IN ASCS

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Tendon injuries are common and current therapies often are unsuccessful. Cell-based therapy using mesenchymal stem cells (MSCs) seems to be the most promising approach to heal tendon. Moreover, providing safe and regulated cell therapy products to patients requires adherence to good manufacturing practices (GMP). Adipose-derived stem cells (n=4) were cultured in 6-well plates coated with type-I collagen in a chemically defined serum-free medium (SF) or a xenogenic-free human pooled platelet lysate medium (hPL). At passage 4, ASCs were induced to tendon lineage for 14 days using 100ng/ml CTGF, 10ng/ml TGFβ3, 50ng/ml BMP12 and 50μg/ml ascorbic acid in the SF (SF-TENO) or in the hPL (hPL-TENO) medium. Cells cultured without any supplements are used as control. Morphological appearance, cell viability and FACS were performed in undifferentiated cells to evaluate the xenogenic-free culture conditions; the gene and protein expression were performed by RT-PCR and immunofluorescence to evaluate to expression of stem cell- and tendon-related markers upon cell differentiation. SF-CTRL and hPL-CTRL showed similar viability and MSC’s surface proteins and expressed the stemness markers NANOG, OCT4 and Ki67. Moreover, both SF-TENO and hPL-
TENO expressed significant higher levels of SCX, COL1A1, COL3A1, COMP, MMP3 and MMP13 genes already at 3d (p<0.05) respect to CTRLs. Scleraxis and collagen were also detected in both SF-TENO and hPL-TENO at protein level in higher amount than CTRLs. In conclusion, ASCs exposed to CTGF, BMP12, TGFb3 and AA in both serum and xenogenic-free media possess similar tenogenic differentiation ability moving forward the GMP-compliant approaches for the clinical use of ASCs.

S43.6 DEVELOPMENT OF SERUM-FREE CULTURE CONDITIONS FOR DIFFERENTIATION OF BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS TO TENOCYTE-LIKE CELLS

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Mesenchymal stromal cells (MSCs) have been one of the most widely studied cell types in preclinical and clinical trials, due to their self-renewing, multipotent capacity, immunomodulatory properties and relative ease of isolation from multiple tissues. Despite limitations and safety concerns, fetal bovine serum (FBS) is still predominantly used for MSC expansion in clinical protocols. In addition, the undefined nature of serum composition and lot-to-lot variability have been linked to reduced reproducibility and efficiency of MSC bioprocessing. Moreover, use of animal serum in human cell culture increases the risk of contamination with adventitious pathogenic microorganisms, such as viruses, prions and bacteria. Hence, a defined serum-free formulation can provide increased safety, better control over physiological responsiveness, consistent performance and reproducible results. Here we present preliminary data on a prototype serum-free medium optimized for in vitro tenogenic differentiation of human bone marrow-derived MSCs. This serum-free formulation is capable of generating tenocyte-like cells in vitro expressing tenogenic markers such as Scx, Tnmd, TnC, Collagen I and Collagen III, whilst repressing expression of specific markers of other mesenchymal lineages.

S43.7 RECONSTRUCTION OF RAT SUPRASPINATUS TENDON INJURY WITH SYNTHETIC SCAFFOLD – EXPERIMENTAL STUDY

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The retear of the rotator cuff (RC) repair is a significant problem. Usually it is the effect of poor quality of the tendon. The aim was to evaluate histologically two types of RC reconstruction with scaffold. We have chosen commercially available scaffold polycaprolactone based poly(urethane urea). Rat model of supraspinatus tendon injury was chosen. There were four study groups: RC tear (no repair) (n=10), RC repair (n=10), RC repair augmented with scaffold (n=10) and RC reconstruction with scaffold interposition between tendon and bone (n=10). The repairs were investigated histologically at 6 and 16 weeks. The results in two groups in which scaffold was used had significantly better scores at 6 weeks comparing to non-scaffold groups (16,4±3, 17,3± 2,8 vs. 12,5±4,4, 13,8±1,4 respectively) and 16 weeks (23±1,9, 22,8±1,6 vs. 13,8±3,3, 14,9± 3,8 respectively). Results in two scaffold groups improved between 6 and 16 weeks. Signs of foreign body reaction against scaffold were not observed. Application of scaffold to strengthen the repair site and bridging of the tendon defect improved healing of the RC repair in animal model at 6 and 16 weeks. The quality of
reconstructed tendon improved over time. No such effect was observed in groups without repairs and isolated repairs were performed.

**S43.8 ENGINEERING OPTIMAL CULTURE CONDITIONS TO MAINTAIN TENOGENIC PHENOTYPE**

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Tenocytes from several mammal species have been shown to be prone to phenotypic drift at early sub-culture passages. In the present study we compared allogenic and xenogenic serum supplementation suitability as a supplement for the *in vitro* expansion of equine tenocytes (eTCs), in combination with the presence or absence of crowding conditions. eTCs were isolated from superficial digital flexor tendon and expanded in normal growth medium containing DMEM, 10% appropriate serum, 1% penicillin/streptomycin solution. Isolation was performed by migration method in growth medium containing the selected serum. Silver staining, densitometry, zymography, immunofluorescence, metabolic activity, proliferation, viability and morphology were performed after 3, 5 and 7 days in culture with a seeding density of 10,000 cells/cm². Treatment conditions were equine serum (ES) or foetal bovine serum (FBS), with or without 75 μg/mL of crowding agent carrageenan (CR). Viability and metabolic activity of eTCs were affected by FBS. eTCs in ES reached higher cell density than in FBS in day 7, especially with CR. Morphology of eTCs was maintained under different sera. Silver staining on pepsin digested cell layers shows that collagen type I deposition rate is remarkably enhanced in the presence of CR in all conditions. Immunofluorescence showed increased expression for collagen I, III, V and VI in both sera in the presence of CR. Deposition of all collagen types but type VI was increased by ES supplementation. We conclude that ES in combination with CR can represent a reliable choice for the ex vivo expansion of eTCs.

**S43.9 LONGITUDINAL CHANGES IN PATELLAR TENDON T2*-METRICS ARE ASSOCIATED WITH TENDON DEGENERATION AND SYMPTOM ONSET WITHIN COLLEGIATE BASKETBALL PLAYERS OVER ONE SEASON OF PLAY**

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Patellar tendinosis (PT) is common and can result in prolonged disability, especially in jumping athletes. Recently developed ultra-short-echo (UTE) MRI sequences allow for quantitative evaluation of tendon biostructure with T2* relaxometry. This study evaluated the relationships between changes over time (COT) in quantitative T2*-metrics, qualitative PT grades, and patient reported symptoms within 10 male basketball players from a single collegiate basketball team. All subjects completed weekly VISA-P symptomology questionnaires over the basketball season. Bilateral 3-Tesla MRIs (GE Healthcare) were obtained at pre- and post-season study visits. High-resolution, PD-weighted, FSE sequences were used to qualitatively grade PT. Quantitative T2*-metrics were evaluated using high-resolution, 3D, multi-echo, UTE-MRI sequences. Bilinear exponential fits of SI to corresponding echo time were used to calculate T2*-metrics. All qualitative and quantitative evaluations were region specific (proximal, middle, distal). Linear mixed effects models assessed associations of side and region with T2*-metrics. Spearman correlations evaluated relationships between
outcome measures. Within and between study visits, significant side-to-side differences in T2*-metrics were found and were significantly impacted by leg dominance (p<0.05). Pre-season T2*-metrics correlated with COT in T2*-metrics, COT in T2*-metrics correlated with COT in qualitative PT grades, and post-season T2*-metrics correlated with max changes in VISA-P scores (p≥0.64). Quantitative T2*-metrics can detect PT and may be capable of predicting the onset of pathology. T2*-metrics could benefit the clinical management of PT: it is sensitive to changes in pathologic severity over time, and therefore can serve as a quantitative metric to guide treatment and evaluate intervention efficacy.

S44.1 AVOIDING COMPLICATIONS IN DISTAL RADIUS FRACTURE FIXATION

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Advancements in treating complications of operatively treated distal radius fractures. We will review tips and tricks to avoid complications associated with operative fixation of these complicated injuries. We will cover treatment of the distal radioulnar joint, associated distal ulna fracture, complications of malreduction and implant prominence. During this session, we will review the latest techniques for treating these complex distal radius fractures and how to avoid associated complications.

S44.2 COMPLEX DISTAL RADIUS FRACTURES

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Advancements in treating complex distal radius fractures. We will review tips and tricks in the treatment of complex articular distal radius fractures. We will discuss the treatment of carpal instability resulting from fracture of the volar marginal fragment. We will cover optimizing surgical exposure to address fractures extending from the radial styloid to the lunate facet. During this session, we will review the latest techniques for treating these complex distal radius fractures.

S44.3 INVESTIGATING THE IMPACT OF DIABETES MELLITUS ON THE OUTCOMES OF HIP FRACTURE SURGERY

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The international literature base demonstrates that individuals living with diabetes mellitus (DM) are at increased risk of mortality and post-operative complications following hip fracture surgery (HFS) than non-diabetics. Studies investigating databases in American, European or Asiatic populations highlight the impact geography can have on the resultant investigation. We aim to quantify the impact DM has on HFS patients in a single university hospital. The HIPE dataset of fragility fractures occurring in Galway University Hospital from 2014-2016 were analysed and cross referenced with hospital laboratory and public databases. A database of 759 individuals was created including 515 females and 237 males, with a mean age of 78+-12.2 years, of which 110 patients had DM. The patient length-of-stay (PLOS) was comparable in all groups with patient age
being the primary influencing factor. An extended PLOS correlated with an increased long-term mortality. A trend toward increased occurrence of sub-trochanteric fractures was observed in diabetics with fewer periprosthetic and intertrochanteric fractures. Patients with DM had a significant increased risk of post-operative mortality compared to non-diabetics. Males with DM where at a greater risk of death after HFS [HR 2.29, 95% CI 1.26-4.17. p=0.006] than females with DM [HR 1.69, 95% CI 0.99-2.91. p=0.056]. The presence of DM did not directly impact a patient’s PLOS or increase the need for a re-operation. DM is associated with increased post-operative patient mortality and may influence the anatomical fracture pattern. This observation will support further investigation into the mechanical and biochemical changes occurring in the femur in individuals living with DM.

S44.4 DISPLACED INTRACAPSULAR HIP FRACTURE IN PATIENTS YOUNGER THAN 65: DOES FIXATION TECHNIQUE MATTER?

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In Displaced Intracapsular Hip Fractures (ICHF) in young active patients, preservation of the femoral head and its blood supply are of high importance and urgent surgical treatment with anatomic reduction and internal fixation is the preferred intervention. Due to the strong varus displacement shear forces exerted across the hip, there are relatively high complication rates after fixation. There is no consensus regarding the optimal fixation device or technique. This retrospective study compared closed reduction internal fixation method using cannulated cancellous screw (CCS) with the Targon Femoral Neck (TFN) hip fixed angle screw. Data regarding, gender, operational data, duration of surgery, complications, NAS (Numerical Analogue Scale) pain score, Modified Harris Hip Score (MHHS) and SF-12 scores were retrieved for patients younger than 65 with displaced ICHF. Eighty-two patients were included in the study, 30 patients treated with CCS were compared to 52 patients treated with TFN. Fracture configuration (Garden and Pauwel classifications), mean time to surgery and complication rate did not differ significantly. Operative time did differ significantly between groups (CCS 56 minutes, TFN 92 minutes, p<0.001). At final follow-up the CCS group reported less pain (NAS 2.3 vs 3.5, p< 0.049) and better Mental Health Composite score of SF-12 (p=0.017) compared to the TFN group. Complication rates for the treatment of displaced ICHF with TFN and CCS showed no significant differences; however, the functional outcomes, as presented by the NAS and Mental Health Composite score of SF-12, showed superiority for CCS treatment. As this fixation method is related to reduce costs, we suggest CCS for the treatment of displaced ICHF in the young population.

S44.5 THE MEDIUM-TERM SURVIVORSHIP OF PATIENTS WITH PERI-PROSTHETIC FRACTURES AROUND TOTAL KNEE ARTHROPLASTY, SURGICALLY MANAGED WITH PERI-ARTICULAR LOCKING PLATES

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With increasing numbers of total joint arthroplasties being performed, peri-prosthetic fracture incidence is rising, and operative management remains the gold standard. Short-term survivorship up to 12 months has been well-documented but medium to long-term is almost unknown. We present survivorship review from a district general hospital, undertaking 800 primary hip and knee arthroplasties per year. Patients with peri-prosthetic fractures and background total knee replacements were identified using our computer database
between 2006-2011. All patients were operated on our site; methods used include open reduction, internal fixation (ORIF) using Axos (Stryker Newbury) locking plates (28), intra-medullary nailing (1) or complex revision (6) depending on fracture and patient factors and surgeon's preference. Mortality was assessed at 30 days, 12 months and 5 years. Thirty-four patients were identified with a 7:1 female to male ratio and mean age of 76. 75% of patients had their primary arthrodesis at our hospital. There was only 1 plate failure noted requiring revision plating. Mortality at 30 days, 12 months and 5 years were 3.2, 12.5% and 50% respectively. When compared to the literature our time interval from index surgery to fracture is considerably longer (115 vs 42 months). Further multi-centre reviews are required to further assess this unexpected finding. Overall mortality is better than our hip fracture cohort, suggesting that good results can be achieved in District Hospital. The longer-term results are encouraging and can act as a guide for patients with this injury. We recommend that patients are managed in consultant-led, multi-disciplinary teams.

S44.6 CHARACTERIZATION OF MACROPHAGE SUBTYPES DURING THE ACUTE INFLAMMATORY PHASE OF FRACTURE HEALING

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Inflammation has been associated with immunological dysfunctions and chronic inflammatory diseases but is important for normal repair processes like bone healing. Macrophages (mØ) are important for bone growth, maintenance, and regeneration. MØ are distinct from other bone cells and play an important role in the inflammatory stage of bone healing. Previous data has shown that ablation of mØs during the inflammatory stage can severely impair bone healing and exacerbate bone loss in osteoporotic models. However, little research has focused on characterizing the mØ subtypes found during the inflammatory stage. We hypothesized that different mØ subtypes are activated during inflammation and release factors to regulate bone repair. Therefore, bone marrow was collected from mice femurs at days 0, 1, 2, 4, and 7 after fracture and mØ were isolated using established methods. MØ subtypes were identified using anti-F4/80, anti-CD80, and anti-CD86 antibodies via flow cytometry and cytokine expression was quantified using Luminex. When compared to unfractured controls, a 40-50% increase in MHC class II+/CD80+ double positive mØs and MHC class II+/CD86+ double positive mØs were found on day 2 post-fracture, which remained elevated through day 4 or 7, respectively. No differences were found in mØ populations between femurs in naïve (unfractured) mice. mØs of the fractured limbs expressed higher levels of cytokines overtime. Our results suggest that different subtypes of mØs are present during the inflammatory stage and may support diverse functions such as effertocytosis, chemotaxis, and tissue anabolism or catabolism, which provides insight into their contribution in normal or uncontrolled inflammatory related processes and conditions.

S44.7 ENDOGENOUS MOBILISATION OF STEM CELLS WITH AMD3100 TO TREAT NON-UNION

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A significant number of fractures develop non-union. Stem cell therapy may be beneficial in their treatment, however this requires acquisition, culture and delivery of stem cells. Stem cell homing and migration is regulated through SDF-1 and its receptor CXCR4. Studies have demonstrated endogenous mobilisation of different populations of stem and progenitor cells by administering growth factors with a pharmacological antagonist of CXCR4, AMD3100. This may therefore be a means to improve compromised fracture healing.
A 1.5mm femoral osteotomy in adult female Wistar rats was stabilised with an external skeletal fixator. After osteotomy, saline/PBS (P) VEGF (V), IGF-1 (I) or GCSF (G) (100ug/kg, 0.5ml/100g i.p.), were administered daily for 4 days. On day 5, a single 5mg/kg i.p. dose of AMD3100 was given. Control group (C) did not receive growth factors or AMD 3100. At 5 weeks, the femur was retrieved and microCT scanned. Compared to group C (n=7), group P (n=5) had a significant increase in bone volume (P=0.01) $8.9\pm2.2\mu m^3$ (control $4.3\pm3.1\mu m^3$) and trabecular thickness (P=0.03). Group I (n=6) also had a significant increase in bone volume (P=0.035) $5.1\pm4.2\mu m^3$ and trabecular thickness $0.062\pm0.008\mu m$ (control $0.042\pm0.01\mu m$) (P=0.01). Group V (n=8), showed a non-significant increase in bone volume; $5.22\pm1.7\mu m^3$ and trabecular thickness $0.048\pm0.007\mu m$. Group G (n=5) showed a significant decrease in bone volume (2.5±2.6$\mu m^3$) (P=0.048). AMD3100 alone and IgF1-AMD3100, showed the greatest increase in bone formation, presumably through mobilisation of beneficial combinations of stem and progenitor cells. GCSF-AMD3100, which is expected to mobilise hematopoietic progenitors inhibited bone healing.

**S44.8 3D PRINTED CUSTOM-MADE TALUS PROSTHESIS COUPLED WITH TOTAL ANKLE ARTHROPLASTY: THE EVOLUTION OF BIOMATERIALS AND TECHNOLOGY**

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Total ankle replacement (TAR) is contraindicated in patients with significant talar collapse due to AVN and in these patients total talus body prosthesis has been proposed to restore ankle joint. To date, five studies have reported implantation of a custom-made talar body in patients with severely damaged talus, showing the limit of short-term damage of tibial and calcaneal thalamic joint surfaces. Four of this kind of implants have been performed. The first two realized with "traditional" technology CAD-CAM has been performed in active patients affected by "missing talus" and now presents a survival follow-up of 15 and 17 years. For the third patient affected by massive talus AVN we designed a 3D printed porous titanium custom talar body prosthesis fixed on the calcaneum and coupled with a TAR, first acquiring high-resolution 3D CT images of the contralateral healthy talus that was "mirroring" obtaining the volume of fractured talus in order to provide the optimal fit. Then the 3D printed implant was manufactured. The fourth concern a TAR septic mobilization with high bone loss of the talus. The "two-stage" reconstruction conducted with the implant of total tibio-talo-calcaneal prosthesis "custom made" built with the same technology 3D, entirely in titanium and using the "trabecular metal" technology for the calcaneous interface. Weightbearing has progressively allowed after 6 weeks. No complications were observed. All the implants are still in place with an overall joint mobility ranging from 40° to 60°. This treatment requires high demanding technical skills and experience with TAR and foot and ankle trauma. The 15 years survival of 2 total talus prosthesis coupled to a TAR manufactured by a CAD-CAM procedure encourages consider this 3D printed custom implant as a new alternative solution for massive AVN and traumatic missing talus in active patients.

**S45.1 THE ROLE OF PHYSIOTHERAPY IN KNEE REPLACEMENT**

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Physiotherapy is generally accepted as an important component in the care pathway surrounding total knee replacement. Therapy interventions can be delivered prior to surgery, as part of the inpatient stay, and post-operation through outpatient appointments. Though ‘physiotherapy’ is generally promoted there is considerable national and international variation in actual therapy provision. Specific rehabilitation protocols are strongly
entrenched at individual physiotherapy departments however the wider efficacy of varying physiotherapeutic interventions is poorly established. This uncertainty as to effectiveness of physiotherapy makes it difficult for commissioning organisations, healthcare providers, and patients to make decisions as to what therapy is 'needed' and therefore the correct level and mechanism of funding for such services. This talk will explore the variation in physiotherapy service provision and evidence for different interventions surrounding total knee replacement.

S45.2 BONE REGENERATION OF UNMET NEED IN LARGE, LATERAL-LOCATED OSTEONECROTIC LESION OF FEMORAL HEAD

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ONFH with large or lateral-located lesion is challenging due to difficulty of regeneration. We introduce novel tissue engineering technique using ex vivo expanded bone marrow stromal cell seeded on calcium metaphosphate (CMP) scaffold to regenerate dead bone for these challenging cases. Ten millilitres of bone marrow was aspirated from iliac crest and mononuclear cells were collected. These cells were expanded and differentiated to osteoblast-lineage cells using osteogenic media and autologous serum for 2-4 weeks ex vivo. Porous bead-form scaffolds were made of CMP and cells were seeded in a density of million/ml³ into 20 to 30 beads for 1 hour. The necrotic area was curetted and the beads were implanted through core tract in 9 hips (Steinberg IIc in 5 hips and IVc in 4 hips which involved greater than 30% of whole head; JIC classification C1 in 4 hips, and C2 in 5 hips which involved weight bearing area). The tract was blocked with a CMP rod. The age of patients ranged from 16 to 37. Associated factors were; steroid in 4, idiopathic in 3, alcoholic in 1 and traumatic in 1 hip, respectively. Kerboul combined necrotic angle was more than 200° in all hips. We compared preoperative and annual radiographs and MRI images to check dome depression of femoral head and signal change of osteonecrotic area. Follow-up period ranged from 8 to 14 years. Two IIc lesions progressed and were converted to THA at two and six years postoperatively. We could get clinical and radiographic success in 7 hips (78%). Follow-up radiographs and MRI showed partial or nearly complete regeneration of necrotic bone, prevention of collapse, and reduction in necrotic lesion. This can be a good strategy for bone regeneration of unmet need as in a human model.

S45.3 7 YEAR OUTCOMES OF THE TRIATHLON KNEE REPLACEMENT: A COHORT STUDY

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The aim of this study was to determine the outcomes and survivorship of the Triathlon knee replacement at 7 years after surgery. A cohort of 266 patients receiving a Triathlon knee replacement were assessed before surgery and at 3 months, 1 year, 2 years, 3 years, 5 years and 7 years post-operation. Patient-reported outcomes were assessed using the WOMAC, KOOS Knee-Related Quality of Life scale, Satisfaction Scale and questions on kneeling ability and whether they regretted having the operation. Data on survivorship was collected from self-report and medical records. At 7 years after surgery, 32 patients were deceased, and 17 patients were withdrawn. Of the 217 patients remaining in the study, 164 (76%) returned a completed study questionnaire. At 7 years after surgery, 92% of patients reported an improvement in their WOMAC Pain score greater than the minimally clinically important improvement (defined as improvement of ≥9 points from before surgery) and 82% reported this in their WOMAC Function score (defined as improvement of ≥12 points). Knee-related
quality of life was good, with a mean score of 66.8 (SD 26.0) (0-100 scale, worst to best). A high percentage of patients (89%) were somewhat or very satisfied with their outcome at 7 years. Survivorship with revision as the endpoint was 96.4% (95% CI 93.2-98.1%) at 7 years post-operation. Five percent of patients regretted having their operation and 68% reported much difficulty or an inability to kneel. In conclusion, this study observed good long-term patient outcomes and survivorship of the Triathlon knee replacement.

S45.4 THE MINIMALLY-INVASIVE MODIFIED PERCUTANEOUS TECHNIQUE VERSUS DISTAL CHEVRON OSTEOTOMY IN THE TREATMENT OF HALLUX VALGUS: A PARALLEL-GROUP, PROSPECTIVE RANDOMISED TRIAL

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Though there are many techniques utilised in the correction of hallux valgus (HV), no single approach has been reported to be ideal for all patients to date. A great deal of controversy remains concerning the type of osteotomy, method of fixation, and inclusion of soft tissue procedures. Herein, we compared the outcomes of two different operative techniques, the minimally-invasive modified percutaneous technique and the distal chevron osteotomy, used to treat mild to moderate hallux valgus. This study was conducted in line with the CONSORT 2010 guidelines. 41 patients (58 feet) with mild to moderate hallux valgus were randomly assigned by computer to two different groups. The first group containing 24 patients (33 feet) was treated by the modified percutaneous technique, whereas the second group included 17 patients (25 feet) treated by distal chevron osteotomy. In the modified percutaneous group, after a mean follow up of 43 months, the mean correction of hallux valgus angle (HVA) was 26.69° (P=0.00001), the mean correction of intermetatarsal angle (IMA) was 9.45° (P=0.00001), and the mean improvement of AOFAS score was 47.94 points (P=0.00001). In the chevron osteotomy group, after a mean follow up of 44 months, the mean correction of hallux valgus angle was 26.72° (P=0.00001), the mean correction of intermetatarsal angle was 9° (P=0.00001), and the mean improvement of AOFAS score was 44.76 points (P=0.00001). In our study, the modified percutaneous technique proved to be equally effective as the distal chevron osteotomy, but with fewer complications and a higher rate of patient satisfaction.

S45.5 LEG-LENGTH RESTORATION IN PRIMARY TOTAL HIP ARTHROPLASTY USING A MULTIMODAL PROTOCOL: A SERIES OF 50 CONSECUTIVE PATIENTS

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Restoration of anatomy is paramount in total hip arthroplasty (THA) to optimise function and stability. Leg-length discrepancy of ≥10mm is poorly tolerated and can be the subject of litigation. We routinely use a multimodal protocol to optimise soft tissue balancing which involves pre-operative templating, leg-length measurement supine and in the lateral position after positioning, and the use of an intra-operative leg-length measurement device to ensure optimisation of leg-length. We have analysed the results of our protocol in restoring leg-length in primary THA. Radiological leg-length was measured in a consecutive series of 50 patients who had THA for unilateral arthritis by an independent observer pre- and post-operatively using validated methods utilising radiological software. The measurements pre- and post-operative were compared. Patients with bilateral hip arthritis and poor imaging were excluded. Leg-length was successfully restored to within 5.0mm of the target leg-length in 84.0% of patients (mean +0.7mm (95% CI +0.2 to +1.1)). The other 14.0% of patients were restored to within 5.1-8.0mm (mean +2.2mm (95% CI -2.7 to +7.1)) and 2.0% of
patients were restored to within 8.1-10.0mm. Leg length was accurately restored across the subset of patients within a narrow range of either side of the mean target leg length. Intra-operative measurement of leg length can be difficult but is vital in ensuring appropriate restoration of leg-length. We recommend a similar multimodal protocol to ensure restoration of leg-length within narrow limits to maximise function and patient satisfaction.

**S45.6 THE USE OF NECK MODULARITY IN THA: A RETROSPECTIVE STUDY ON 1,033 IMPLANTS WITH A MAXIMUM FOLLOW-UP OF 15 YEARS**

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Neck modularity has been proposed to improve THA accuracy, thanks to the close restoration of anatomy, however it has been associated with issues like early breakages or corrosion. Our Hospital has been using neck modularity since the 90s, so we analyzed retrospectively implants performed between January 2000 and December 2014. The minimum follow up was 1Y. The cohort was composed of 1,033 THAs or 951 patients (82 bilateral), of which 643 females and 390 males. Average patient age was 67.7Y. THA indications were primary Osteoarthritis (80.9%), Fracture (9.0%), Congenital Dysplasia or Congenital Luxation (4.2%), Osteonecrosis (3.2%), other causes (2.7%). The stems used were all cementless, 381 anatomically shaped (36.9%), 635 straight (61.5%), 17 short MIS (1.6%). All necks used were made of Titanium alloy. 419 implants (40.5%) were manufactured by Wright Medical, while 614 (59.5%) were produced by Adler Ortho. A total of 37 revisions has been reported, mainly due to periprosthetic fractures (32.4%), luxation (24.3%), implant mobilization (18.9%) and implant breakage (16.2%). We have recorded 3 modular neck breakages. 4 patients required re-revisions, because of luxations (3) and neck breakage (1). The overall survival rate was 96.4%. We did not observe any component corrosion, probably thanks to the exclusive use of Titanium necks. We had a neck breakages rate of 0.29% and a luxation rate of 0.87%, lower than normally reported in the literature. In conclusion, our experience suggests as neck modularity could be a safe and effective way to reconstruct the proximal femur in THA patients.

**S45.7 OUTCOMES FOLLOWING ORTHOPAEDIC SURGICAL WOUND CLOSURE WITH SUTURE COMPARED WITH NON-ABSORBABLE STAPLES IN ADULTS. A SYSTEMATIC REVIEW AND META-ANALYSIS**

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Orthopaedic surgical site infections (SSI’s) prolong total hospital stays by a median of 2 weeks per patient, approximately double re-hospitalization rates, and increase healthcare costs by more than 300%. Patients with orthopaedic SSI’s have significant reductions in their health-related quality of life. We performed a systematic review and meta-analysis to compare differences in outcomes between use of sutures and non-absorbable staples for closure of orthopaedic surgical wounds in adults. The primary outcomes were rates of superficial and deep SSI. Secondary outcomes included wound dehiscence, length of hospital stay, patient satisfaction and pain during removal of closure material. Data sources including PubMed, EMBASE, Scopus, Web of Science, Cochrane Library, clinicaltrials.gov, National Institute for Health and Research, UK clinical trials gateway were searched for randomised controlled trials (RCT’s) meeting inclusion criteria. Sixteen RCT’s published between 1987 and 2017 were included. Overall, wound infection outcomes (superficial and deep infections combined) showed no statistically significant difference between closure with staples compared
with sutures with a relative risk of 1.17 (95% CI 0.59-2.30, p=0.66). A subgroup was performed specific to hip wound infection outcomes. Interestingly, a sensitivity analysis demonstrated sutures to be statistically favourable (p=0.04) in terms of hip wound infection outcomes. There was no statistically significant difference among secondary outcomes between sutures and staple groups. Overall it appears the choice of sutures or staples in closure of orthopaedic wounds has no effect on wound complications. However, caution is needed in applying the findings to different population groups due to heterogeneity across studies.

**S45.8 APPLICATION OF THE OTTOWA RULES IN AN ACCIDENT AND EMERGENCY DEPARTMENT**

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The Ottowa Rules were developed in 1992 by Stiell et al. to “assist clinicians in being more selective in their use of radiography” in suspected ankle fractures. They have a sensitivity of almost 100% and should reduce the number of unnecessary radiographs by 30-40%. We aimed to determine the application of the Ottowa Rules in ankle plain radiograph requests in Accident and Emergency (A&E) and determine the number of unnecessary plain radiographs requests. We carried out a retrospective analysis of 366 ankle plain radiographs, request forms and reports, in A&E over 3 months. We implemented a reminder on the electronic requesting system to prompt clinicians to apply the Ottowa rules and analysed a further 226 scans over the next month. Unnecessary scans were calculated by determining the false negatives, i.e. requests did not fulfill Ottowa Rules and scan showed no fracture. Of the 336 original requests, 45% fulfilled the Ottowa Rules and 43% of all scans were unnecessary. Following our intervention, only 42% of requested scans fulfilled the Ottowa rules and 52% were unnecessary. We concluded that Ottowa rules are knowingly not applied in A&E. Reasons may include low cost and ease of scan, patient and clinician reassurance and perceived low risk of scanning. There are a huge number of unnecessary ankle plain radiographs and soft interventions do not impact on this. We have implemented a simplified version of the text reminder and are re-auditing the data.

**S45.9 UTILIZATION OF PRE-OPERATIVE ULTRASOUND TO DELINEATE ZONE OF NERVE INJURY**

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The treatment of extremity ballistic injury is challenging in that the zone of injury can be extensive and determining the surgical exposure can be difficult. We describe a method of pre-operative evaluation of the zone of injury in conjunction with the regional anesthesiologist utilizing ultrasound to determine the presence of nerve disruption. This non-invasive method of examination may elucidate whether significant nerve exists and may also serve to pinpoint the location of injury. Such information allows the surgeon to more effectively and efficiently surgically expose the zone of injury and understand the boundaries of the nerve outside the zone of injury. Moreover, such preoperative evaluation may at times obviate the need for exploratory surgery at all. It is important for the anesthesiologist and surgeon to work together with respect to the ability to both interpret the ultrasound images and to clinically correlate the findings. The zone of tissue disruption in ballistic injuries is extremely variable. It is beneficial to both the surgeon and patient to engage in a collaborative effort with an experienced regional anesthesiologist who is well-versed in interpretation of ultrasound images and tissue plane disruption in an effort to minimize surgical time and the potential unintended consequences of unnecessary exploration. We present a series of cases representing instances wherein the zone of injury was
small, extensive, and a unique situation in which there was in fact no injury present despite clinical symptoms and MRI consistent with radial nerve disruption.

**S46.1 ELECTROMECHANICAL MICROENVIRONMENTS FOR NOVEL TISSUE ENGINEERING STRATEGIES**

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Tissue engineering and regenerative medicine are increasingly taking advantage of active materials, allowing to provide specific clues to the cells. In particular, the use of electroactive polymers that deliver electrical signals to the cells upon mechanical solicitation, open new scientific and technological opportunities, as they in fact mimic signals and effects present in living tissues, allowing the development of suitable microenvironments for tissue regeneration. In fact, electrical and electromechanical clues are among the most relevant ones in determining tissue functionality in tissues such as muscle and bone, among others, indicating their requirement for proper tissue regeneration. Piezoelectric polymers have already shown strong potential for novel tissue engineering strategies, once they can account for the existence of piezoelectricity within some specific tissues and also can modulate the electrical signals existing in tissue development and function. In this context, this talk reports on piezoelectric and magnetoelectric materials used for tissue engineering applications. The most used materials and morphologies for tissue engineering strategies are reported, together with the need of novel bioreactor designs allowing to take full advantage of those materials. Further, the main achievements, challenges and future needs for research and actual therapies will be presented and discussed.

**S46.2 SMART BIOMATERIALS IN MUSCULOSKELETAL TISSUE ENGINEERING**

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By definition, a smart biomaterial is a material, such as a ceramic, alloy, gel or polymer, that can convert energy from one form into another by responding to a change in a stimulus in its environment. These stimuli may involve temperature, pH, moisture, or electric and magnetic fields. In particular, thermoresponsive biomaterials have been successfully employed to host mammalian cells with a view to musculoskeletal tissue engineering. The presentation provides an overview of the use of thermosensitive polymers for the non-enzymatic stem cell harvesting, cell sheet engineering, three-dimensional scaffolds fabrications and organ-printing materials.

**S46.3 IN VITRO 3D MODEL FOR BONE TISSUE: A BIOELECTRONICS APPROACH**

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One of the latest trends in the field of tissue engineering is the development of *in vitro* 3D systems mimicking the target tissue or organ and thus recapitulating the tridimensional structure and microenvironment...
experienced by cells in vivo. Interestingly, certain tissues are known to be regulated by endogenous bioelectrical cues, in addition to chemical and mechanical cues. One such tissue is the bone. It has, indeed, been demonstrated to exhibit piezoelectric properties in vivo, with electrical signaling playing a role in its formation during the early embryo developmental stages. Electrical stimulation has been proven to sustain cell proliferation and to boost the expression of relevant genes and induce higher levels of enzymatic activities related to bone matrix deposition. Herein, we describe the development of a 3D model of bone tissue based on the conductive polymer PEDOT:PSS and human adipose derived stem cells. 3D electroactive porous scaffolds have been produced using the ice-templating technique, and different compositions (different ratios of conductive polymer to Collagen Type 1) have been explored. The developed scaffolds as well as cells interaction and response have been characterized. Overall, the results obtained so far highlight the usefulness of the porous conductive scaffolds as an in vitro platform for the development of 3D models for bone tissue engineering.

**S46.4 NEAR INFRARED-RESPONSIVE HYDROGELS FOR THE SPATIOTEMPORAL CONTROL OF BONE MORPHOGENETIC PROTEIN 2 BIOAVAILABILITY IN CRITICAL-SIZED BONE DEFECTS**


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There is a growing interest in the development of tissue engineering (TE) therapies to repair damaged bone. Among the scaffolds for TE applications, injectable hydrogels have demonstrated great potential as three-dimensional cell cultures in bone TE, owing to their high water content, porous structure that allows cell transplantation and proliferation, similarity to the natural extracellular matrix and ability to match irregular defects. We investigated whether fibrin-based hydrogels capable of transducing near infrared (NIR) energy into heat can be employed to lead bone repair. Hollow gold nanoparticles with a plasmon surface band absorption at ~750 nm, a NIR wavelength within the so called “tissue optical window”, were used as fillers in injectable fibrin-based hydrogels. These composites were loaded with genetically-modified cells harbouring a heat-activated and rapamycin-dependent gene circuit to regulate transgenic expression of the reporter gene firefly luciferase (fLuc). NIR-responsive cell constructs were injected to fill a 4 mm diameter critical-sized defect (CSD) in the parietal bone of mouse calvaria. NIR-irradiation in the presence of rapamycin triggered a pattern of fLuc activity that faithfully matched the illuminated area of the implanted hydrogel. Having shown that this platform can control the expression of a transgene product, we tested its effectiveness on regulating the secretion of transgenic bone morphogenetic protein 2 (BMP-2) from NIR-responsive hydrogels implanted in CSD. The spatiotemporal pattern of transgenic BMP-2 secretion induced by NIR-irradiation in the presence of rapamycin significantly stimulated bone regeneration from the edge of osteotomy in the CSD practiced, validating the therapeutic approach.

**S46.5 BIOCOMPATIBILITY AND BIODISTRIBUTION OF MAGNETIC SILICA NANOPARTICLES FOR IMPLANT DIRECTED MAGNETIC DRUG TARGETING**

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The field of nanoparticle related research for the diagnosis and therapy of diseases evolves rapidly. Magnetic nanoparticles in combination with magnetizable implant materials for the treatment of implant related infections present a possible implementation in orthopedics. Magnetic nanoporous silica nanoparticles (MNPSNPs) were developed and equipped with fluorescent dyes. *In vitro*/*in vivo* biocompatibility and *in vivo* biodistribution were examined to appraise their potential applicability. Cell culture tests with NIH-3T3 and HepG2 cell lines indicated a good *in vitro* biocompatibility. Ferritic and titanium alloy (control) plates were implanted subcutaneously at the hind legs of Balb/c mice. Immediately after i.v. or s.c. injection of MNPSNPs, the caudal half of the mice was placed between the poles of an electromagnet. Exposure to the electromagnetic field of approx. 1.7 T was maintained for 10 minutes. 10 animals each were euthanized at days 0, 1, 7, 21 or 42, respectively. Quantity of MNPSNPs in liver, spleen, kidney, lung and skin/muscle samples was assessed by fluorescent microscopic methods. MNPSNP existence on the implant surface was also appraised after several steps of detachment. MNPSNPs showed a time-dependent accumulation in the organs after i.v. injection with initial accumulation in the lungs followed by redistribution to liver and spleen. After s.c. injection no systemic distribution but local appearance of MNPSNPs could be found. First histological evaluation showed no pathological changes after i.v. injection. With good *in vivo* biocompatibility, future focus will be laid on increasing circle life time of MNPSNPs and evaluation in an infection model.

S46.6 THERMORESPONSIVE NANOFIBERS FOR ENGINEERING EXTRACELLULAR MATRIX-RICH TISSUE SUBSTITUTES

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Tissue engineering by self-assembly offers the possibility to fabricate contiguous cell sheets that are stabilised by intact cell-cell contacts and endogenously produced extracellular matrix (ECM) However, these systems lack the possibility to introduce topographical cues, that are fundamental for the organisation of many types of tissues. Herein we venture to fabricate aligned electrospun thermoresponsive nanofibres to sustain growth and detachment of ECM-rich living substitutes in the presence of a MMC microenvironment. A copolymer of 85% poly-N-isopropylacrylamide and 15% N-tert-butylacrylamide (pNIPAAm/NTBA) were used. To create aligned nanofibers, the polymer was electrospun and collected on a mandrel rotating at 2000 rpm. Human adipose derived stem cells (hADSC) were treated with media containing macromolecular crowders to enhance matrix deposition. Cell viability and morphology were assessed, and immunocytochemistry was conducted to estimate matrix deposition and composition. Non-invasive cell detachment was enabled by decreasing the temperature of culture to 10 °C for 20 minutes. The electrospinning process resulted in the production of pNIPAm/NTBA fibres in the diameter range from 1 to 2 μm and an overall alignment of 80%. Cell viability revealed that hADSCs were able to grow on the scaffold. The cells aligned on the fibres after 3 days and they were able to detach as intact cell sheets in presence of MMC. Moreover, it was demonstrated that MMC, by a volume extrusion effect, enhances collagen type I deposition, one of the main components of the ECM. Collectively the pNIPAm/NTBA fibres were able to successfully sustain growth and detachment of ECM-rich cell sheets.

S47.1 ASSESSMENT OF VERTEBRA STRENGTH USING CT-IMAGE BASED FINITE ELEMENT METHOD

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Compressive fracture of osteoporotic vertebrae has been one of the most important health problems in aged societies because severely injured spin might be a reason of bedridden for elderly people. Osteoporosis has been widely assessed by averaged bone mineral density of vertebrae measured using DEXA, however, BMD sometimes does not reflect the strength of vertebrae. CT imaged based finite element method (CT-FEM) has been applied to evaluate the strength of vertebrae based on the biomechanics theory and approved by a part of the highly advanced medical treatment in Japan. In the present study, compressive strength of more than 100 vertebrae were evaluated using CT-FEM, and the correlation between BMD and the strength was thoroughly investigated. It was found that some vertebrae with high BMD could have low strength which may cause fracture easily. Thus, a controversial point of the BMD based diagnosis of osteoporosis was clearly indicated. In this invited talk, some basic theories of CT-FEM and fracture assessment and some key results from the recent study will be presented.

**S47.2 EVALUATION OF THE EFFECT OF HIP SURGERIES USING FINITE ELEMENT ANALYSIS**

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Biomechanical analysis is important to evaluate the effect of orthopaedic surgeries. CT-image based finite element method (CT-FEM) is one of the most important techniques in the computational biomechanics field. We have been applied CT-FEM to evaluate resorptive bone remodeling, secondary to stress shielding, after total hip arthroplasty (THA). We compared the equivalent stress and strain energy density to postoperative BMD (bone mineral density) change in the femur after THA, and a significant correlation was observed between the rate of changes in BMD after THA and equivalent stress. For periacetabular osteotomy cases, we investigated mechanical stress in the hip joint before and after surgery. Mechanical stress in the hip joint decreased significantly after osteotomy and correlated with the degree of the acetabular coverage. For arthroscopic osteochondroplasty cases, we examined mechanical strength of the proximal femur after cam resection using CT-FEM. The results suggested that both the depth and area of the resection at the distal part of femoral head-neck junction correlated strongly with fracture risk after osteochondroplasty. This talk consists of our results of clinical application studies using CT-FEM, and importance of application of CT-FEM to biomechanical studies to assess the effect of orthopaedic surgeries.

**S47.3 ALTERATION OF INTRAARTICULAR STRESS DISTRIBUTION AFTER LATARJET PROCEDURE: A SIMULATION STUDY USING 3D FINITE ELEMENT METHOD**


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Latarjet procedure (transfer of coracoid process to the anterior glenoid rim) has been widely used for severe anterior shoulder instability. The purpose of the present study was to investigate the intraarticular stress distribution after this procedure to clarify the pathomechanism of its postoperative complications. CT-DICOM data of the contralateral healthy shoulder in 10 patients with unilateral anterior shoulder instability (9 males and 1 female, age: 17-49) was used for the present study. Three-dimensional finite element models of the glenohumeral joint was developed using software, Mechanical Finder (RCCM, Japan). In each shoulder, a 25% bony defect was created in the anterior glenoid cavity, where coracoid process was transferred using two
half-threaded screws. The arm position was determined as 0-degree and 90-degree abduction. While medial margin of the scapula was completely constrained, a standard compressive load (50 N) toward the centre of the glenoid was applied to the lateral wall of the greater tuberosity. A tensile load (20N) was also applied to the tip of coracoid process along the direction of conjoint tendon. Then, elastic analysis was performed, and the distribution pattern of Drucker-Prager equivalent stress was investigated in each model. The proximal half of the coracoid represented significantly lower equivalent stress than the distal half (p < 0.05). In particular, the lowest mean equivalent stress was seen in its proximal-medial-superficial part. On the other hand, a high stress concentration newly appeared in the antero-inferior aspect of the humeral head exactly on the site of coracoid bone graft. We assumed that the reduction of mean equivalent stress in the proximal half of the coracoid was caused by the stress shielding, which may constitute one of the pathogenetic factors of its osteolysis. A high stress concentration in the humeral head may eventually lead shoulder joint to osteoarthritis.

S47.4 MECHANICS OF A SMITH’S FRACTURE CAUSED BY FALLING ON THE PALM OF THE HAND

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Smith’s fractures generally occur when falling on a flexed wrist; however, orthopedic trauma surgeons often encounter distal radius fractures with volar displacement in patients who have allegedly fallen on the palm of their hands. This study aimed to reveal both the basic and clinical pathogenesis of Smith’s fracture through a step-by-step investigation. We enrolled 17 patients with Smith’s fractures, of which 71% fell on the palm and only 6% on the dorsum of the hand. First, we interviewed the outpatients to determine the mechanics of the injury and the position of their arm during injury. Second, we created a three-dimensional (3D) finite element model to predict the arm’s position when the Smith’s fracture occurred, which finite element analysis revealed as a 30° angle between the long axis of the forearm and the ground in the sagittal plane. Third, using this predicted position, we conducted experiments on 10 fresh frozen cadavers to prove the possibility of causing a Smith’s fracture by falling on the palm of the hand. The results showed Smith-type fractures in seven of 10 wrists, whereas Colles-type fractures did not occur. Finally, we analyzed stress distribution in the distal radius when a Smith’s fracture occurs using the 3D finite element model. In conclusion, this study demonstrates that Smith’s fractures can also occur by falling on the palm of the hand.

S47.5 COMPARISON OF BIOMECHANICAL EVALUATIONS BETWEEN EXTRAMEDULLARY AND INTRAMEDULLARY REDUCTIONS IN UNSTABLE INTERTROCHANTERIC FRACTURE FIXATION WITH INTRAMEDULLARY NAILING


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The reduction for unstable femoral intertrochanteric fracture should be extramedullary, which means that the proximal fragment protrudes for the distal fragment. However, only few articles have compared extramedullary and intramedullary reductions in a biomechanical study. Thus, we created unstable femoral intertrochanteric fracture models using imitational bone (extramedullary and intramedullary groups, each with 12 cases) and evaluated their biomechanical stabilities. The fracture type was 31-A2 according to the AO-OTA Classification of Fractures and Dislocations and greatly lacked bone on the posterior side. We performed compression examination and evaluated stiffness. The implant used for fixation was TFNA (DePuy Synthes). We applied axial compression with 20 adduction in the standing position. Statistical analysis was performed.
using the Mann-Whitney U test. No significant difference in initial loading force was found between the two groups. However, the axial stiffness of the extramedullary bone showed a significant increase (p < 0.05) in high loading force (800–1000 N). This means that the stability of the extramedullary reduction was superior to that of the intramedullary reduction in terms of high loading force in the standing position. We suggest that antero-medial bony buttress is important for unstable femoral intertrochanteric fractures. These data indicate that extramedullary reduction and fixation for unstable femoral intertrochanteric fractures increase stability.

**S47.6 THE POSTERIOR ACETABULAR UPTAKE (CONTRE-COUP REGION) by 18F-FLUORIDE PET/CT IN FAI SYNDROME CASES**

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The pathology of the posterior acetabular lesion in femoroacetabular impingement (FAI) syndrome, so called “contre-coup region”, is still unclear. 18F-fluoride positron emission tomography (PET) is a functional imaging modality, which reflects the osteoblast activity. Recent technological advances in PET combined with computed tomography (CT) imaging allowed us to obtain detailed 3-dimensional (3D) morphological information. We evaluated the abnormal uptake of 18F-fluoride PET/CT on posterior acetabular lesion in FAI syndrome cases. We enrolled forty-one hips from 41 patients who were diagnosed as FAI syndrome and were performed 18F-fluoride PET/CT between October 2014 and October 2016. In each hip, the maximum standardized uptake value (SUVmax) on the posterior acetabular was measured. The cases were divided into 4 groups; cam-type (11 cases), pincer-type (7), combined-type (11), dysplastic developmental hip (DDH) with cam morphology (12). The average SUVmax of the pincer-type was significantly smaller than that of the other 3 groups (p < .05). The percentage of the cases with SUVmax ≥ 6 was 81.8% in cam-type, 28.6% in pincer-type, 90.9% in combined-type, 91.7% in DDH with cam morphology. Furthermore, the average degree of α angle of the cases of SUVmax ≥ 6 was significantly higher than that of the cases of SUVmax < 6 (p = .005). Although actual biomechanical mechanism in contre-coup region is still controversial, this result indicated that the cam morphology related to the posterior acetabular lesion with accelerated bone metabolism.

**S48.1 CIRCADIAN RHYTHMS IN THE MUSCULOSKELETAL SYSTEM: IMPLICATIONS IN HEALTH AND DISEASE**

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Osteoarthritis is the most prevalent joint disease, causing severe pain, deformity and a loss of mobility. Low back pain (LBP), frequently associated with degeneration of the intervertebral disc (IVD), is the No.1 cause of Years Lived with Disability. Age is a major risk factor for both conditions. However, the reasons why susceptibility to these conditions increases with age are poorly understood. The circadian (24 hourly) clocks in the brain and periphery direct key aspects of physiology through rhythmic control of tissue-specific sets of downstream genes. Work from our group focuses on the roles of circadian clocks in the articular cartilage and IVD. We show that the daily rhythm in these tissues becomes dampened and out-of-phase during ageing. Further, our data identify circadian clock disruption in cartilage and IVD as a new target of inflammation. Moreover, we show that mice with targeted knockout of an essential clock gene (BMAL1) in chondrocytes and disc cells have profound, yet tissue-specific degeneration in the articular cartilage and IVD. These findings
implicate the local skeletal clock as a key regulatory mechanism for tissue homeostasis. This new avenue of research holds potential to better understand, and eventually treat these debilitating conditions.

**S48.2 STEM CELLS AND BIOMATERIALS FOR THE REGENERATIVE MEDICINE OF INTERVERTEBRAL DISC: “WHEN DEVELOPMENTAL BIOLOGISTS MEET TISSUE ENGINEERS”**

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Degeneration of intervertebral disc (IVD) Nucleus Pulposus (NP) is a major cause of low back pain (LBP). Healthy NP contains two cell types: notochordal cells (NTC) and nucleopulpocytes (NPCytes). While NTC are embryonic notochord derived cells that are regarded as the resident stem cells of NP, NPCytes are considered the mature NP cells responsible for extracellular matrix (ECM) synthesis. During IVD aging, some still unknown cues drive NTC disappearance. This loss of NTC alters their dialog with NPCytes thereby jeopardizing cell viability and ECM homeostasis, which in turn drives NP degeneration. In this context, NP regeneration by re-establishing this NTC/NPCytes dialog has been contemplated with clinical interest. We will first share our view of the mesenchymal stem cells (MSC)-based therapies that have been preclinically and clinically assessed in LBP. We will then comment on the biomaterial-assisted MSC therapies that recently enter the scene of IVD regeneration. Finally, we will present our REMEDIV project that aims at developing a NP substitute containing stem cells-derived NPCytes and NTC within an injectable hydrogel. We will share our results regarding the generation of NPCytes from adipose-derived MSC and our recent unpublished evidences that human induced-pluripotent stem cells can be differentiated into NTC. Finally, we will consider our ability to transplant these regenerative cells using hydrogels in various animal models. Whether this concept could open new therapeutic windows in the management of discogenic low back pain will finally be discussed.

**S48.3 CELLULAR SENESCENCE IN INTERVERTEBRAL DISC DEGENERATION IS ASSOCIATED WITH DNA DAMAGE AND CYTOPLASMIC DNA**

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Intervertebral disc (IVD) degeneration is a major cause of low back pain (LBP). Degenerate discs are associated with accelerated cellular senescence. Cell senescence is associated with a secretory phenotype characterised by increased production of catabolic enzymes and cytokines. However, to date, the mechanism of cell senescence within disc degeneration is unclear. Senescence can be induced by increased replication or induced by stress such as reactive oxygen species or cytokines. This study investigated the association of cellular senescence with markers of DNA damage and presence of cytoplasmic DNA (which in cancer cells has been shown to be a key regulator of the secretory phenotype), to determine mechanisms of senescence in disc degeneration. Immunohistochemistry for the senescence marker: p16INK4A was firstly utilised to screen human intervertebral discs for discs displaying at least 30% immunopositivity. These discs were then subsequently analysed for immunopositivity for DNA damage markers γH2AX and cGAS and the presence of cytoplasmic DNA. The number of immunopositive cells for p16 INK4A positively correlated with the expression of γH2AX and cGAS. Senescent cells were also associated with the presence of cytoplasmic DNA.
These new findings elucidated a role of cGAS and γH2AX as a link from genotoxic stress to cytokine expression which is associated with senescent cells. The findings indicate that cellular senescence in vivo is associated with DNA damage and presence of cytoplasmic DNA. Whether this DNA damage is a result of replicative senescence or stress induced is currently being investigated in vitro.

S48.4 INVESTIGATING TROPHIC FACTOR EXPRESSION IN PATIENT MATCHED ADIPOSE & BONE-MARROW DERIVED MESENCHYMAL STEM CELLS IN RESPONSE TO INFLAMMATORY CYTOKINES: IMPLICATIONS FOR INTERVERTEBRAL DISC REGENERATIVE THERAPIES

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Clinical trials are underway to elucidate a successful MSC-based therapy for the repair and regeneration of intervertebral disc (IVD) tissue. Currently, there is a lack of knowledge surrounding the relationship between naïve MSCs and the inflammatory microenvironment of the degenerate disc. To inform a phase II clinical trial, this study tests the hypothesis that cytokines, IL-1β and TNFα regulate the expression of neuropeptides and neurotrophic factors from MSCs, thus exacerbating pain in those patients that have the presence of sensory nerve fibres within the IVD. Patient-matched MSCs derived from bone marrow (BM) or adipose (AD) tissue were stimulated with IL-1β (10ng/ml) or TNFα (10ng/ml) for 48 hours in either 21% or 5% O2. qRT-PCR was performed to assess expression of trophic factors involved in the survival of nerves (NGF & BDNF), blood vessels (VEGF) as well as pain related peptides (SP & CGRP) and inflammatory factors. Conditioned culture medium was analysed using ELISAs to identify secretion of soluble factors. IL-1β did not regulate neurotrophic factor expression from BM-MSCs under normoxic or hypoxic conditions. However, TNFα increased NGF, BDNF, SP and CGRP under normoxic conditions. In ADMSCs, VEGF was increased following IL-1β and TNFα stimulation; with TNFα also increasing NGF and CGRP under normoxic conditions. When exposed to hypoxia, the trophic effect of TNFα on human BM-MSCs was reduced. Overall this data suggests a role for priming or pre-stimulation of naïve MSCs prior to implantation to prevent exacerbation of pain from sensory nerve fibres.

S48.5 OPTIMISATION OF A COMBINED ADIPOSE STEM CELL-GDF6 THERAPY FOR INTERVERTEBRAL DISC REGENERATION

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Low back pain (LBP), caused by intervertebral disc (IVD) degeneration represents one of the most significant socioeconomic conditions facing Western economies. Novel regenerative therapies, however, have the potential to restore function and relieve pain. We have previously shown that stimulation of adipose-derived stem cells (ASCs) with growth differentiation factor-6 (GDF6) promotes differentiation to nucleus pulposus (NP) cells of the IVD, offering a potential treatment for LBP. The aims of this study were to i) elucidate GDF6 cell surface receptor profile and signalling pathways to better understand mechanism of action; and (ii) develop a microparticle (MP) delivery system for GDF6 stimulation of ASCs. GDF6 receptor expression by ASCs (N=6) was profiled through western blot, immunofluorescence (IF) and flow cytometry. Signal transduction through Smad1/5/9 and non-Smad pathways following GDF6 (100ng/ml) stimulation was assessed using western blotting and confirmed using pathway specific blockers and type II receptor sub-unit knockdown using
CRISPR. Release kinetics of GDF6 from MPs was calculated (BCA assay, ELISAs) and ASC differentiation to NP cells was assessed. BMPR profiling revealed high BMPR2 expression on ASCs. GDF6 stimulation of ASCs resulted in significant increases in Smad1/5/9 and Erk phosphorylation, but not p38 signalling. Blocking GDF6 signalling confirmed differentiation to NP cells required Smad phosphorylation, but not Erk. GDF6 release from MPs was controlled over 14 days in vitro and demonstrated comparable NP-like differentiation to exogenous GDF6 delivery. This study elucidates the signalling mechanisms responsible for GDF6-induced ASC differentiation to NP cells and also demonstrates an effective and controllable release vehicle for GDF6.

S49.1 THE CLINICAL REALITY OF PREVENTING, DIAGNOSING AND TREATING FRACTURE-RELATED INFECTIONS (FRI): FOCUS ON BIOMATERIALS FOR ANTIBIOTIC DELIVERY

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The most challenging complications in orthopaedic trauma surgery are fracture-related infections (FRI). The incidence ranges from approximately 1% after closed fractures or joint replacement, to more than 30% in complex open limb fractures. Despite tremendous efforts with prolonged antibiotic therapy and multiple revision surgeries, these complications are associated with considerable rates of recurrent infections as well as permanent functional impairment. The primary aim for the clinician is to prevent infection, because once established, an infection is difficult to eradicate. The main reason for this is biofilm formation on the implanted device, which allows pathogens to protect themselves from host immune response and antimicrobial therapy. In open fractures with a considerable wound contamination and soft-tissue damage, systemically-delivered antibiotics may not reach sufficient local concentrations to eradicate the bacteria. Locally delivered antibiotics can overcome this problem by providing high local concentrations. Currently, several antibiotic loaded biomaterials for local infection prophylaxis and/or treatment are available. In this talk, next to the diagnostic challenges of FRIs, the currently available antimicrobial-loaded biomaterials will be described. Against a backdrop of increasing infection and antimicrobial resistance, the prudent use and availability of such materials will become even more important.

S49.2 THE DESIGN AND PRECLINICAL EVALUATION OF ANTIBIOTIC RELEASING BIOMATERIALS

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Device-associated infection remains a serious clinical problem in orthopaedic and trauma surgery. The emergence of resistant organisms such as methicillin resistant Staphylococcus aureus (MRSA) has further exacerbated this problem by limiting the range of treatment options. Currently, systemic antibiotic therapy is the cornerstone of treatment, alongside surgical resection of infected tissues and implant removal. The potential for antibiotic loaded biomaterials to support the prevention and treatment of infection is significant, although the currently available options are limited in number and often re-purposed from other applications e.g. antibiotic loading of bone cement. The first part of the talk will cover the basic concepts involved in antibiotic treatment, with an emphasis on the ideal antibiotic release kinetics from biomaterials, and how bacterial biofilms and antibiotic resistance influence antimicrobial efficacy. The next generation of biomaterials for antibiotic delivery should be specifically designed with this knowledge in mind. Regulatory approval of antimicrobial combination devices, however, is an evolving process as regulatory bodies seek more robust and
clinically relevant efficacy data. Approval will require preclinical efficacy using standardized animal models that recapitulate the key features of the clinical disease. The second part of this talk will cover best practice in this important stage of development.

S49.3 HEAT STABILITY OF THE ANTIMICROBIAL ACTIVITY OF ANTIBIOTICS AFTER HIGH TEMPERATURE EXPOSURE

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Thermostability is a key property in determining the suitability of local delivery of antibiotics in the treatment of orthopaedic infections. Herein, we aimed to assess the thermal stability and antibacterial activity of ciprofloxacin, ceftriaxone, gentamycine and vancomycine in high temperature conditions. Using a standardized E-test method, minimally inhibited concentration of each antibiotic substance against *Staphylococcus aureus* cultures were determined. The solutions of antimicrobial drugs ciprofloxacin 2 mg/ml, ceftriaxone 200 mg/ml, gentamycine 40 mg/ml and vancomycine 200 mg/ml were diluted twofold in deionised water. Acquired solutions were divided into three aliquots. The first aliquot was held at 40°C for 30 min in a waterbath, the second and the third aliquots were exposed to 80 and 100°C for 30 min in hot-air sterilizer, respectively. The treated solutions were tested for residual activity against *S. aureus* using a standardized disk diffusion method. Mediums with untreated antibiotic solutions and *S. aureus* were used as control. Plates were incubated at 37°C, at which time zones of inhibition (ZoI) were measured to the nearest whole millimeter for 14 days. The investigation indicated that the temperature elevation impacted considerably on antimicrobial activity and antibiotic stability overall. The *in vitro* temperature-response curves showed that ZoI diameter decreases logarithmically with elevated temperatures. Gentamicin was the only drug that was found to be affected to some extent. Results from the study provides a valuable dataset for orthopaedic surgeons considering local application of antibiotics and methods of antibiotic impregnation.

S49.4 NOVEL ANTIBACTERIAL SILVER-BASED NANOCOATINGS FOR BIOMEDICAL DEVICES

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Implant-related infections pose a severe economical and societal burden, hence solutions capable of exerting suitable efficacy while not causing toxicity and/or development of resistant bacterial strains are needed. Thus, inorganic antibacterial coatings, and in particular silver coatings, have been extensively studied and used in the clinical practice. However, some drawbacks such as scarce adhesion to the substrate, delamination, or scarce control over silver release have been evidenced. Here, antibacterial nanostructured silver thin films have been developed by a novel plasma-assisted technique. The technique allows deposition on several substrates, including heat sensitive materials and objects of complex shape. Thanks to nanostructured surface, a tuned release can be achieved, preventing citotoxicity. Composition (grazing incidence XRD, XPS) and morphology (SEM, AFM, ASTM) of the obtained coatings were characterized, then, their efficacy was validated *in vitro* against relevant bacterial strains (gram+ *S. Aureus* and gram– *E. Coli*). Live/dead kit and confocal microscopy were used to evaluate antibacterial efficacy. Super resolution imaging in the Structured Illumination Microscopy (SIM) setup was used to investigate damage to the bacterial wall. Results indicate that the coatings are composed of nanosized aggregates of metallic silver, indicating a perfect transfer of composition from the deposition target to the coating. Because of the sub-micrometric thickness, they do not alter the micro- and
macro-morphology and surface finishing of the implants, developed by the manufacturers to ensure optimal integration in the host bone. Finally, remarkable efficacy was found against both gram+ and gram- bacteria, indicating that the developed coatings are promising for antibacterial applications.

S49.5 METALLURGICAL GALLIUM ADDITIONS DEMONSTRATE A STRONG TIME-INCREASING ANTIBACTERIAL ACTIVITY WITHOUT ANY CELLULAR TOXICITY

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Orthopedic metallic medical devices are essential in the treatment of a wide range of skeletal diseases and disabilities. However, they are often related with surgery complications due to acute prosthetic joint infections (PJI) causing devastating complications. Gallium (Ga) antibacterial activity has been recently demonstrated: in aqueous solutions, Ga ionize in a trivalent form Ga³⁺ that can replace Fe³⁺ in bacterial metabolism thus leading to bacteria death. However, it is not yet clear whether such effect is typical to Ga³⁺ release, and how this would affect longer term performance. Here we investigated Ga addition into titanium alloys using metallurgical methods. The study has confirmed that metallurgical addition of gallium even in small amounts (1-2% wt.) to titanium alloys have highly efficient antibacterial function without any visible cytostatic or cytotoxic effects. The presence of gallium within the metal matrix might ensure that antibacterial effect will persist for a long time towards multi-drug resistant S. aureus, which might not be possible if gallium or other metal are only in thin degradable coatings or similar formulations. A 5-logs decrease in CFU number was detected for alloys with 2% Ga and more after 72 h (alamar blue and CFU count assays). The alloys also show to be in vitro cytocompatible with both mature U2OS osteoblasts and progenitor pre-osteoblasts hFOB. Since gallium is metallurgically analogous to aluminium in titanium alloys, it might be used without affecting other alloy properties.

S49.6 CELL DEATH AND IL-1β RELEASE INDUCED BY TI PARTICLES DEPENDS ON LYSOSOMAL MEMBRANE DISRUPTION


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Aseptic loosening is a major cause of revision surgeries and occurs when osteolysis is stimulated around the implant by pro-inflammatory cytokines including IL-1β. Production of active IL-1β in response to orthopedic wear particles depends on processing by the NLRP3 inflammasome which requires priming followed by activation. We found that pathogen associated molecular patterns (PAMPs) adherent to wear particles are necessary to prime the NLRP3 inflammasome. In contrast, in pre-primed macrophages, particles themselves are sufficient to activate the NLRP3 inflammasome and induce secretion of active IL-1β. Particles themselves also induce cell death, kinetically preceding the release of active IL-1β. Phagocytosis of particles is required to initiate both responses as the phagocytosis inhibitor cytochalasin blocks cell death and IL-1β release. Lysosome membrane destabilization is also critical as inhibition of lysosomal function with bafilomycin or chloroquine significantly abrogated the release of active IL-1β and cell death in response to wear particles. The pan-cathepsin inhibitors Ca-074-Me or K777 also inhibit cell death and IL-1β release indicating that cathepsin release from lysosomes is also a necessary step in the particle-induced response. Our results open the possibility of clinical intervention with lysosomal or cathepsin inhibitors to treat aseptic loosening as these
drugs have better specificity and less in vivo toxicity than the phagocytosis inhibitors. Testing of these inhibitors in vivo in models of particle induced osteolysis is a key future direction.

**S50.1 ENGINEERING MICROENVIRONMENTS FOR REGENERATION OF BONE CRITICAL SIZE DEFECTS USING HIGHLY EFFICIENCY PRESENTATION OF BMP-2**

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While new biomaterials for regenerative therapies are being reported in the literature, clinical translation is slow. Existing regenerative approaches rely on high doses of growth factors, such as BMP-2 in bone regeneration, which can cause serious side effects. We describe an ultra-low-dose growth factor technology yielding high bioactivity based on a simple polymer, poly (ethyl acrylate) (PEA), and report its translation to a clinical veterinary setting. This polymer-based technology triggers spontaneous fibronectin organization and stimulates growth factor signaling, enabling synergistic integrin and BMP-2 receptor activation in mesenchymal stem cells. To translate this technology, we use plasma-polymerized PEA on 2D and 3D substrates to enhance cell signaling in vitro, showing the complete healing of a critical-size bone injury in mice in vivo. We demonstrate its safety and efficacy in a Münsterländer dog with a non-healing humerus fracture, establishing the clinical translation of advanced ultra-low-dose growth factor treatment.

**S50.2 THE FUNCTIONAL RESPONSE OF MESENCHYMAL STEM CELLS TO ELECTRON-BEAM PATTERNED ELASTOMERIC SURFACES PRESENTING MICRON TO NANOSCALE HETEROGENEOUS RIGIDITY**

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Cells directly probe and respond to the physicomechanical properties of their extracellular environment, a dynamic process which has been shown to play a key role in regulating both cellular adhesive processes and differential function. Recent studies indicate that stem cells show lineage-specific differentiation when cultured on substrates approximating the stiffness profiles of specific tissues. Although tissues are associated with ranging Young’s modulus values for bulk rigidity, at the sub-cellular level, and particularly at the micro- and nanoscales, tissues are comprised of heterogeneous distributions of rigidity.

Lithographic processes have been widely explored in cell biology for the generation of analytical substrates to probe cellular physicomechanical responses. In this work, we show for the first time that that direct-write e-beam exposure can significantly alter the rigidity of elastomeric PDMS substrates and develop a new class of two-dimensional elastomeric substrates with controlled patterned rigidity ranging from the micron to the nanoscale. The mechano-response of human mesenchymal stem cells to e-beam patterned substrates was subsequently probed in vitro and significant modulation of focal adhesion formation and osteochondral lineage commitment was observed as a function of both feature diameter and rigidity, establishing the groundwork for a new generation of biomimetic material interfaces.

**S50.3 45S5 AND 1393 BIOACTIVE GLASSES DIFFERENTIALLY REGULATE BEHAVIOR AS WELL AS ANGIogenic AND OSTEOGENIC RESPONSE OF HUMAN MSCs**

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Promising work on bioactive glasses (BAGs) in bone defect regeneration has led to their clinical implementation. However, the effects of the ionic dissolution products of different types and the physical interaction modalities of BAGs on the behavior and function of mesenchymal stromal cells (MSCs) of human patients have not received sufficient attention. Recently, we showed that the *in vitro* response of hMSC to micron-sized, monodispersed BAGs is dependent on dosage, composition, and mode of interaction\(^1\). Two commercially available and widely used types of BAGs, namely the silicate BAGs 45S5 and 1393, were used to study hMSC cell behavior. Interestingly, exposure to 1393 BAG resulted in superior metabolic activity, proliferation, and cell spreading compared to 45S5 BAG in similar dosage, suggesting that additional cellular functions could also be differentially modulated by both glasses\(^1\). In the context of bone regeneration, the hMSCs’ potential to secrete angiogenic factors as well as deposit mineralized matrix upon exposure to BAG dissolution products was investigated in the present study. Aside from dose-dependent effects of both glasses, 45S5 BAG induced a significant pro-angiogenic response, demonstrated by robust tube formation in HUVECs in the presence of MSC conditioned media. 1393 BAG, on the other hand, stimulated osteogenesis by upregulating osteogenic gene expression and mineralized matrix deposition. Based on these results, combining the pro-angiogenic 45S5 BAG and the pro-osteogenic 1393 BAG might be an attractive strategy to target the multiple processes underlying bone regeneration. These results highlight how different BAGs can be utilized to promote MSC-mediated bone regeneration.

S50.4 OPTIMIZATION OF MESENCHYMAL STEM CELL SEEDING ON FIBROIN-COATED MICROCARRIERS BASED ON DESIGN OF EXPERIMENT APPROACH

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Among the innovative therapeutic techniques in orthopedics, a considerable interest arose around Mesenchymal Stem Cells (MSCs) - based therapies for one-step clinical applications. In order to achieve a better cell targeting at the injury site, these applications would need a specific cell delivery system. Hence, in this study a protocol for an efficient cell delivery based on the rapid cell adhesion on the surface of lyophilized fibroin-coated alginate microcarriers (L-FAMs) was optimized by the Design of Experiment (DoE) method in accordance with the minimum requirements for one-step clinical application. Specific parameters (seeding time, intermittent or not dynamic culture, stirring speed and volume of cell suspension) were combined in 13 different protocols, tested on human Adipose derived stem cells - ASCs (n=3). Cell adhesion rate in term of DNA quantification and metabolic activity of cells adhered on L-FAMs, and their qualitative observations by Calcein Staining were evaluated. The data showed that a suspension of 3.75 x 10^5 cells/ml and 10 mg/ml of FAMs, 12.3 rpm of stirring speed and 85.6 minutes of seeding time are the most performing combination of parameters. The final protocol was then tested and validated on both hASCs (n=3) and human bone marrow derived stem cells - BMSCs (n=3). The results confirmed a high adhesion rate of cells, homogenously arranged on the surface of L-FAMs without cell cluster formation. Even though further optimizations are still needed, the present protocol may represent the proof of concept for the introduction of L-FAMs as carriers in one-step intraoperative applications.
S50.5 BONE INSPIRED CALCIUM PHOSPHATE/BIOPOLYMERS COATED MEMBRANE: A VERSATILE TOOL FOR BONE REGENERATION

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Bone regenerative medicine aims at designing biomimetic biomaterials able to guide stem cells fate towards osteoblast lineage and prevent orthopaedic common pathogen adhesion. Owing to bone inorganic/organic composition, we herein report, using a versatile process based on simultaneous spray coating of interacting species, a calcium phosphate (CaP) / chitosan (CHI) / hyaluronic acid (HA) functionalized collagen membrane as a new strategy for bone regenerative medicine. Physicochemical characterizations of CaP-CHI-HA coating were performed by scanning electron microscopy, X-ray photoelectron and infrared spectroscopies and high-resolution transmission electron microscopy, revealing the formation of a thin coating mainly composed of non-stoichiometric crystalline hydroxyapatite dispersed into polymorphic organic film. Biocompatibility of CaP-CHI-HA coated membrane, evaluated after 7 days in contact with human mesenchymal stem cells (MSCs), showed spread, elongated and aligned cells. Metabolic activity and DNA quantification studies showed an increase in MSCs proliferation on coated membrane compared to uncoated membrane over the study time. Similarly, cytokines (IL-6, IL-8, osteoprotegerin) and growth factors (VEGF, bFGF) release in supernatant, as well as endothelial cells recruitment, were significantly increased in presence of CaP-CHI-HA coated membrane. Thus, CaP-CHI-HA coated membrane provides a suitable environment for MSCs to induce bone healing. Moreover, pro-inflammatory cytokines (IL-1β and TNF-α) secretion by human monocytes was significantly reduced on CaP-CHI-HA coating compared to LPS stimulation. CaP-CHI-HA coating also reduced significantly *Staphylococcus aureus* and *Pseudomonas aeruginosa* adhesion on the membrane, conferring a bacterial anti-adhesive surface. Based on our results, CaP-CHI-HA functionalized collagen membrane provides an interesting material for bone regeneration.

S50.6 DESIGN AND EVALUATION OF ELECTROSPUN STRUCTURED POLYCAPROLACTONE BIOMATERIALS FOR ANNULUS FIBROSUS REPAIR


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Extensive annulus fibrosus (AF) radial tears lead to intervertebral disc (IVD) herniation. While unrepaired defects in the AF are associated with postoperative reherniation and high IVD degeneration prevalence, current surgical strategies are limited to symptomatic treatment of pain and disregard the structural integrity of the AF. For all these reasons, this study is focused on i) designing polycaprolactone (PCL) electrospun implants that mimic the multi-lamellar fibrous structure of the native tissue and ii) assessing their ability to properly close and repair an AF defect in a sheep *in vivo* model. Oriented PCL mats were produced by electrospinning with average fiber diameters of 1.3μm and a tensile modulus (55±1MPa) matching the one of a native human AF lamella (~47MPa). *In vitro* experiments demonstrated a spontaneous colonization of PCL mats by human and ovine AF cells. *In vivo* study was carried out on 6 sheep in which 5 lumbar discs were exposed using a left retroperitoneal approach. Defects (2x5mm, 2mm depth) were created in the outer annulus, with randomized distribution of conditions including 10-layer oriented or non-oriented mats, untreated and healthy groups. X-ray and MRI examinations were performed every month until explantations at 1, 3 and 6 months, followed by
immuno-histological analysis. Data showed no dislocation of the implants, cell infiltration between the PCL mats and within the mats, and a continuous type I collagen tissue formation between the implants and the surrounding AF tissue. These results highlight that multi-layer PCL electrospun mat is a promising biomaterial for AF repair.

S51.1 TUMOR MICROENVIRONMENT AND THE BEHAVIOR OF BONE CANCER

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The initiation and progression of malignant tumors are supported by their microenvironment: cancer cells per se cannot explain growth and formation of the primary or metastasis, and a combination of proliferating tumor cells, cancer stem cells, immune cells, mesenchymal stromal cells and/or cancer-associated fibroblasts all contribute to the tumor bulk. The interaction between these multiple players, under different microenvironmental conditions of biochemical and physical stimuli (i.e. oxygen tension, pH, matrix mechanics), regulates the production and biological activity of several soluble factors, extracellular matrix components, and extracellular vesicles that are needed for growth, maintenance, chemoresistance and metastatization of cancer. Both in osteosarcoma and bone metastases from carcinomas this aspect has been only recently explored. In this lecture, I will discuss the role of tumor microenvironment, with a particular focus on the mesenchymal stroma, contributing to bone tumor progression through inherent. The most recent advances in the molecular cues triggered by cytokines, soluble factors, and metabolites that are partially beginning to unravel the axis between stromal elements of mesenchymal origin and bone cancer cells, under different microenvironmental conditions, will be reviewed providing insights likely to be used for novel therapeutic approaches.

S51.2 OSTEOMYELITIS TREATMENT; OPTIONS, RESULTS AND LEVEL OF EVIDENCE

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Osteomyelitis is an infection of bone or bone marrow with a concomitant inflammation involving the bone marrow and the surrounding tissues. Chronic osteomyelitis is historically treated in a two-stage fashion with antibiotic-loaded polymethylmethacrylate as local antibacterial therapy. Two-stage surgeries are associated with high morbidity, long hospitalization and high treatment costs. Next to antibiotic releasing biomaterials, S53P4 bioactive glass is a biomaterial that enables one-stage surgery in local treatment of chronic osteomyelitis. S53P4 bioactive glass is gaining interests in recent years in clinical treatment of chronic osteomyelitis in a one-stage fashion due to its antibacterial and bone regenerating capacities. By changing local pH and osmotic pressure S53P4 bioactive glass attack bacteria in a different way as compared to antibiotics. In this presentation, we will present current clinical treatment options for osteomyelitis, clinical results and level of evidence of various biomaterials used in osteomyelitis treatment. In addition, the clinical results and health-economic results of S53P4 bioactive glass will be detailed. Thereafter a summary of the current standing across the board in osteomyelitis treatment will be provided.

S51.3 HDAC INHIBITORS SYNERGIZE WITH STANDARD-OF-CARE MAP CHEMOTHERAPEUTICS TO BLOCK GROWTH OF OSTEOSARCOMA SARCOSPHERES

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Our goal is to repurpose drugs to block the growth of lung metastases, the lethal process in osteosarcoma. We therefore screened the NCI-panel of 114 FDA-approved oncology drugs to identify agents that potently reduce growth of osteosarcoma spheroids (sarcospheres). We first developed a system to routinely generate large numbers of highly-uniform spherical sarcospheres (1/well) with a 400um diameter, to most closely simulate micrometastases. Our primary drug screen (Z’-factor=0.70+0.10) utilized sarcospheres from three highly-metastatic human osteosarcoma cell lines (LM7, 143B, and MG63.3) in the presence and absence of MAP chemotherapeutics. Dose-response experiments with 13 of the most effective drugs confirmed initial results and allowed comparison with each drug’s toxicity on normal human osteoblasts and normal small airway epithelial cells. Romidepsin, a HDAC inhibitor (HDACi), had the most favorable toxicity/efficacy ratios (TD50/IC50=57-580, depending on cell line). The only other HDACi in the panel of FDA-approved drugs (vorinostat) also ranked highly in the screen. Since newer HDACi’s may have improved toxicity/efficacy ratios, we compared romidepsin and vorinostat with the three other HDACi’s that are FDA-approved (belinostat, panobinostat, and valproic acid) plus one that is in clinical trials (entinostat). Romidepsin (Cmax/IC50=36-360) and belinostat (Cmax/IC50=14-20) reduced sarcosphere growth at clinically-achievable levels, in the presence or absence of MAP. Importantly, both romidepsin and belinostat were synergistic with MAP (BLISS scores=5-15). Propidium iodide staining showed that both romidepsin and MAP substantially induced cell death throughout the sarcospheres. Our results strongly support future studies to determine effects of romidepsin and belinostat on growth of lung metastases in vivo.

S51.4 HIGH RATE OF Tibial Debonding AND Failure IN A Modern Knee Replacement: A Cause FOR CONCERN

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Total knee arthroplasty (TKA) is a common orthopaedic procedure with over 1,500 done in 2016 in Ireland alone. 96% of all TKAs are due to pain in the knee associated with osteoarthritis. According to the UK National Joint Registry (NJR), there is a 0.47%, 1.81%, 2.63% and 4.34% probability risk of undergoing a revision TKA within one, three, five and ten years respectively post-index surgery. A variety of reasons for failure of TKA have been described in the literature including infection, aseptic loosening, pain, instability, implant wear, mal-alignment, osteolysis, dislocation, peri-prosthetic fracture and implant fracture. The NexGen Posterior Stabilised Fixed has NJR revision rates of 0.44%, 1.61% and 2.54% at years one, three and five respectively. A retrospective review was carried out of 350 NexGen TKAs that were performed directly by, or under the supervision of, a fellowship trained arthroplasty surgeon in a dedicated orthopaedic hospital between April 2013 and December 2015. 26 (7.4%) of these were revised as of 31 December 2017. Three were for septic arthritis with the remaining 23 (6.6%) for aseptic loosening. Patients typically started to experience symptoms of medial tibial pain with supra-patellar swelling from a combination of effusion and synovial thickening at 12-24 months. Inflammatory markers were normal in all cases. Radiographs of symptomatic knee replacements showed bone loss on the medial tibia with a tilt of the tibial component into a varus alignment. The high number of revisions of this particular prosthetic has led to its use being discontinued at this centre.

S51.5 Profiles OF Monocyte Subsets AND Synovial Macrophage Phenotypes During the Course OF Osteoarthritis IN THE Destabilisation OF the Medial Meniscus Mouse Model
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Although osteoarthritis (OA) is characterized by articular cartilage damage, synovial inflammation is a prominent feature contributing to disease progression. In addition to synovial tissue resident macrophages, infiltrating macrophages and monocytes, their lineage precursors, may also contribute to pathological processes. In mice, peripheral blood monocytes may be categorized according to pro-inflammatory/classical and patrolling/non-classical subsets. The aim of this study was to identify profiles of peripheral blood monocyte subsets as well as different synovial macrophage phenotypes during disease development. OA was induced in knees of C57BL/6 mice by destabilization of the medial meniscus (DMM). Blood was harvested from the facial vein 7 days prior to and 1, 7, 14, 28, and 56 days post induction of OA. Separate mice were sham-operated as a control. Monocyte subsets and synovial macrophage populations were identified by flow cytometry. Levels of classical monocytes were significantly higher at day 14 (p<0.001) and day 28 (p=0.031) in peripheral blood of DMM-operated mice compared to control. Furthermore, the percentage of non-classical monocytes was significantly lower in DMM-mice at day 14 (p=0.026). At day 56 post OA-induction, an increase in total synovial macrophages (CD11b+F4/80+ cells) was observed between DMM and sham operated knees (p=0.026). The ratio between pro-inflammatory (CD11b+F4/80+CD86+) and tissue repair (CD11b+F4/80+CD206+) synovial macrophage subsets tended to be higher in DMM knees, however this finding was not statistically significant (p>0.05). In light of the present findings, further investigation is required to elucidate the relationship of peripheral blood monocyte subsets to synovial inflammation and features of OA pathogenesis.

S52.2 ADVANCED MUSCULOSKELETAL MODELLING USING PATIENT-SPECIFIC DATA THE NEXT FRONTIER IN OSTEOARTHRITIS PREVENTION

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Osteoarthritis is a multifactorial disease in which altered mechanical loading is one of the agreed contributing factors. Whereas in the past, altered mechanical loading was merely deferred from static, image-based evaluations of malalignment, the recent use of 3D motion capture allowed dynamic evaluation of joint loading in terms of dynamic alignment (e.g. varus trust) and even joint loading strategy (merely using proxy measures like knee adduction moment.) Combining these measurements with musculoskeletal models, the overall loading distribution in the joint due to muscle action underlying the patient’s motion pattern can be quantified. Using this approach, our group showed the potential of this technique to differentiate between control subjects and subjects with early medial knee OA before the presence of radiographic evidence of structural joint degradation. Nevertheless, no changes in loading distribution could be detected in a cohort of subjects suffering of local cartilage defects in an otherwise healthy knee joint, indicating that patients did not present active unloading strategies despite the presence of clinical symptoms. Furthermore, subject-specific strategies aiming contributing to modified loading of the hip joint have been evaluated.

**S52.3 IN VIVO KINEMATIC COMPARISON BETWEEN BI-CRUCIATE STABILIZED AND CRUCIATE RETAINING TKA DURING DEEP KNEE BENDING**


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Bi-cruciate stabilized (BCS) TKA is the prosthesis that aims to substitute bi-cruciate ligament with post-cam engagement. We estimated to describe the *in vivo* kinematics during deep knee bending in BCS and Cruciate retaining (CR) TKA with the same articular geometry. We analyzed 26 knees who agreed to the current investigation under institutional review board approval. 17 knees were implanted with BCS (Journey II BCS, Smith & Nephew. Memphis, US) and 9 knees with CR (Journey IICR). Each patient was asked to perform deep knee bending under weight-bearing condition. To estimate the spatial position and orientation of the TKA, 2D/3D registration technique with single fluoroscopy was used. We evaluated anteroposterior (AP) translation of the nearest point from femoral component to tibial axial plane for medial and lateral sides, femoral external rotation relative to tibial component and post-cam engagement in BCS. Measurement results were analyzed using Wilcoxon test. Values of P<0.05 were considered statistically significant. Medial AP translation indicated 11.7±5.1% posterior movement in BCS and 4.0±6.6% anterior movement in CR from minimum flexion to 130°. Lateral AP translation indicated 28.9±11.4% posterior movement in BCS and 18.3±6.2% posterior movement in CR from minimum flexion to 130°. Femoral external rotation were observed in both group and the amount of rotation were 5.2°±4.5° in BCS and 8.2°±4.0° in CR. Anterior post-cam engagement was not observed in all cases (76.5%). But medial AP translation in BCS was anteriorly in shallow flexion angles compared to CR. It suggested that anterior post-cam engagement couldn’t work in valid.

**S52.4 IN VIVO THREE-DIMENSIONAL KINEMATIC COMPARISON OF NORMAL KNEES BETWEEN FLEXION ACTIVITIES AND EXTENSION ACTIVITIES**


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There are few studies that have compared between continuous flexion activities and extension activities of normal knees. The purpose of this study is to compare in vivo kinematic comparison of normal knees between flexion activities and extension activities. Total of 8 normal male knees were investigated. We evaluated in vivo three-dimensional kinematics using 2D/3D registration technique. We compared femoral rotation angle relative to tibia, anterior/posterior (AP) translation of medial femoral sulcus (medial side) and lateral femoral epicondyle (lateral side) onto tibial plane perpendicular to tibial functional axis between flexion activities (F groups) and extension activities (E groups). Femoral external rotation was observed with the knee bending during both groups. The external rotation angle of F group was larger than that of E group significantly from 20 to 30 degrees with flexion (p<0.05). Regarding medial side, anterior translation was observed up to 40 degrees in F group. From 40 to 140 degrees, posterior translation was observed. In E group, anterior translation was observed from 140 to 40 degrees with extension. From 40 degrees, posterior translation was observed. From 30 to 40 degrees, F group located anterior than E group (p<0.05). Regarding lateral side, posterior translation was observed with flexion in F group. On the other hand, anterior translation was observed with extension in E group. Regarding AP location with flexion angle, there was no significant difference between two groups. In conclusion, there were different kinematics between flexion activities and extension activities.

S52.5 MECHANICAL VERSUS KINEMATIC ALIGNMENT IN TOTAL KNEE ARTHROPLASTY: DOES THE BONE DENSITY AT THE IMPLANT-TIBIA INTERFACE DIFFER?

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Tibial bone density may affect implant stability and functional outcomes following total knee replacement (TKR). Our aim was to characterise the bone density profile at the implant-tibia interface following TKR in mechanical versus kinematic alignment. Pre-operative computed tomography scans for 10 patients were obtained. Using surgical planning software, tibial cuts were made for TKR either neutral (mechanical) or 3 degrees varus (kinematic) alignment. Signal intensity, in Hounsfield Units (HU), was measured at 25,600 points throughout an axial slice at the implant-tibia interface and density profiles compared along defined radial axes from the centre of the tibia towards the cortices. From the tibial centre towards the lateral cortex, trabecular bone density for kinematic and mechanical TKR are similar in the inner 50% but differ significantly beyond this (p=0.012). There were two distinct density peaks, with peak trabecular bone density being higher in kinematic TKR (p<0.001) and peak cortical bone density being higher in mechanical TKR (p<0.01). The difference in peak cortical to peak trabecular signal was 43 HU and 185 HU respectively (p<0.001). On the medial side there was no significant difference in density profile and a linear increase from centre to cortex. In the lateral proximal tibia, peak cortical and peak trabecular bone densities differ between kinematic TKR and mechanical TKR. Laterally, mechanical TKR may be more dependent upon cortical bone for support compared to kinematic TKR, where trabecular bone density is higher. This may have implications for surgical planning and implant design.

S53.1 ENIGMA OF NUCLEUS PULPOSUS CELL METABOLISM

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A defining characteristic of the Nucleus Pulposus (NP) and the inner AF is the very limited vascular supply and low pH that imposes metabolic constraints on the disc cells. Interference with the normal physiology of the NP niche, by activities linked to changes in oxygen diffusion across the endplate leads to dysregulated niche function. Hypoxia Inducible Factor-1 (HIF-1) and HIF-2 are robustly and constitutively expressed by cells of the NP. Our recent work has shown that expression of HIF-1 is indispensable for NP cell survival *in vivo* and suggests an important role of HIF-1 in NP cell metabolic program. This talk will discuss central role of HIF-1 as metabolic and pH homeostatic regulator of NP cells and possible implication for a therapeutic strategy to treat disc degeneration.

**S53.2 DEVELOPMENT OF CELL BASED REGENERATIVE THERAPIES FOR INTERVERTEBRAL DISC DEGENERATION**

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Current medical treatments for IVD degeneration rely on conservative therapies or surgery. Surgical treatments (e.g. spinal fusion,) have shown satisfactory results in alleviating pain, but long-term clinical outcomes remain poor. Thus, there is an urgent need for alternative cell based regenerative therapies focussed on correcting the underlying pathogenesis of IVD degeneration. However, for these to be successful an appropriate cell source for implantation, together with a suitable growth factor to direct cell differentiation and formation of a functional matrix must be identified. Additionally, extensive *in vitro* studies are needed to establish and support further pre-clinical and potential commercial development. We have demonstrated that stimulation of both BM-MSCs and AD-MSCs with GDF6 results in improved differentiation to a nucleus pulposus (NP)-like phenotype and synthesis of proteoglycan rich matrix with micromechanical properties akin to the healthy IVD. Significantly, these studies have highlighted that AD-MSCs are the more appropriate cell source. Furthermore, our studies have shown that GDF6 has anabolic effects on degenerate human NP cells, stimulating adoption of a more normal NP phenotype and increasing appropriate matrix synthesis. This suggests that delivery of GDF6 as part of an MSC-based therapy may be beneficial both in directing lineage-specific MSC differentiation, but also in restoring a more anabolic phenotype in native NP cells, thereby having a dual regenerative effect.

**S53.3 ANALOGOUS IN VITRO MODEL OF MSC INJECTION INTO INTERVERTEBRAL DISCS**

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Early clinical studies investigating the effects of delivery of mesenchymal stromal cells (MSCs) to degenerated intervertebral discs have shown promising results, but with an incomplete understanding of the therapeutic mechanism(s) of action. To address this, we have developed a 3D co-culture system to unravel the biological interaction between MSCs and nucleus pulposus (NP) cells. Alginate constructs were created using a biphasic configuration consisting of a cylindrical shell with an encapsulated bead. Human NP cells were seeded in monolayer or encapsulated within alginate and cultured in hypoxia with variations of pH, osmolarity and growth factors (n = 6) to replicate healthy or degenerative conditions. Wells and gels were fixed and stained for ECM content, and retrieved cells and media were analysed for ECM and inflammatory factor expression. Encapsulated hNPCs showed no migration from either alginate structure and full bead separation was achieved over 14 days, reinforcing the construct as a separable 3D co-culture method. Addition of the degenerative
growth factors TNFα and IL-1β as well as the adjustment of media pH to degenerative levels (pH 6.8) caused the hNPCs to decrease in size and proliferate significantly higher than control levels. TGF-β3 addition showed higher incidence of aggrecan deposition over addition of IL-1β. Addition of FGF2 altered cell morphology and ECM deposition including formation of pseudo lamellae, indicating a phenotype shift toward annulus fibrosis cells as shown in late-stage degenerative disc disease. The data from this study will be used in future MSC:NPC co-cultures to determine immunoregulatory interactions in a degenerative environment.

S53.4 HISTOLOGICAL ANALYSIS OF BONE REGENERATION WITH DIFFERENT DOSES OF RHBMP-2 IN AN OVINE LUMBAR INTERBODY FUSION MODEL

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The objective of this study was to investigate the effects of different doses rhBMP-2 on bone healing in an ovine lumbar interbody fusion model. In this study 22 sheep underwent two level lumbar interbody fusion using a ventrolateral approach with secondary dorsal fixation at L1/2 and L3/4. After randomization in one level a PEEK-cage was implanted filled with one of three doses rhBMP-2 (0.5mg; 1mg; 2mg) delivered on an ACS. The other level received an empty PEEK-cage or ACS filled cage. Animals were sacrificed after 3 and 6 months and decalcified histology was performed. This included histomorphological analysis as well as histomorphometry of the tissues within the cage.

At 3 months after surgery the groups treated with rhBMP-2 showed higher amounts of bone tissue within the cage. At 6 months the amounts of bone tissue increased in all groups, were still lower in the groups without growth factor. At 3 months there was only one active osteolysis in the cage/ACS. 7 of 8 segments of the rhBMP-2 groups had a compromised bone structure around the implant. These areas were filled with fibrous tissue and fibrocartilage. This finding was not detected in the groups without rhBMP-2 at 3 months. At 6 months most of the segments with an empty cage or cage/ACS showed a chronic inflammation. Predominant cells were macrophages and giant cells. The groups treated with rhBMP-2 showed only a few mild chronic inflammatory reactions. The well-known dose dependent effect of rhBMP-2 on bone healing could also be recognized in our study. Attention has to be payed to the proinflammatory properties of the growth factor. Consistent with other studies we found 2 strong inflammatory reactions, each one in the lowest and highest dose group. Also, the potential for causing transient bone resorptions, according to the results of others, was demonstrated. At 3 months 7 of 8 segments treated with rhBMP-2 showed compromised peri-implant bone. Osteoblasts, but not osteoclasts, were seen in the periphery of these areas. It can be concluded that there where bone resorptions which already merged into an increased osteoblastic activity. Usually resorptions occur between 2 and 12 weeks and are followed by a period of increased osteoblastic activity. This finding wasn’t recognized at 6 months anymore. Striking is that at 6 months most of the segments without rhBMP-2 showed a compromised bone structure around the implant with a mild to mainly moderate chronic inflammatory reaction. This cannot be attributed to the growth factor. Also, the ACS is degraded at 6 months and is unlikely a possible explanation. Therefore, the cage as a reason must be considered and it has to be questioned whether PEEK is the optimal material for interbody cages.

S53.5 PARAVERTEBRAL INJECTION OF BOTULINUM TOXIN-A REDUCES LUMBAR VERTEBRAL BONE QUALITY IN A RAT MODEL

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Aging has been associated with decreases in muscle strength and bone quality. In elderly patients, paravertebral muscle atrophy is accompanied by vertebral osteoporosis. The purpose of this study was to use paravertebral injection of botulinum toxin-A (BTX) to investigate the effects of paravertebral muscle atrophy on lumbar vertebral bone quality. Forty 16-week-old female SD rats were randomly divided into four groups: (1) a control group (CNT); (2) a resection of erector spinae muscles group (RESM); (3) a botulinum toxin-A group (BTX) that was treated with local injection of 5U BTX into the paravertebral muscles bilaterally; and (4) a positive control group (OVX) that underwent bilateral ovariectomy. At 3 months post-surgery the lumbar vertebrae (L3 – L6) were collected. The BMDs of the RESM and BTX groups were significantly lower than that of the CNT group (P < 0.01). Micro-CT scans showed that rats in the three experimental groups had fewer trabeculae and trabecular connections than rats in the CNT group. The bone loss trend of the trabecular networks was most obvious in the OVX rats. Vertebral compression testing revealed that the three experimental groups had significantly lower maximum load, energy absorption, maximum stress, and elastic modulus values than the CNT group (P < 0.01), and these parameters were lowest in the OVX group (P < 0.05). Our results demonstrate that the new paravertebral muscle atrophy model using local BTX injection causes sufficient muscle atrophy and dysfunction to result in local lumbar vertebral bone loss and quality deterioration.

S54.1 FIGHTING ORTHOPEDIC IMPLANT INFECTIONS WITHOUT ANTIBIOTICS BUT WITH NANOMEDICINE

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By modifying only the nanofeatures on material surfaces without changing surface chemistry, it is possible to increase tissue growth of any human tissue by controlling the endogenous adsorption of adhesive proteins onto the material surface. In addition, our group has shown that these same nanofeatures and nano-modifications can reduce bacterial growth without using antibiotics, which may further accelerate the growth of antibiotic resistant microbes. Inflammation can also be decreased through the use of nanomaterials. Finally, nanomedicine has been shown to stimulate the growth and differentiation of stem cells, which may someday be used to treat incurable disorders, such as neural damage. This strategy also accelerates FDA approval and commercialization efforts since new chemistries are not proposed, rather chemistries already approved by the FDA with altered nanoscale features. This invited talk will highlight some of the advancements and emphasize current ceramic nanomaterials approved by the FDA for human implantation. It will also emphasize the future of nanomaterials in medicine, such as their use in personalized medicine as internal sensors to detect and fight alterations in health.

S54.2 SELF-ASSEMBLED NANO-STRUCTURES FOR RNA DELIVERY AGAINST JOINT INFLAMMATION

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When joints sustain injury, the release of inflammation cytokines can cleavage matrix proteins and result in cartilage degradation and the subsequent osteoarthritis. RNA therapeutics emerging recently is a very promising approach to efficiently and specifically inhibit disease gene expression. However, the major challenge is how to deliver therapeutic RNA into joint and cartilage. Janus base nanotubes are self-assembled from synthetic Janus bases inspired from DNA base pairs. Based on the charge interaction, we are able to
“sandwich” small RNAs among Janus base nanotubes to form tiny, nano-rod shaped delivery vehicles. Such vehicles can be engineered into different sizes and shapes. We have found that short and slim morphologies can greatly increase their penetration to extracellular matrix and delivery into “difficult-to-reach” tissues, such as cartilage and brain. Moreover, by delivering therapeutic siRNA, we have demonstrated its high-efficacy in inhibiting expression of an inflammatory regulator, Interleukin-1 receptor (IL-1R) in articular cartilage. Moreover, the inhibition effect is long-lasting so that joint inflammation and cartilage degradation caused by meniscus injury are greatly inhibited in a mouse model. Therefore, the Janus base nanotubes present a great potential in engineering into nano-structures for RNA delivery. Such approach may become an effective therapeutic against joint inflammation and arthritis.

S54.3 INFLUENCE OF LASER-STRUCTURED SURFACES ON BACTERIAL LOAD OF IMPLANT MATERIALS

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Implant infection is an increasing problem in orthopedic surgery, especially due to progressive antibiotic resistance and an aging population with rising numbers of implantations. As a consequence, new strategies for infection prevention are necessary. In the previous study it was hypothesized that laser-structured implant surfaces favor cellular adhesion while hindering bacterial ongrowth and therewith contribute to reduce implant infections. Cuboid titanium implants (0.8 x 0.8 x 12 mm³, n=34) were used. Seventeen were laser-structured by ultra-short pulsed laser ablation to create a spike structure; the others were polished and served as controls. In general anesthesia, implants were inserted in rat tibiae and infected with a S. aureus suspension. During a 21 day postoperative follow-up, daily clinical control was performed. Radiographs were taken at day 14 and day 21. After euthanasia, bacterial load and biofilm formation on the implant surface was evaluated semi-quantitatively by confocal laser scanning microscopy and computational acquisition of bacteria and cells by Imaris®-software. Additionally, histology of the surrounding bone was performed. Clinically, no differences were observed between the groups. However, contrary to our hypothesis, bacterial load was increased in the laser-structured implant group although cellular adhesion was even more pronounced. Radiographical and histological evaluations showed increased bone alterations in the group with laser-structured implants compared to the control group. These findings did not confirm prior in vitro studies, where a reduction of bacterial load was found for similar surfaces and demonstrate the necessity of in vivo trials prior to the clinical use of new materials.

S54.4 EFFECT OF LOW INTENSITY PULSED ULTRASOUND THERAPY ON STAPHYLOCOCCUS AUREUS BIOFILMS


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Staphylococcus aureus is responsible for 60-70% infections of surgical implants and prostheses in Orthopaedic surgery, costing the NHS £120-200 million per annum. Its ability to develop resistance or tolerance to a diverse range of antimicrobial compounds, threatens to halt routine elective implant surgery. One strategy to overcome this problem is to look beyond traditional antimicrobial drug therapies and investigate other treatment modalities. Biophysical modalities, such as ultrasound, are poorly explored, but preliminary work has shown
potential benefit, especially when combined with existing antibiotics. Using a methicillin-sensitive \(S. \textit{aureus}\) reference strain and the dissolvable bead assay, biofilms were challenged by a low-intensity ultrasound (1.5MHz, 30mW/cm\(^2\), pulse duration 200μs/1KHz) for 20 minutes and gentamicin. The outcome measures were colony-forming units/mL (CFU/mL) and the minimum biofilm eradication concentration (MBEC) of gentamicin. The mean number of \(S. \textit{aureus}\) within control biofilms was \(1.04 \times 10^9\) CFU/mL. There was no clinically or statistically significant (\(p=0.531\)) reduction in viable \(S. \textit{aureus}\) following ultrasound therapy alone. The MBEC of gentamicin for this \(S. \textit{aureus}\) strain was 256 mg/L. The MBEC of gentamicin with the addition of ultrasound was 64mg/L. Low intensity pulsed ultrasound was associated with a 4-fold reduction in the effective biofilm eradication concentration of gentamicin; bringing the MBEC of gentamicin to within clinically achievable concentrations.

**S54.5 SILVER ION DOPED CALCIUM PHOSPHATE BASED CERAMIC (SILVERON®) COATED EXTERNAL FIXATOR PINS FOR PREVENTING IMPLANT RELATED INFECTION**

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Long-term survival and favourable outcome of implant use are determined by bone-implant osseointegration and absence of infection near the implants. As with most diseases, prevention is the preferred approach. Silver ion doped calcium phosphate based ceramic coating (Silveron®) for implant coating has been shown previously to be a potent antimicrobial agent as indicated by \textit{in vitro} testing. The present study reports on clinical experience using silver ion doped calcium phosphate based ceramic coated external fixator pins as surgical treatment in the management of chronic osteomyelitis and open fractures. Ten patients had external fixators: six for open fractures of ankle, three for chronic osteomyelitis of the femur, one for tibia pseudoarthrosis. The electrospray method was used for coating the external fixator pins with silver ion doped calcium phosphate-based ceramics. A radiofrequency energy source was used to sinter the coated pins. Microbiological, roentgenographic, toxic and biochemical analyses of patients were carried out. Wound debridement, and subsequent wound care resulted in control of the infection in three chronic osteomyelitis and in healing of seven fractures after follow-up ranging from three to six months. In total 67 pins were used in 10 patients but only one pin was positive microbiologically in one patient. Collectively, these data clearly illustrate that the toxic effects of silver were not observed at the doses used. Silver ion doped calcium phosphate based ceramic coating (Silveron®) can be used to prevent infection associated with the implant.

**S55.1 A NOVEL ANATOMICAL PATELLAR PLATE FOR TRANSVERSE PATELLAR FRACTURE - A BIOMECHANICAL IN-VITRO STUDY**

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Transverse patella fractures are commonly encountered in trauma surgery, open reduction and internal fixation are considered the gold standard treatment modality that could permit early knee motion and immediate rehabilitation. Many fixation methods had been defined and compared to each other’s in many clinical and biomechanical studies. The aim of this study was to assess the safety and stability of our novel anatomical patella plate and to compare its stability with tension band-wire technique. A total of 12 cadaveric preserved knees (six right and six left patellae) with close patellar size were chosen to form two groups of six samples. Each group received either plate or tension band-wiring fixation for an experimentally created patella fracture. Cyclic load of an average of 350 N was applied for all specimens and after accomplishing 50 cycles...
the displacements of all fracture edges were recorded. After completing 50 cycles in each group, the average fracture edges displacement measured in the plate group was $1.98 \pm 0.299$ mm, whereas the average fracture edges displacement measured in the tension band-wire group was $2.85 \pm 0.768$ mm ($p = 0.016$). In the operative treatment of displaced transverse patellar fractures, the strength of fixation obtained by titanium curved plates is highly stronger when compared to the fixation with a tension band-wire technique. Fixation with titanium curved plates provides satisfactory stability at the fracture site which allow withstanding the cyclic loads during the postoperative rehabilitation.

**S55.2 CURRENT CONCEPTS IN DIFFERENT SCAFFOLD MATERIALS AND PREPARATION METHODS FOR BONE TISSUE ENGINEERING**

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Scaffold-based bone tissue engineering holds great promise for the future of osseous defects therapies. Prepare the suitable scaffold properties are physiochemical modifications in terms of porosity, mechanical strength, cell adhesion, biocompatibility, cell proliferation, mineralization and osteogenic differentiation are required. We produce various bone tissue scaffolds with different techniques such as lyophilization, 3D printing and electrospinning. We wish to overview all the different novel scaffold methods and materials. To improve scaffolds poor mechanical properties, while preserving the porous structure, it is possible to coat the scaffold with synthetic or natural polymers. An increasing interest in developing materials in bone tissue engineering is directed to the organic/inorganic composites that mimic natural bone. Specifically, bone tissue is a composite of an organic and inorganic matrix. Using PLLA, loofah, chitin and cellulose biomaterials we produced bone tissue scaffold with lyophilization technique. Also, using fish scale powder and wet electrospun Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) a sponge structure had created. Using MRI image data and 3D printer method, a bone tissue scaffold is created by PLA filament. Their mechanical properties had analysed with compression tests and their biocompatibilities had investigated. In order to provide novel strategies for future treatment of bone tumours, the properties of the scaffold, including its in vitro extended-release properties, the inhibition effects of chemotherapeutic agent on the bone tumours and its bone repair capacities were investigated in vitro by using MG63 cells. To develop chemotherapeutic agent-encapsulated poly(lactic-co-glycolic acid) (PLGA) nanoparticles in a porous nano-hydroxyapatite scaffold we aimed to use double emulsion method.

**S55.3 CREATING MENISCUS CELL POPULATION WITH MESENCHYMAL STEM CELLS AND DIFFERENTIATED CELL COCKTAIL**

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Meniscus is mainly composed of three different cell types; chondrocytes(Ch) situate in the superficial zone, whereas fibroblast-like cells locate in the peripheral region having long cell extensions in contact with different parts of the matrix, fibrochondrocytes(FC), is from the inner part of the meniscus and show a clear cell associated matrix. The aim of this study is to develop meniscus cell population using with mesenchymal stem cells (MSCs). For this purpose, MSCs were isolated from rabbit bone marrow and verified by flow cytometry analyses using cell surface markers (CD73APC, CD90FITC, CD34PE, CD45PE/Cy5.5). The results indicate that CD73 and CD90-positive cells were 92.8%, and CD 45 and CD 34-negative cells were 52.4%.
Differentiation potential of MSCs were also evaluated by differentiating into Ch, osteoblasts (Ob), adipocytes (Ad), fibroblasts (Fb). Histology stainings showed that differentiated Ch can produce proteoglycans, Ob have mineralization property, Fb have spindle shape and Ad have oil drops morphology. Afterwards Fb, Ch and undifferentiated MSCs (for formation of the FC) were seeded in same plate in cocktail medium and Fb, Ch, seeded individually, were used as control group. Proliferation activity of the cells was analyzed by XTT assay at 3th, 7th and 14th days. In addition, cells were analyzed by flow cytometry with identical surface markers at 3th, 7th and 14th days. Results show that cell cocktail have the greatest proliferation ability with a greater speed than the individual Ch or Fb cultures. In addition, FC formation was identified by histological staining. In conclusion, meniscus specific cell population has been successfully generated from the cell cocktail containing rabbit MSCs.

S55.4 EFFECTS OF WEAK GLUTEAL MUSCLES AND INCREASED FEMORAL OFFSET ON MUSCLE ACTIVATIONS AND STRESSES ON FEMUR AFTER TOTAL HIP REPLACEMENT

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After total hip replacement, force generating capacity of gluteal muscles is an important parameter on joint contact forces and primary fixation of total hip replacement. Femoral offset is an option to optimize muscle moment arms, especially main abductor Gluteus Medius and Minimus. To investigate relationship with weak gluteal muscles (Gluteus Medius and Minimus) and increased femoral offset, we built a musculoskeletal model. Creating of three-dimensional femur geometry and scaling of the musculoskeletal model according to the subject were performed with computed tomography data. Obtained gait kinematic and kinetic data were applied and to mimic gluteal muscle weakness, the force generating capacities of Gluteus Medius and Minimus reduced (%20-%80). Analysis were done for both anatomical and +10mm offset. Then, muscle and joint reaction forces obtained from musculoskeletal analysis transferred to CT based finite element model to evaluate changes in maximum principle stresses on femur. According to the results of the musculoskeletal analysis, the weakness of the gluteal muscles caused an increase in the activation of Gluteus Maximus, Rectus Femoris and Tensor Fasciae Latae. Effects of +10 mm femoral offset on total abductor muscle activity increased with reduced muscle strength. As a result of the finite element analysis, no significant difference was observed for maximum principle stresses on femur with varying muscle activities. The results of these analyses are important to understand weakness of gluteal muscles and for planning hip surgery.

S55.5 PREPARATION AND CHARACTERIZATION OF NOVEL POLYDIMETHYLSILOXANE CELL SUBSTRATES TO ENHANCE OSTEOBLAST BEHAVIOR IN VITRO

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Cell micro-environment and biochemical, physical and mechanical signals coming from their micro-environment orientate specific functions of cells. In this study, we prepared novel hydrophilic and hydrophobic amino acids conjugated self-assembled molecules (AA-SAMs) modified Polydimethylsiloxane (PDMS) in order to observe the effect of hydrophathy on osteoblasts behaviour. PDMS cell substrates were prepared with a prepolymer cross linker ratio of 10:1. Hydrophobic leucine amino acid (Leu-SAM) and hydrophilic histidine amino acid (His-SAM) conjugated SAMs were produced and characterized by using 1H Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FTIR) Spectrophotometers. AA-SAMs have ethoxy surface active head group to form SAMs on plasma oxygenated PDMS and functional head group to interact
with cells. Hydrophilic 3-Aminopropyltriethoxysilane (APTS) modification was also done as a control group. Modifications of PDMS substrates were confirmed by using water contact angle measurements and X-ray Photoelectron Spectroscopy (XPS) analysis. In order to investigate cellular behaviour, as a preliminary experiment, human osteoblasts were cultured on PDMS substrates at 15,000 cells/cm² in 48 well plates with DMEM-F12 (Sigma Aldrich, D6421) medium supplemented with 10% FBS. Cell viability and proliferation were assessed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay after 1, 4 and 7 days. MTT assay showed a significant increase in cell proliferation in both AA-SAMs modified PDMS, in comparison to plain PDMS (p < 0.01). Among AA-SAMs and hydrophilic APTES, hydrophilic His-SAM modification was observed to provide a better cellular metabolic activity (p < 0.01). Hence, these novel AA-SAMs modified PDMS substrates are promising cell substrates to enhance osteoblast behaviour in vitro.

PS2.1 REGENERATIVE ENGINEERING: OPPORTUNITIES IN A NEW CONVERGENCE FIELD

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We define regenerative engineering as a convergence of advanced materials science, stem cell science, physics, developmental biology, and clinical translation. Stem cells play an important role. Work in the area of musculoskeletal tissue regeneration has focused on a number of paradigms. Polymer and polymer-ceramic systems can be utilized for the regeneration of bone. Direct induction can be controlled through material characteristics. Through the use of inducerons, small molecules fostering induction, the design of regeneration-inducing materials can be realized. We believe the medicinal use of stem cells will be of critical importance in the design of next generation systems answering grand challenges to musculoskeletal regeneration.
S56.1 NON-VIRAL GENE ACTIVATED SCAFFOLDS FOR ENHANCED BONE & CARTILAGE REPAIR

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Recent advances in tissue engineering have made progress towards the development of biomaterials with the capability for delivery of growth factors to promote enhanced tissue repair. However, controlling the release of these growth factors is a major challenge and the associated high costs and side effects of uncontrolled delivery has proved increasingly problematic in clinical orthopaedics. Gene therapy might be a valuable tool to avoid these limitations. While non-viral vectors are typically inefficient at transfecting cells, our group have had significant success in this area using a scaffold-mediated gene therapy approach for regenerative applications. These gene activated scaffold platforms not only act as a template for cell infiltration and tissue formation, but also as a ‘factory’ to provoke autologous host cells to take up specific genes and then engineer therapeutic proteins in a sustained but eventually transient fashion. Alternatively, scaffold-mediated delivery of siRNAs and miRNAs can be used to silence specific genes associated with pathological states in orthopaedics. This presentation will provide an overview of some of this research with a particular focus on gene-activated biomaterials for promoting stable cartilage formation in joint repair and on scaffold-based delivery of therapeutics for enhancing vascularization & bone repair.

S56.2 TRANSCRIPT-ACTIVATED MATRIX: SCAFFOLD BASED DELIVERY OF mRNA FOR ENHANCED BONE REPAIR

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Tissue regeneration using growth factors has disadvantages while needing to use supraphysiological growth factor concentrations. Gene therapy has been proposed as alternative. Unfortunately, drawbacks such as the use of viruses and the inefficiency of non-viral systems jeopardize clinical translation. mRNA-based transcript therapy is a novel approach that may solve plasmid DNA-based gene therapy limitations. mRNA molecules can be chemically modified in order to improve stability and immunogenicity. Chemically modified mRNA (cmRNA) is much more efficient than pDNA in delivering genes into the cell. The combination of biomaterials with cmRNA is interesting for the tissue engineering and regenerative medicine field. The resulting construct, known as Transcript-Activated Matrix, may act as a cmRNA delivery platform while supporting cell proliferation, extracellular matrix deposition and ultimately de novo tissue formation. Our work and the work of others demonstrated that the use of Transcript-Activated Matrix prolonged transgene expression and enhanced protein translation. This presentation will provide an overview of ongoing research from our group on cmRNA for improving bone repair with a particular focus on Transcript-Activated Matrix for enhancing osteogenesis. Results of our investigation in vitro with stem cells, ex vivo using tissue culture and in vivo using rat models will be presented.

S56.3 MANIPULATING BONE METABOLISM - OLD DRUGS, NEW TRICKS

C.M. Murphy
Healthy bone metabolism is a tightly coupled dynamic process that relies on a balance between bone resorption (catabolism) by osteoclasts and bone formation (anabolism) by osteoblasts. Traditionally, tissue-engineering approaches for non-union fracture repair employ local anabolic therapeutic delivery strategies that target mesenchymal stem cells (MSCs) and osteoblasts to induce bone formation, however, the challenge of healing non-union defects depends on the cause of defect e.g. trauma or disease, and targeting bone formation alone is often not sufficient. Our research focuses on utilising both anabolic therapeutics, including recombinant human bone morphogenic protein (rhBMP) -2 and parathyroid hormone (PTH)(1-34), and anti-catabolic bisphosphonates (BPs) to target bone metabolism. A major challenge with harnessing a combined dosing regimen is controlling the release of the individual therapeutics to target cells. We have developed a number of polymer-ceramic based biomaterial delivery systems, including injectable and implantable scaffolds, for the controlled release of rhBMP-2 and the BP zoledronic acid (ZA) and demonstrated their efficacy in vivo. A dual therapeutic load provided a synergistic enhancement of bone regeneration, demonstrating significantly increased bone formation and remodelling compared to anabolic therapies alone. Utilising hydroxyapatite as the ceramic phase in our scaffolds further increased bone formation, demonstrating the polymer-ceramic scaffolds to be osteoconductive in the absence of therapeutics. In addition, we have demonstrated the manipulation of bone metabolism through a specific dosing regimen of PTH(1-34), a therapeutic traditionally used as an anabolic, to induce bone remodelling and drive healing in BP loaded fractures. Our research to date has shown that optimising the delivery and regimen of anabolic and anti-catabolic therapeutics to control bone metabolism, augments the bone regenerative potential of these therapeutics in orthopaedic applications.

S56.4 IN-VIVO DEMONSTRATION OF THE SUITABILITY OF PIEZOELECTRIC STIMULI FOR TISSUE ENGINEERING

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The potential of piezoelectric biomaterials for bone tissue engineering is demonstrated. This work proves that the use of piezoelectric poly(vinylidene fluoride) (PVDF), able to provide electrical stimuli upon mechanical solicitation to the growing bone cells, enhances the bone regeneration in vivo. Poled and non-poled PVDF films, with and without macroscopic piezoelectric response, respectively and randomly oriented piezoelectric electrospun fiber mats have been used as substitutes for bone to test their osteogenic properties in Wistar rats by analyzing new bone formation in 3 mm bilateral femur defects in vivo. After 4 weeks, the qualification of the regenerated bone was performed according the H&E staining. Defect implanted with poled PVDF films demonstrated significantly more defect closure and bone remodeling, showing the large potential of piezoelectric biomaterials for bone repair, as well as for other electromechanical responsive tissues such as muscle and tendon.

S57.1 MESENCHYMAL STEM CELL ENCAPSULATION IN ALGINATE MICRO-PARTICLES FOR INTRA-ARTICULAR INJECTION IN OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative and inflammatory joint disease that affects the whole joint. Mesenchymal stem cells ability to secrete anti-inflammatory and immuno-modulatory factors represents an attractive tool in the treatment of OA. Considering the risk of cell leakage and the massive cell death upon intra-articular injection, we developed a micromolding protocol of encapsulation that allows to obtain particles that (i) could be injected with a 26G needle into a mouse joint and (ii) could provide a 3D microenvironment supporting cell biological activity. Polydimethylsiloxane (PDMS) chips containing circular micromolds were manufactured and a solution of alginate (2% w/v) containing human adipose stem cells (3 millions/mL) was deposited on the chips. Cell loading into the micromolds was performed either by sedimentation or by centrifugation. Following Ca2+ crosslinking, alginate particles (diameter 150±0.7μm) were obtained. The number of cells per particle was 5 times higher when the micromolds were loaded by centrifugation. Cell number and metabolic activity remained stable for 7 days after encapsulation and injection through a 26G needle had no impact on cell viability. When cells were stimulated with TNF-alpha and INF-gamma, prostaglandin E2 (PGE2) concentration in the supernatant was multiplied by 13 and 7 and indoleamine2,3-dioxygenase (IDO) activity was 2 and 4 times higher when cell loading was performed by sedimentation or centrifugation, respectively. We have demonstrated that encapsulated cells were able to sense and respond to an inflammatory stimulus and their therapeutic potential will be evaluated in a murine model of osteoarthritis.

**S57.2 LOADING-INDUCED BONE FORMATION: A ROLE FOR THE SKELETAL STEM CELL PRIMARY CILIUM**

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Osteoporosis affects millions globally and current anti-catabolic treatments are limited by significant side-effects. Osteoporosis arises when skeletal stem cells (SSC) no longer sufficiently replenish osteoblasts, leading to net bone loss. A key regulator of SSC behaviour is physical loading, yet the mechanisms by which SSCs sense and respond to changes in their mechanical environment are virtually unknown. Primary cilia are nearly ubiquitous ‘antennae-like’ cellular organelles that have very recently emerged as extracellular chemo/mechano-sensors and thus, are strong candidates to play an important role in regulating SSC responses in bone. This paper will demonstrate that the SSC primary cilium plays an important role in loading-induced bone formation via initial chemosensation and transduction of the potent chemokine TGFβ1 regulating SSC recruitment to the bone surface and secondly it will be shown that the primary cilium is a cAMP responsive mechanosensor directly regulating SSC mechanotransduction via localisation of adenyl cyclase 6 to the ciliary microdomain. Finally, it will be shown that targeting the cilium therapeutically can be an effective approach to enhance both biochemical and biophysically induced SSC osteogenesis contributing to bone formation, demonstrating a novel anabolic therapy for bone loss diseases such as osteoporosis.

**S57.3 DEVELOPMENT OF A NOVEL QUALITY ASSESSMENT TOOL FOR MESENCHYMAL STEM CELLS**

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Clinical translation of MSC therapies in orthopaedics has been hampered by heterogeneity and a lack of standardised and validated testing protocols for quality assurance. Although minimal criteria have been proposed, it is apparent that these do not predict performance in vivo. We used a combinatorial antibody profiling tool to probe the surface immunophenotype of human bone marrow derived MSCs and used this to define new marker panels. Cells were cultured from three marrow donors using specified expansion conditions and probed by high throughput flow cytometry using a panel of 230 antibodies. Analysis of expression of the surface proteins revealed significant variation in response to culture conditions and considerably less variation between donors. Of the panel of 230 markers 107 were negative, 24 had high expression in all samples, 1 had low expression and 98 displayed significant differences between cell preparations. Cluster analyses revealed that marker expression in one culture condition varied considerably from the other two. Phenotypic characterization of the cell preparations, assessed by analysis of differentiation propensity, showed similar patterns of variability between these samples. This suggests that the selected panel may be used as phenotypic MSC markers. Ongoing work involves the generation of novel antibody arrays which will be used as quality tests in a manufacturing environment. These tests will be used for in-process and product release applications for enhanced cell manufacturing and improved clinical outcomes.

**S57.4 TENOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS (MSC): A CO-CULTURE APPROACH**

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Mesenchymal stem cells (MSCs), characterised by their self-renewal and multidifferentiation potential, are a favoured cell population for future tissue engineering applications. Differentiation of MSCs towards a specific lineage has been extensively studied, mainly through the use of growth factors or conditioned media. However, growth factor supplementation is a mono-domain approach and considering the number of permutations, it is unlikely to find the optimal cocktail. Although PRPs are used extensively, its use is controversial, and standardization is impossible. Conditions media have various limitations, including how much, when and how effective it is at the time that it would be aspirated. Thus, co-culture systems are at forefront of scientific research and technological innovation. Co-culture system gives access to the complete cell secretome and offers the advantages of autologous therapy. However, several weeks of co-culture are necessary to observe stem cell differentiation. We hypothesize that, by using macromolecular crowding, which has been shown to recapitulate the dense in vivo microenvironment of the extracellular area and enhance matrix deposition in vitro with its excluded volume effect, it will accelerate stem cell differentiation towards tenogenic lineage. Further, we will assess if tendon specific extracellular matrix deposited by tenocytes is sufficient for stem cell differentiation without the necessity of cell contact between tenocytes and stem cells.

**S57.5 ADIPOSE MESENCHYMAL STROMAL CELLS CHARACTERISTICS ARE DIFFERENTLY MODULATED BY OSTEOARTHRITIC MILIEU**

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Mesenchymal stromal cells (MSCs) are promising candidates for cell therapy in osteoarthritis (OA) patients since they exert anti-inflammatory, immunomodulatory, anti-fibrotic, and anti-hypertrophic effects in the joint tissues. However, little is known about the OA milieu factors that could enhance the migration and tissue specific engraftment of exogenously injected MSC for successful regenerative cell therapy. GMP-clinical grade adipose stromal cells (ASC) were evaluated both in normoxic and hypoxic (2%O₂) conditions, with or without OA synovium milieu. We found that both OA synovial fluids and OA synoviocytes derived conditioned medium (CM) contain approximately the same amounts of different cytokines/chemokines (i.e., IL6, CXCL8, CXCL10, CXCL12, CCL2, CCL3, CCL4, CCL5, CCL11). ASC migration was significantly increased by both OA synovium milieu and not affected by normoxic or hypoxic condition. We identify that ASC migration was mainly influenced by different macrophage chemokines (i.e., CCL2, CCL3, CCL4). In hypoxic condition basal GMP-ASC showed an increase of CXCR3 and CCR3, a decrease of CCR1 and CCR5 receptors, while CXCR1, CXCR4, CXCR7, CCR2 and IL6R were not modulated. The addition of OA synovium milieu induced CCR3, CXCR3 and IL6R and decreased CCR1 and not affected CCR2, CCR5, CXCR1, CXCR4, CXCR7 in hypoxic condition. Our data demonstrated that GMP-ASC chemotaxis was mainly induced by macrophage chemokines. Moreover, we evidenced that hypoxia, as a better condition to mimic the OA milieu, affected some GMP-ASC cytokine/chemokine receptors, suggesting the involvement of specific chemokine-receptor axis.

S57.6 DEVELOPMENT OF A HUMAN IN VITRO MODEL OF THE NEUROMUSCULAR JUNCTION, USING IPSC-DERIVED MOTOR NEURONS AND 3D TISSUE ENGINEERING CONSTRUCTS

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Human in vitro models of the neuromuscular junction (NMJ) are currently moving from embryonic stem cells to induced Pluripotent Stem Cells (iPSCs). With this, a robust model could be optimised for physiology and pathophysiology studies, as well as representing a drug screening platform. For this reason, the work presented here represents the optimisation of a human co-culture model of skeletal muscle (hSkM)/iPSC-derived motor neurons (MNs) both in monolayer and in 3D tissue engineering collagen constructs. Firstly, human iPSC-derived motor neurons (MNs) were characterised over a period of 35 days to test their cholinergic potential. Then, primary human skeletal muscle (hSkM) and MNs were co-cultured on different substrates (gelatin and SureBond+ReadySet (Axol Bioscience)) and differentiated in various combinations of media to allow both myotube formation and neurite extension. Morphological (β-III Tubulin and Rhodamine Phalloidin) and interaction (α-Bungarotoxin and Synaptic Vesicle 2) immunofluorescent stainings were used to evaluate cell differentiation and co-localisation of pre and post-synaptic markers. Results from this study showed that the MNs presented a cholinergic phenotype up to 21 days; hSkM and MNs co-existed in culture and differentiated in neuronal Maintenance Medium (MM, Axol Bioscience); the 3D constructs allowed alignment and maturation of the muscle tissue, while providing a matrix for neurite extension and NMJ formation. This model has the potential to become a valid tool for in vitro drug screening while reducing the use of animals in research and providing the scientific community with a platform for personalised medicine.

S58.1 THE BIOLOGICAL INSIGHTS OF DISTRACTION HISTOGENESIS AND NOVEL CLINICAL APPLICATIONS

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Distraction histogenesis (DH) techniques have been widely accepted and practiced in orthopaedics, traumatology, and craniofacial surgery over the last two decades. Using DH methods, many previously untreatable conditions have been successfully managed with outstanding clinical outcomes. The biological mechanisms underlying DH have been studied and the tension-stress principles of tissue regeneration are attributed to upregulated gene expression, enhanced cell proliferation, angiogenesis and tissue remodelling and endogenous stem cell mobilization. The new methods of enhancing bone consolidation in DH are proposed and need further clinical studies. The novel applications of DH have now been extended for the treatment of vascular diseases, cranial defect (with neuronal disorders), hip and spinal deformity corrections and soft-tissue defects in addition to various bone defects and deformities. There are more surprises and novel mechanisms yet to be discovered for these novel applications of DH.

S58.2 WHY AND HOW DO LOCKING PLATES FAIL?

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Locking plates have led to important changes in bone fracture management, allowing flexible biological fracture fixation based on the principle of an internal fixator. The technique of locking plate fixation differs fundamentally from conventional plating and has its indications and limitations. Most of the typical locking plate failure patterns are related to basic technical errors, such as under-sizing of the implant, too short working length, and imperfect application of locking screws. After analysis of the fracture morphology and intrinsic stability following fracture reduction, a meticulous preoperative planning is mandatory under consideration of the principles of the internal fixator technique to avoid technical errors and inaccuracies leading to early implant failure.

S58.3 GLOBAL BURDEN OF MUSCULOSKELETAL DISEASES: A GLOBAL CRISIS IN NEED OF FUNDING

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Musculoskeletal diseases are leading causes of disability, morbidity and economic loss across the globe today. Yet for much of the world’s population access to cheap, safe and effective intervention is lacking, while others choose not to accept best practice and best evidence, or significantly more expensive treatment. Great advances have been made in some diseases like rheumatoid arthritis, but the cost of many new treatments is unaffordable, and individuals, insurance and governments struggle to, or cannot fund it. Anchor bias and politics determines national policies and research funding, often favouring other illnesses while musculoskeletal disorders lack the support proportional to their frequency and impact. This is not appreciated by policy makers and governments, and the consequences of lack of care or poor-quality care. The need has never been greater for a treatment for osteoarthritis, the most common disease in the world; but the search for a cure needs funding, and if discovered, who will pay for it?

S58.4 BIOACTIVITY OF METAL WEAR IN A MURINE IN VIVO MODEL

The biological reaction in metallosis and pseudotumor generation after metal on metal total hip arthroplasty or corroding metal implants remains unsettled. Clinically, still lethal cases appear with massive bone loss and metal ions are suspected to be responsible for this inflammatory reaction, solid metal wear particles instead are usually not observed in the common literature. The aim of this study was to compare the biological reactions of metal ions and metal wear particles in a murine in vivo model. Metal ions (CoCr), metal particles (CoCr), polyethylene particles (UHMWPE) and phosphate buffered saline (PBS) were injected into the left knee joint of female BALB/c mice. 7 days after injection, the microcirculation was observed using intravitral fluorescence microscopy, followed by euthanasia of the animals. After the assessment of the knee diameter, the knees underwent histological evaluations of the synovial layer. Throughout all recorded data, CoCr particles caused higher inflammatory reactions compared to metal ions and UHMWPE particles. The mice treated with the solid particles showed enlarged knee diameters, more intensive leukocyte–endothelial cell interactions and an elevated functional capillary density. Pseudotumor-like tissue formations in the synovial layer of the mice were only seen after the exposition to solid CoCr particles. Even if the focus of several national guidelines concerning metallosis and pseudotumor generation is on metal ions, the present data reveal that solid CoCr particles have the strongest inflammatory activity compared with metal ions and UHMWPE particles in vivo.

S59.1 TOWARDS A BIOPHYSICAL FRAMEWORK FOR MESENCHYMAL STEM CELLS FATE CHOICES
V. Prasad Shastri, M. Sarem

Mesenchymal stem cells (MSCs) have been long studied for their role in skeletal development. MSCs are unique in adult physiology in that they exhibit pluripotency and differentiate into cells that can evolve into various skeletal tissue as a result have been extensively employed as a viable alternative to terminally differentiated cells in engineering of cartilage and bone tissue ex vivo and in vivo. In spite of decades of effort in this direction, our understanding of what drives MSC fate choices is rather narrow in that it places heavy emphasis on a role for morphogens and cytokines (TGF-beta super family, FGF-2). In recent years it has become evident that MSCs also play an important role in wound healing, immunomodulation (immune suppression) and in tumour progression. However, what becomes of an MSC when it arrives at or exits an environment is less understood. We hypothesize that activation of differentiation programs in MSCs have an autocrine and paracrine component involving interplay between MSC-MSC (cell-cell contact) and MSC-(environment), and in this signalling paradigm the biophysical aspects of their microenvironment play a dominant role. We have tested this premise in several aspects of MSC behaviour (proliferation, migration, differentiation, chondrogenesis) and have gathered compelling evidence for biophysics and mechanobiology in MSC fate decisions. This talk will present some of our latest findings in this broad arena.

S59.2 ENGINEERING THE SHAPE AND BEHAVIOUR OF MESENCHYMAL STROMAL CELLS THROUGH BIOPHYSICAL CUES
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As stem cells and primary cells hold potential for improving disease outcomes and patient lives, methods for steering cell fate are of considerable importance. In this context, an emerging method is directing cell function through controlling cellular shape. The talk will discuss how cell functions are based on mechano-transduction events related to the balance of intra- and extracellular forces. The talk will explore the multiple biophysical cues that affect cell shape and present methods for directly generating cell shape, e.g. micro-contact printing used for directing the differentiation lineage of stem cells. Based on our own work, the talk will introduce the novel concept that specific biomaterial types and stiffnesses can be chosen for generating specific cellular “baseline shapes” and associated function. As our cells are exposed to continuously changing biomechanical forces, the talk will also report how specific forces can be used for engineering shape. The talk will explore how biomaterial stiffness and biomechanical forces act together on cellular shape, and whether one of the two stimuli is able to override the other. The novel insights reported here are fundamental for designing cell shape-instructive 3D biomaterials in the context of steering cell function in situ for regenerative medicine.

S59.3 3D OSTEOCHONDRAL MICROPHYSIOLOGICAL SYSTEMS: FROM CARTILAGE-BONE CROSSTALK, TO SCREENING REGENERATIVE APPROACHES, TO SPACE RESEARCH

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Cartilage-bone interactions play a critical role in joint diseases and the osteochondral junction has been identified as a locus of osteoarthritis development. However, it is challenging to study osteochondral (OC) interaction in vitro, since cartilage and bone require very different environments. We developed a new medium-to-high throughput osteochondral microphysiological system bioreactor to culture biphasic native or engineered constructs and that can be used to study any musculoskeletal tissue interfaces. We developed engineered constructs from hMSCs on a porous polymeric matrix with a gradient in pore size to assess the supportive effect of the local topology on cartilaginous and osseous differentiation. Furthermore, we developed a triphasic, vascualized osteochondral constructs based on porous polycaprolactone and methacrylated gelatin scaffolds to study the specific effects of vasculature on cartilage and bone. We also cultured native OC tissues from postmenopausal women, exposing either cartilage or bone to sex hormones studying their protective effects. Finally, our bioreactor is being implemented for use on the International Space Station to study countermeasures against microgravity bone loss. Overall, our bioreactor maintains media separation for in vitro culture and engineering of OC tissues and constructs of progressively greater complexity, and it preserves the possibility of direct cartilage-bone crosstalk opening new opportunities to study interactions across the osteochondral junction.

S59.1 4 ART NANOPATTERNING TO DESIGN ADVANCED BIOACTIVE INTERFACES FOR TISSUE REGENERATION

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The successful application of smart implantable devices requires materials used to easily adapt and respond to their microenvironment via physical and chemical cues. Nanotopography, a known important factor in cellular processes (i.e. cellular adhesion, proliferation, and, differentiation), has become a central approach to imparting clinically relevant materials with bioactive and biomimetic properties. This work focuses on the use of Directed irradiation synthesis (DIS), to create nanostructures on dissimilar materials including surfaces of metals, semiconductors, and polymers. DIS is a novel method that allows for the tuning of both surface nanoscale topography and surface chemistry through the tailoring of ion beam parameters, including energy and fluence. The application of DIS to direct cellular interactions on Ti6Al4V, MgAZ31, and PEEK is presented. Topography and chemistry changes at the nanoscale were characterized by SEM, XPS, AFM, and Contact Angle. *In vitro* tests were performed using macrophages (JJ741A) and human aortic and bone marrow mesenchymal stem cell (MSCs). DIS promotes an advanced cell adhesion state where cells are orientated following the designed nanofeatures in all irradiated specimens. A delay on immune response due to low levels of TNFa and higher levels of IL10 on irradiated Ti6Al4V were observed. Modified PEEK showed 3-fold higher ALP content at 7 days compared to pristine samples, and porous MgAZ31 treated with DIS revealed lower corrosion state and increased cell proliferation of HBMMSCs. Controlling the nanopatterning in biomaterials using DIS enables the design of bioactive surfaces to highly promote implant integration and tissue regeneration.

**S60.1 THE FUTURE OF BIOACTIVE GLASSES IN BONE TISSUE ENGINEERING**

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Bioactive glasses were first discovered in the late 1960s by Larry Hench. In the 1980s and 1990s bioactive glasses experienced a surge of research interest, an interest which has since declined. This talk will examine the current status of bioactive glasses and discuss future roles and applications for bioactive glasses in regenerative medicine, specifically those related to orthopaedic tissue engineering. Bioactive materials are often considered as those that have the ability to bond to mineralised bone tissue in the physiological environment, however, this talk, as well as examining this aspect, will consider the broader sense of bioactive as ‘having or eliciting a biological effect’. It will examine the role of bioactive glasses as active drug carriers and the influence which enhanced nanotechnology will have on the application of bioactive glasses *in vivo*.

**S60.2 SILATED HYDROGELS IN BONE REGENERATIVE MEDICINE**

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20 years ago, we designed injectable bioactive suspensions in water of calcium phosphate ceramics for bone and periapical regenerations. Because of leakage of these suspensions, we focused on injectable hydrogels before to set in situ by chemical crosslinking to form 3D scaffolds. We set up a platform to develop a series of innovative hydrogels for bone, cartilages and periodontal tissue regeneration. We based our strategy on polysaccharides macromolecules because they are renewable materials, that originate from biological sources and generally are biocompatible, non-toxic and biodegradable. We developed a family of silated macromolecules able to react forming biocompatible hydrogels. The silated polymers are self-setting hydrogels able to covalently crosslink under pH variation, without addition of toxic crosslinking agent. All
these macromolecules could be combined in multicomponent hydrogels, representing a strategy for improving mechanical properties of biomaterials or to tailor particular properties to meet specific needs. For mineral scaffolding, we realized composites of calcium phosphates particles or cements with hydrogel, increasing the ductility and creating macroporous scaffold to propose foam bone cements well adapted to bone biomaterials and Bone tissue engineering. Perspectives are 3D printing and bio printing techniques. We will use our hydrogels platform to prepare tunable (bio)inks in skeletal medicine.

S60.3 DEVELOPING AND TESTING NOVEL DELIVERY SYSTEMS FOR GLUTAMATE RECEPTOR ANTAGONISTS FOR THE TREATMENT OF JOINT PAIN AND DISEASE

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The AMPA/kainate glutamate receptor (GluR) antagonist NBQX reduced bone destruction when injected intra-articularly, in rat antigen induced arthritis (AIA) and is similarly protective in rodent models of osteoarthritis. NBQX reduced bone turnover \textit{in vivo} and reduced mineralization in human primary osteoblasts (HOBs) \textit{in vitro}. We are developing sustained release GluR antagonist delivery methods, to improve therapeutic effect. DNQX loaded Poly(lactic-co-glycolic acid) (PLGA) nanoparticles were synthesized via double emulsion. DNQX loaded thermosetting hydrogels were synthesised by dissolving Pluronic-F127 (22\% w/v) and Carbopol 934 (0.5\% w/v) in dH\textsubscript{2}O, homogenising with DNQX/NBQX and set in dialysis cassettes at 37\°C. Supernatants from nanoparticles and hydrogels suspended in PBS (37\°C) were analysed using high performance liquid chromatography to determine drug release. Y201 MSCs were differentiated to osteoblasts (DMEM+10\% FBS, Dexamethasone, β-Glycerophosphate and Ascorbic acid-2-phosphate) in sustained presence/absence of NBQX (200μM) or DNQX (200 and 400μM). Alizarin red staining quantified mineralisation at 14 days. Nanoparticles encapsulated 2.5mM DNQX (encapsulation efficiency=22\%) and released encapsulated drug over 4 weeks. Hydrogels released 2.5mM DNQX load over 24 hours in 37\°C PBS. Y201 alizarin red staining was significantly reduced by both DNQX (p<0.01) and NBQX (p<0.05), compared to untreated controls. PLGA nanoparticles and hydrogels revealed different sustained release profiles. Sustained treatment with GluR antagonists reduced mineralisation in Y201 derived osteoblasts, consistent with effects of NBQX in HOBs. Sustained release of NBQX and DNQX in nanoparticles and hydrogels may improve efficacy of AMPA/kainate GluR antagonists in reducing bone remodelling and enhancing their bone protective potential in the treatment of joint disease.

S60.4 A NOVEL INJECTABLE BONE ALLOGENIC SUBSTITUTE FOR SKELETON REGENERATIVE MEDICINE

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Skeletal sequels of traumatisms, diseases or surgery often lead to bone defects that fail to self-repair. Although the gold standard for bone reconstruction remains the autologous bone graft (ABG), it however exhibits some drawbacks and bone substitutes developed to replace ABG are still far for having its bone regeneration capacity. Herein, we aim to assess a new injectable allogeneic bone substitute (AlloBS) for bone reconstruction. Decellularized and viro-inactivated human femoral heads were crushed then sifted to obtain...
cortico-spongious powders (CSP). CSP were then partly demineralized and heated, resulting in AlloBS composed of particles consisting in a mineralized core surrounded by demineralized bone matrix, engulfed in a collagen I gelatin. Calvarial defects (5mm in diameter, n=6/condition) in syngeneic Lewis1A rats were filled with CSP, AlloBS±TBM (total bone marrow), BCP (biphasic calcium phosphate)±TBM or left unfilled (control). After 7 weeks, the mineral volume/total volume (MV/TV) ratios were measured by μCT and Movat’s pentachrome staining were performed on undemineralized frontal sections. The MV/TV ratios in defects filled with CSP, AlloBS or BCP were equivalent, whereas the MV/TV ratio was higher in AlloBS+TBM compared to CSP, AlloBS or BCP (p<0.01; Mann-Whitney). Histological analyses exhibited a collagen-rich matrix in all the defects, and osteoid at the surface of all implanted biomaterials. Our data indicates that AlloBS is a promising candidate for bone reconstruction, with ease of manipulation, injectability and substantial osteogenic capacity. Further experiments in larger animal models are under consideration to assess whether AlloBS may be a relevant clinical alternative to ABG.

S60.5 CHARACTERIZATION OF OVINE COLLAGEN OBTAINED FROM DIFFERENT TIMES OF HYDROLYSIS


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The hydrolysed collagen has a molecular weight of 3-6 KDa, is soluble in water, colourless and odourless. Hydrolysed collagen was obtained by proteolysis of the native ovine collagen. The enzymatic treatment was carried out with Heliozym under alkaline treatment (pH 8) for different periods of time (0 min, 10 min, 20 min, 30 min, 1 h, 2h, 3h y 4h) at 60°C. The hydroxyproline concentration increased significantly from time 0 min (11.44±2.81 mg/L) to the 4 h (24.47±1.60 mg/L); this change in concentration was observed in the FTIR spectra at a length of 1,037 cm⁻¹ for OH group as well a change in the Amide I (1641 cm⁻¹). The viscosity showed significant differences (P≤ 0.05) between the different hydrolysis times. This parameter was correlated with the molecular weight; when the viscosity was 0 cP the molecular weight showed the lowest value at 5.62 KDa. The antioxidant activity for ABTS radical scavenging showed significant differences (P≤ 0.05) between the times of hydrolysis, the greater the time, the higher the inhibition resulting with 67.61% at the end of the treatment. The DPPH radical scavenging resulted with 27.89 % at the beginning of the hydrolysis. However, the end of the hydrolysis (4h) showed inhibition at 52.75%. The antioxidant activity increased when molecular weight decreased, and this is related with the amino acids present in the peptides obtained for the hydrolysis of the collagen.

S60.6 PRO-ANGIOGENIC NEAR INFRARED-RESPONSIVE HYDROGELS FOR PATTERNING THE EXPRESSION OF THERAPEUTIC TRANSGENES

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As near-infrared (NIR) photothermal agents, copper sulfide nanoparticles (CuSNP) offer several advantages over plasmonic gold nanoparticles (GNP), the most widely used photothermal nanotransducers in biomedical applications. CuSNP exhibit strong optical absorption at NIR wavelengths (650-1100 nm) and convert it into heat due excitation of electronic transitions or plasmonic photoexcitation. In contrast with GNP, CuSNP are
degradable, readily prepared, inexpensive to produce, efficiently cleared from the body and their photothermal efficiency is less sensitive to the dielectric constant of the surrounding medium. We explored the feasibility of CuSNP to function as degradable NIR nanotransducers within fibrin-based cellular scaffolds, paying great attention to the stability and photothermal efficiency of the composite. We tested in vitro and in vivo whether NIR-responsive fibrin hydrogels comprising CuSNP (CuSNP hydrogels) are reliable platforms for triggering transgene expression in cells harboring a gene circuit activatable by heat and dependent of rapamycin. NIR laser irradiation of the CuSNP hydrogels increased local temperature and, in the presence of rapamycin, triggers the gene switch based on the promoter of the highly heat-inducible HSP70B gene (HSPA7). After implantation of such a cell-containing CuSNP hydrogel, transgenic expression can be remotely triggered by NIR-irradiation. Interestingly, we found that CuSNP hydrogels induce remodeling activity in stem cells and stimulate an angiogenic response. In short, CuSNP hydrogels offer compelling features for tissue engineering applications, as fully degradable implants with enhanced integration capacity in host tissues that can provide for remote control in the deployment of therapeutic gene products.

S61.1 MULTI-SCALE, MULTIDISCIPLINARY RESEARCH INTO BONE MECHANOBIOLOGY DURING NORMAL PHYSIOLOGY AND OSTEOPOROSIS TO ENHANCE BONE REGENERATION AND THERAPEUTIC APPROACHES

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While the phenomena of bone adaption to mechanical loading has been long observed, the mechanisms governing bone mechanotransduction during health and disease are not well understood. Our multidisciplinary experimental and computational research strives to enhance understanding of bone mechanobiology, and in particular how this process is affected at the onset of osteoporosis. We have provided an enhanced understanding of bone cell mechanosensation. We have characterised the local mechanical environment of MSCs, osteoblasts and osteocytes in vivo. Most importantly, we have discovered that the matrix composition, expression of mechanosensors and the mechanical environment of osteocytes is altered during osteoporosis. Interestingly, a mechanobiological response restores the homeostatic mechanical environment of the cells in the longer term. Our recent in vitro studies have revealed that estrogen withdrawal from bone cells alters calcium signalling, mineralisation, biochemical responses and osteogenic gene expression when these cells are exposed to an applied fluid shear stress. Our ongoing research is investigating mechanobiology-based therapeutic approaches for treatment of bone pathologies, by (1) targeting mechanoregulatory signalling pathways and (2) developing in vitro tissue regeneration strategies that seek to optimise the mechanical environment (through matrix stiffness, bioreactors) to stimulate osteogenesis.

S61.2 CHARACTERISATION AND DELIVERY OF PRO-OSTEOGENIC VESICLES: A NEW ACELLULAR APPROACH TO BONE TISSUE ENGINEERING?

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By combining cells, biological factors, and biomaterials the field of tissue engineering has generated technologies capable of supporting regeneration. However, the regulatory hurdles associated with the use of cell-based therapies often hinder translation. Consequently, to meet the growing demand for regenerative technologies new approaches are needed. Emerging evidence suggests that cell-derived extracellular vesicles (EVs) are critical in cell-cell communication and regulation of bone formation. This talk will explore the role
of osteoblast EVs in directing stem-cell differentiation in-vitro. EVs were isolated from cell culture media by ultracentrifugation and profiled for size and composition using a range of techniques. Notably, proteomic analysis revealed the presence of calcium channelling annexins and bridging collagens that may be key to their role in mineralisation. To minimise the concentration of EVs required to induce a pro-osteogenic effect we propose that they may be locally delivered. Opportunities to incorporate these pro-osteogenic EVs into injectable biomaterials will be discussed, in particular the formulation of microcapsules and fluid-gels. In summary, incorporation of EVs in tissue-engineered scaffolds has the potential to deliver all the advantages of a cell-based therapy but without using viable cells. The advantages of this approach may represent a new phase of tissue engineering.

S61.3 COMPARISON OF SYSTEMIC AND LOCAL ADMINISTRATION OF BISPHOSPHONATES IN AN ANIMAL BONE DEFECT MODEL: THE IMPORTANCE OF LOCAL DRUG DELIVERY

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Nitrogen-containing bisphosphonates such as Zoledronic Acid (ZA) are used clinically for the treatment of skeletal diseases related with increased bone resorption. The gold standard is to administrate the drug through a systemic pathway, however this is often associated with high dosages, risk of side-effects, reduced site-specific drug delivery and hence, limited drug-effectiveness. A controlled local drug delivery, via a biomimetic bone graft, could be beneficial by direct and time-regulated application of significantly lower drug dosage at the site of interest. Thus, higher efficacy and reduced side-effects could be expected. In this experimental in vivo study, we examined the effect of ZA when used together with a Calcium Sulphate/Hydroxyapatite biomaterial in a femoral condyle bone defect in rats and compared local to systemic administration. The following groups were used: group1: empty defect (no biomaterial & no treatment), group2: biomaterial alone, group3: biomaterial + systemic ZA (0.1mg ZA/kg – single subcutaneous injection), group4-6: biomaterial conjugated with ZA at different concentrations, (0.07 to 0.70 mg ZA/mL of paste, corresponding to 0.0024 to 0.024 mg ZA/kg). The animals were sacrificed at 6 weeks and toxicological examination was performed. Bone regeneration was evaluated using qualitative and quantitative micro-CT analysis and Histomorphometry. The results showed a significant difference between the groups, suggesting that ZA has an overall effect on bone healing. The most pronounced effect was seen with the local application of approximately 10 times less ZA-dosage when compared to systemic use (p<0.001). This study demonstrates the importance of local ZA administration in bone regeneration.

S61.4 ESTROGEN DEPLETION ALTERS OSTEOGENIC DIFFERENTIATION AND MATRIX PRODUCTION BY MECHANICALLY STIMULATED OSTEOBLASTS IN VITRO

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Osteoporosis has long been associated with weak bones but recent studies have shown that bone tissue mineral becomes more heterogeneous and the expression of mechanosensors are altered during estrogen deficiency in an animal model of osteoporosis. However, whether these changes occur as a primary response to estrogen deficiency is unknown. In this study we investigate whether matrix production and mineralisation by mechanically-stimulated osteoblasts are impaired as a direct consequence of estrogen depletion. Osteoblast-
like MC3T3-E1 cells were cultured for 14 days with $10^{-8}$M of 17β-estradiol and subsequently cultured with osteogenic media only, or supplemented with estrogen or an estrogen antagonist (Fulvestrant, $10^{-7}$M). Physiological shear stress (1Pa) was applied using an orbital shaker (290rpm, 40min/day), which allows long-term culture and induces oscillatory flow on cells. Osteoblasts phenotype, extracellular matrix (ECM), mineralisation and mechanosensors were tracked by qRT-PCR (Runx2, Col1a1, Col1a2, Cox2, Bglap2, FN1), by biochemical assays (ALP activity, DNA and calcium content), by immunostaining (integrin $\alpha_v$, BSP2, fibronectin) and by labelling with calcein the calcium. The results of this study demonstrate that after 7 days, estrogen depleted cells had less integrin $\alpha_v$ mechanosensors compared to those that received continuous estrogen treatment. By 14 days the ECM formation (calcium, fibronectin) by osteoblasts was altered under estrogen depletion, when compared to cells that were cultured continuously with estrogen. This study provides evidence of changes in osteoblast behaviour under estrogen depletion, which might explain the alteration in tissue mineral content and the decrease of integrins observed previously in ovariectomized rats \textit{in vivo}.

S61.5 ENDOPLASMIC RETICULUM MEDIATES MITOCHONDRIAL TRANSFER IN THE OSTEOCYTE DENDRITIC NETWORK


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Osteocytes are terminally differentiated long-lived cells and account for greater than 95% of the bone cell population. It has been established that osteocytes are connected through their highly developed dendritic network, which is necessary for the maintenance of optimal bone homeostasis. However, little is known on how osteocytes use the network to coordinate their cellular function and communication that requires energy and protein turnover. Here using super-resolution confocal imaging on both live and fixed osteocytes, we demonstrated conclusively that mitochondria are widely distributed and dynamically shared between osteocytes. Using confocal live cell imaging analysis we showed that inhibiting the contact between mitochondria and endoplasmic reticulum (ER) by the knockdown of MFN2 in osteocytes impedes the transfer of mitochondria suggesting the involvement of ER contact with mitochondria in the transfer process. Moreover, we showed that transport of mitochondria between osteocytes within the network enables rescue of osteocytes with dysfunction of mitochondria. Using the 3D tetra culture system with confocal imaging, we identify the transfer of mitochondria from healthy osteocytes enables recovery of mitochondria activities in osteocytes that devoid of mitochondrial DNA by ethidium bromide. The results indicated that when osteocytes are depleted of functional mitochondria, normal parental osteocytes can transfer mitochondria to these stressed osteocytes to provide them with energy. Collectively we show for the first time that the utilisation of mitochondrial transfer enables osteocytes to function with a network and coordinate their cellular activities in response to different energy demands.

S61.6 EFFECT OF LONG-TERM NICOTINE EXPOSURE ON BONE MINERAL DENSITY

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To prevent bone loss, OPG/RANK/RANKL signalling pathway is a key in keeping the balance between the action of osteoblasts and osteoclasts. Aim of this study is to assess the influence of long-term nicotine exposure...
on bone mineral density (BMD) scores, RANKL and OPG levels of plasma and RANKL and OPG immunoreactivities of tissue in rats. In this study, totally 36 Swiss Albino rats (70±10 g) were used in three groups. Whereas normal drinking water was given for the control group (n:12), 0.4 mg/kg/day and 6.0 mg/kg/day nicotine was added to drinking water for low-dose nicotine (LDN) group (n:12) and high-dose nicotine (HDN) group (n:12), respectively for 12 months. At the end of 12th month, BMD scores were measured via X-ray absorptiometry and then bone turnover was assessed via measuring both RANKL, OPG levels in plasma and RANKL, OPG immunoreactivities in tail vertebrae of all rats. Lumbar spine and femoral regions BMD scores of the control group and the nicotine groups were not significantly difference. In HDN group, OPG levels of plasma were found significantly higher when compared with the control and LDN groups (p=0.001) unlike RANKL levels of plasma. RANKL and OPG immunoreactivities of tissue were found significantly lower in both LDN and HDN groups (p<0.001, p=0.004, respectively) in comparison to control group. No correlation was found between plasma levels and tissue immunoreactivities of RANKL and OPG. As a result, this study indicates that nicotine is not primarily responsible for the decline of BMD frequently seen in smokers.

S61.7 POST-TRAUMATIC OSTEONECROSIS OF THE TIBIAL PLAFOND, A CLINICAL ENTITY TO RECOGNIZE. A CASE SERIES AND LITERATURE REVIEW

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Osteonecrosis is a potentially devastating condition with poorly defined pathogenesis that can affect several anatomical areas with or without a previous traumatic insult. Post traumatic osteonecrosis (PON) in the foot and ankle has been commonly described in the talus and navicular but rarely in the distal tibia. PON of the distal tibia is a rarely reported and infrequent complication of fracture dislocations of the ankle. Its scarcity can lead to misdiagnosis and inappropriate management due to a lack of clinical knowledge or suspicion with resultant severe functional compromise. We aim to highlight the clinical and radiological features of PON of the distal tibia and report the findings in a series of four patients following a fracture dislocation of the ankle. Three patients sustained a SER4 fracture dislocation and one patient sustained a PER4 fracture dislocation in keeping with standard patterns of injury seen in most trauma units. In each case, PON of the distal tibia presented with progressive anterolateral tibial plafond collapse and valgus deformity of the ankle. The radiological features previously reported in the literature are based on plain film x-ray, CT and MRI but no description of SPECT-CT findings. One of the patients in the series underwent SPECT-CT following clinical suspicion of PON and thus we describe the findings not previously reported. Our objective is to highlight this rare condition as a potential cause for ongoing pain following fracture dislocation of the ankle as well as advocating the use of SPECT/CT as a useful imaging modality to aid in the diagnosis.

S61.8 EXPERIMENTAL STUDY TO INVESTIGATE A POTENTIAL MODEL FOR IMPROVED OSSEOINTEGRATION IN SICKLE CELL BONE DISEASE PATIENTS WITH AVASCULAR NECROSIS

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It is well documented that implant loosening rate in sickle cell disease patients is higher than that seen in patients with hip arthroplasty from other indications. The Hypoxic inducible factor (HIF) - is activated in the microcellular hypoxic environment and this through a cascade of other enzymatic reactions promotes the
activity of other factors and further help enhance angiogenesis and osteogenesis. The aim of this study was to investigate and propose a potential model for investigating osseointegration in a hypoxic microcellular environment using osteoblasts (MG63).

Human MG63 osteoblastic cells were cultured under normoxia and hypoxic conditions (20%; and 1% oxygen saturation) for 72 hours under two different conditions: with and without cobalt chloride. The samples cultured under normoxic conditions without cobalt chloride acted as control. Using qualitative polymerase chain reaction (qPCR) - HIF expression was assessed under the above conditions in relation to the control. The results showed there was significant expression of the HIF 1 alpha protein under hypoxic condition with cobalt chloride in comparison with the control samples - all at 72 hours incubation. Mann-Whitney U test was used to deduce level of significance of fold change. (p=0.002; <0.05). This was deemed as being a significant difference in the level of expression of HIF compared to the control.

The results showed that the hypoxic inducible factor can be expressed using the above tested experimental invitro-model with significant results which can be a foundation for further research into improving hip implant prosthesis design to help enhance osseo-integration in sickle cell disease patient with AVN.

S61.9 RESTORING THE SUPERIOR BONE HEALING CAPACITY OF CHILDREN IN ADULTS BY DESIGNING SCAFFOLD-BASED THERAPIES THAT HARNESS AGE-ALTERED JNK3 ACTIVATION IN STEM CELLS

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Side-effects associated to the use of bone morphogenetic proteins into scaffold-based devices for bone repair highlight the necessity for identifying new therapeutic targets that potentially improve bone healing in adults. In this sense, we recently demonstrated the age-associated decrease in the mechanosensitivity of bone marrow mesenchymal stromal/stem cells (MSCs) and identified c-Jun N-terminal kinase 3 (JNK3) as a mechanically-activated modulator of the superior osteogenic potential of MSCs derived from children (C-MSCs) in comparison to adults (A-MSCs). Building on this work, the aim of this study was to design a JNK3-activated collagen-nanohydroxyapatite (coll-nHA) scaffold that restore the child bone healing capacity in adults. Results revealed that JNK3 activator (JNK3*) enhanced A-MSC alkaline phosphatase (ALP) activity to the same extent of C-MSCs by facilitating the activation of JNK3. Moreover, A-MSCs cultured on the coll-JNK3* scaffold (collagen-scaffold containing JNK3*) showed positive uptake of the JNK3*, upregulation of early osteogenic markers as well as increased ALP activity and mineralization. More importantly, rat critical calvarial defects treated with coll-JNK3* for 28 days showed a significantly higher 18.07% bone volume fraction in comparison to rats treated with Coll-nHA -6.04% - and empty defects -2.58%. Which correlated with the presence of a larger amount of blood vessels and mineralized tissue in samples treated with coll-JNK3* when compared with coll-nHA and empty defects. In conclusion, the coll-JNK3* capacity to enhance osteogenesis and bone healing by activating JNK3 highlights how by understanding the stem cell mechanobiology we can improve the development of next generation therapeutics for tissue repair.

S62.1 MSC BASED THERAPY FOR SEVERE OSTEOARTHRITIS OF THE KNEE: THE ADIPOA EXPERIENCE

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Adipose derived mesenchymal stromal cells (ASC) are adult stem cells exhibiting functional properties that have opened the way for cell-based clinical therapies. Primarily, their capacity of multilineage differentiation has been explored in a number of strategies for skeletal tissue regeneration. More recently, MSCs have been reported to exhibit immunosuppressive as well as healing capacities, to improve angiogenesis and prevent apoptosis or fibrosis through the secretion of paracrine mediators. Among the degenerative diseases associated with aging, osteoarthritis is the most common pathology and affects 16% of the female population over 65 years. Up to now, no therapeutic option exists to obtain a sustainable improvement of joint function beside knee arthroplasty. This prompted us to propose adipose derived stem cells as a possible cell therapy. We performed pre-clinical models of osteoarthritis and showed that a local injection of ASC showed a reduction of synovitis, reduction of osteophytes, joint stabilization, reducing the score of cartilage lesions. This work was completed by toxicology data showing the excellent tolerance of the local injection of ADSC and biodistribution showing the persistence of cells after 6 months in murine models. The aim of the ADIPOA trial is to demonstrate the efficacy of adipose derived stem cells therapy in knee osteoarthritis (OA) in a phase 2/3 controlled multicenter study controlled against standard of care. Safety and feasibility as well as dose response was previously assessed in the ADIPOA FP7 project. The bi-centric phase I clinical trial in Montpellier (France) and Würzburg (Germany) included 18 patients with moderate to severe knee OA, each patient received a single injection of autologous ADSC, in a open scale up dose trial, starting form 2 \times 10^6 cells to 50 \times 10^6 cells. The 10^7 dose appears to be well tolerated and showed preliminary response in terms of decreasing local inflammation. This first study confirmed the feasibility and safety of local injection of ADSC in knee OA and suggested the most effective dose (10^7 autologous ADSC). This work constituted a significant step forward treating this disease with ADSC to demonstrate safety of the procedure. We conduct a prospective multicenter randomized Phase 2/3 study with 86 patients with moderate to severe knee OA to demonstrate superiority of stem cell-based therapy compared to standard of care (SOC) in terms in reduction in clinical symptoms (WOMAC score) and structural benefit (assessed by T1rhoMRI that allow quantification of cartilage proteoglycan content). This project will offer EU a unique leadership in OA with strong positions in EU and US due to patents and quality of the methodology to demonstrate efficiency of ADSC. ADIPOA brings together a unique combination of expertises and leaders in clinical rheumatology, MRI specialists, Stem cell Institutes, national GMP grade adipose derived stem cell production platform (ECELLFRANCE) and SME specialized in cell therapy trials in the EU. The production of the cells will be granted to EFS through ECELLFRANCE national platform, which have the GMP facility and will work as a contracting manufacturing organization. The expertise, leadership and critical mass achieved by this Consortium should enable breakthroughs in ASC engineering directly amenable for clinical applications in OA.

**S62.2 MSC/ASC SAFETY AND POTENCY ASSAYS: WHERE WE STAND?**

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Among all stem cell based clinical trials in the world, most of them are related to Mesenchymal Stem Cells whatever the tissue origin. Over time, the uses of cultured cells have increased greatly, particularly since 2009. Cells derived from adipose tissue are also increasingly used in trials compared with bone marrow cells. No real specificity emerged as to the therapeutic uses of the different types of stem cells and the more than half the MSC studies concerned allogeneic MSCs. With the maturation of this field, the requirements of relevant safety and potency cell control assays are now absolutely required for the future phase III and IV but quite different according to the autologous or allogenic setting. If for autologous setting, such assays have to be defined to identify MSC batches not to inject (for safety or lack of efficacy), in allogenic setting, potency assays are required to select the best donor with the maximum of safety. Up to now, most of assays are based...
on pre-clinical animal studies but need to be largely improved for a better relevance and accuracy. Their development stumbles on two difficulties: MSC themselves and our limited knowledge of their pleiotropic action mechanisms in conjunction with MTI regulatory rules. This indicates that we have to move from simple tests to multi-modal and combinatorial approaches. We propose to discuss and illustrate these different points in view of the different clinical trials and how they inform us.

S62.3 SWITCHING OF THE PRO-INFLAMMATORY PROFILE OF SYNOVIAL OSTEOARTHRITIC MACROPHAGES BY ADIPOSE MESENCHYMAL STROMAL CELLS

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Synovitis has been shown to play a role in pathophysiology of OA promoting cartilage destruction and pain. Synovium is mainly composed of synovial fibroblast (SF) and macrophage (SM) that guide synovial inflammation. Adipose stromal cells (ASC) promising candidate for cell therapy in OA are able to counteract inflammation. Two different subsets of macrophages have been described showing a pro-inflammatory (M1) and an anti-inflammatory (M2) phenotype. Macrophage markers: CD68, CD80 (M1-like) and CD206 (M2-like) were evaluated in osteoarthritic synovial tissue. GMP-clinical grade ASC were isolated from subcutaneous adipose tissue and M1-macrophages were differentiated from CD14+ obtained from peripheral blood of healthy donors. ASC were co-cultured in direct and indirect contact with activated (GM-CSF+IFNγ)-M1 macrophages for 48h. At the end of this co-culture we analyzed IL1β, TNFα, IL6, MIP1α/CCL3, S100A8, S100A9, IL10, CD163 and CD206 by qRT-PCR or immunoassay. PGE2 blocking experiments were performed. In moderate grade OA synovium we found similar percentages of CD80 and CD206. M1-activated macrophage factors IL1β, TNFα, IL6, MIP1α/CCL3, S100A8 and S100A9 were down-modulated both co-culture conditions. Moreover, ASC induced the typical M2 macrophage markers IL10, CD163 and CD206 by qRT-PCR or immunoassay. PGE2 blocking experiments showed that TNFα, IL6, IL10, CD163 and CD206 were significantly modulated by PGE2. We confirmed the involvement of PGE2/COX2 also in CD14+ OA synovial macrophages. In conclusion we demonstrated that ASC are responsible for the switching of activated-M1-like to a M2-like anti-inflammatory phenotype, mainly through PGE2. This suggested a specific role of ASC as important determinants in therapeutic dampening of synovial inflammation in OA.

S62.4 EXOSOMES AND MICROPARTICLES RELEASED BY MESENCHYMAL STEM CELLS EXERT A CHONDROPROTECTIVE EFFECT IN OSTEOARTHritis

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Mesenchymal stem cells (MSC) are multipotent cells that possess regenerative functions that are of interest for in osteoarticular diseases such as osteoarthritis (OA). These functions are thought to be primarily mediated by mediators released within extracellular vesicles (EV). The aim of this study was to compare the immunomodulatory effects of two major types of EV, exosomes and microparticles, secreted by MSCs. EV subsets were isolated from murine primary MSCs by ultracentrifugation. Size and structure were evaluated by Dynamic Light Scattering and electron microscopy. Expression of membrane and endosomal markers was tested by flow cytometry. Proliferation of murine splenocytes was quantified after 72h of incubation with EVs after CFSE-labelling. Phenotypic analysis of T lymphocyte subpopulations was also performed by flow cytometry. In vivo, EVs were injected in the knee joint in the collagenase-induced osteoarthritis (CIOA) model.
and histological score was performed. *In vitro* functional analysis indicated that addition of microparticles or exosomes in proliferative assays inhibited the proliferation of total splenocytes in a dose-dependent manner. Analysis of T cell subpopulations revealed a decrease in CD8^+IFNγ^+ lymphocytes and an increase in both CD4^+IL10^+ T1r and CD4^+CD25^+FOXP3^+ Treg cells. This immunomodulatory function of EVs was also observed *in vivo* in the CIOA model. In summary, our data indicated that the immunosuppressive effect of MSCs is in part mediated by exosomes and microparticles that play *in vivo* a major role in MSC-mediated therapeutic effect by reducing osteoarthritic symptoms.

**S62.5 STIMULATION OF CALVARIAL BONE HEALING WITH HUMAN BONE MARROW STROMAL CELLS VERSUS INHIBITION WITH ADIPOSE TISSUE-DERIVED STROMAL CELLS ON NANOSTRUCTURED β-TCP-COLLAGEN**


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Bioactive functional scaffolds are essential for support of cell-based strategies to improve bone regeneration. Adipose-tissue-derived-stromal-cells (ASC) are more accessible multipotent cells with faster proliferation than bone-marrow-derived-stromal-cells (BMSC) having potential to replace BMSC for therapeutic stimulation of bone-defect healing. Their osteogenic potential is, however lower compared to BMSC, a deficit that may be overcome in growth factor-rich orthotopic bone defects with enhanced bone-conductive scaffolds. Objective of this study was to compare the therapeutic potency of human ASC and BMSC for bone regeneration on a novel nanoparticulate β-TCP/collagen-carrier (β-TNC). Cytotoxicity of β-TCP nanoparticles and multilineage differentiation of cells were characterized *in vitro*. Cell-seeded β-TNC versus cell-free controls were implanted into 4 mm calvarial bone-defects in immunodeficient mice and bone healing was quantified by μCT at 4 and 8 weeks. Tissue-quality and cell-origin were assessed by histology. β-TNC was non-toxic, radiolucent and biocompatible, lent excellent support for human cell persistence and allowed formation of human bone tissue by BMSC but not ASC. Opposite to BMSC, ASC-grafting significantly inhibited calvarial bone healing compared to controls. Bone formation progressed significantly from 4 to 8 weeks only in BMSC and controls yielding 5.6-fold more mineralized tissue in BMSC versus ASC-treated defects. Conclusively, β-TNC was simple to generate, biocompatible, osteoconductive, and stimulated osteogenicity of BMSC to enhance calvarial defect healing while ASC had negative effects. Thus, an orthotopic environment and β-TNC could not compensate for cell-autonomous deficits of ASC which should systematically be considered when choosing the right cell source for tissue engineering-based stimulation of bone regeneration.

**S62.6 THE EFFECT OF BFGF ANF HYPOXIC PRE-CONDITIONING ON CXCR4 AND SDF-1 EXPRESSION AS TARGETS FOR HOMING ENHANCEMENT OF CANINE AT-MSCS**

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Mesenchymal stem/stromal cells (MSC) have the ability to home and migrate towards injured and inflamed tissues which can be useful as a minimally invasive systemic approach to deliver MSC to the site of damaged articular surface in arthritis in human and veterinary patients. From a molecular point of view, the CXCR4/SDF-1 plays an important role in this phenomenon and can be used as a target to enhance the
therapeutic efficacy of culture expanded MSC. It has been demonstrated that extensive in vitro expansion down-regulates CXCR4 expression in human, murine and canine MSCs hindering their therapeutic efficacy. Therefore, the aim of the present study was to assess the effect of hypoxia and basic fibroblast growth factor (bFGF) pre-conditioning on CXCR4 and SDF-1 expression in canine adipose derived MSC (cAT-MSC). MSC were isolated from subcutaneous adipose tissue of two adult Beagle dogs (n=2; 3-5 years old, 9-12kg) and cultured under standard conditions (5%CO2, 37°C). Cells at passage 3 were then cultured in hypoxia (2%O2) and normoxia, with supplementation of 1 and 5 ng/ml bFGF for 24h. MTT assay, flow cytometry, immunohistochemistry and qRT-PCR analysis were conducted to assess respectively the modulation effect on cell proliferation, CXCR4 protein expression and CXCR4 and SDF-1 gene expression. Cell proliferation increased proportionally with the increasing bFGF concentrations, with a statistically significant higher proliferative rate in normoxic conditions (p<0.05). The gene expression of CXCR4 and SDF-1 increased in hypoxic conditions with bFGF supplementation (p<0.05). bFGF supplementation increased cytoplasmatic expression of CXCR4 in hypoxic conditions (p<0.05), however the surface expression remained low in all culture conditions. The described pre-conditioning method can be used for the enhancement of the therapeutic potential of systemically administered canine AT-MSC and can have a relevant translational character for the optimization of culturing protocols of human adipose derived MSC.

S62.7 ROLE OF BONE MINERAL PHASE IN PATHWAY CHOICE FOR BONE FORMATION BY HUMAN MESENCHYAL STEM/STROMAL CELLS

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Bone formation proceeds through two distinct processes. One involves the deposition of bone by osteoblasts (intramembranous ossification) and another through the remodeling of an intermediate cartilaginous matrix formed by chondrogenic differentiation of mesenchymal stem/stromal cells (MSCs) aggregates – a process called endochondral ossification (EO). EO is responsible for formation of long bones during development and most prevalent during fracture repair upon callus formation. In adult bone injuries MSCs from periosteum form bone via EO whereas MSCs from bone marrow are primarily differentiate to osteoblast in vivo. We hypothesized that the unique biophysical and biochemical properties of bone mineral phase has a role in programming MSCs. Using a biomimetic bone like apatite (BBHAp) as surrogate for bone mineral phase, we studied the chondrogenic differentiation of human marrow derived MSCs and observed that the BBHAp dictates MSCs fate and strictly dictates the pathway of bone formation in vivo. Through exhaustive dissection of the signaling pathways at play, a prominent role of PTH1R in modulating the effects imposed by the BBHAp has been unraveled. These fundamental insights gained in how bone microenvironment might alter fate of MSCs has important implications for bone repair and regeneration therapies.

S62.8 LOOK AT THE FUTURE OF CELLULAR THERAPIES - FROM THE PERSPECTIVE OF LABORATORY INVOLVED IN DEVELOPMENT AND CLINICAL TESTING OF ATIMP

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Cell-based therapies have taken the emerging field in many clinical directions. Among them, orthopaedic surgery is one of the most promising directions – due to the clinical needs, and because of the availability of the advanced cell-based constructs dedicated to bone and cartilage regeneration. The current practical clinical input is, however, below expectations – because of numerous difficulties which have their source in scientific, practical, finance and legal issues. Regarding legal issues, Advanced Therapy Investigational Medicinal Products (ATIMP) are regulated by three different legal orders. As medicines (according to the EU law, ATIMP is a pharmaceutical) – they are subject to pharmaceutical law; as cell-containing specimens – to cell and tissue banking regulations; as tested by registered clinical trials - they are subject to Good Clinical Practice rules and regulations. Formal requirements coming from these three areas are completely different, sometimes contradictory and incompatible with the specific nature of cell-based products. At the same time they involves the need for huge financial expenditures. We discuss these issues from the perspective of the university laboratory, which currently conducts clinical trials of the ATIMPs for three different clinical indications and, at the same time, has experience in the basic and applied scientific work at the laboratory level – towards improvement of osteogenic capacity of stem cells. With the undoubtful need of well documented scientific results, which is accompanied by complicated and imperfect regulations, we think that the scientific community focused around cellular therapies is now facing challenges that may determine the future of this field.

**S62.9 IMMUNOMODULATORY PARACRINE EFFECT OF IMMOBILIZED MESENCHYMAL STEM CELLS IN A HYALURONIC ACID HYDROGEL ON CHONDROCYTES IN VITRO**

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Osteoarthritis (OA) is an inflammatory degenerative disease that affects every fourth person with irreversible damage to the articular. Mesenchymal stem cells (MSCs) have been shown to affect host cells by paracrine stimulation in regenerative environments. Here we apply hyaluronic acid (HA), an essential part of the extracellular matrix in cartilage, for MSC immobilization. The aim was to investigate long-term MSC survival and paracrine effect on chondrocytes in an inflammatory co-culture environment. We hypothesized that MSCs immobilized in a HA hydrogel could provide a long-term immunomodulatory effect on chondrocytes in vitro. Human MSCs were seeded in a HA hydrogel and co-cultured with non-osteoarthritic human chondrocytes in biphasic wells inhibiting cellular contact. An inflammatory environment was induced by IL1-beta and compared with standard culture medium. Relative gene expressions of collagen types I, II and X, aggrecan, SOX9, MMP-13 and ADAMTS-5) were examined at day 3, 7, 14 and 28. Significant up-regulation of SOX9 at day 7, 14 and 28 and a significant down-regulation of ADAMTS-5 (day 14 and 28) was observed with co-culture of HA-immobilized MSCs and MSCs compared with controls with or without HA (without MSCs)No changes in expression was observed for aggrecan and collagen type 1. We showed that MSC affect the expression of SOX9 and ADAMTS-5 in a paracrine manner when co-cultured with chondrocytes in an inflammatory environment. MSCs immobilized in HA hydrogels survived and were contained in the hydrogel for up to 28 days. This suggests that HA-immobilized MSCs could potentially be used as adjuvant treatment of OA.

**S63.1 EARLY INTERVENTION THERAPIES FOR CARTILAGE LESION REPAIR**

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Cartilage lesions occur as a result of joint trauma, and progressively degenerate over time leading to osteoarthritis (OA). Early intervention therapies to repair the initial tissue damage have the potential to delay or prevent the onset of OA. We have developed two acellular treatments; 1) an injectable proteoglycan-like self-assembling hydrogel for the repair of ICRS grade 1 lesions, and 2) a decellularised xenogeneic osteochondral scaffold for surgical grafting in grade 2-4 lesions. We produced an in vitro glycosaminoglycan depleted grade 1 lesion model using porcine cartilage. Peptide-chondroitin sulphate mixture was injected and spontaneously gelled in situ. Cartilage resistance to deformation was increased by 50%. Decellularised porcine osteochondral scaffolds which maintain the native tissue composition and architecture whilst being immunocompatible were successfully developed and are currently undergoing in vivo assessment in an ovine critical size condylar defect model. Incorporation of the subchondral bone in osteochondral scaffolds is intended to improve osseointegration; implanted decellularised bone-only scaffolds in sheep exhibited superb osteoinductive and osteoconductive properties in a proof-of-concept study. We envisage that our early intervention therapies will be employed clinically to maintain or restore functional hyaline-like cartilage across the whole range of early chondral pathologies and prevent the onset of OA.

S63.2 PRE-CLINICAL TESTING OF BIOLOGICAL THERAPIES FOR THE INTERVERTEBRAL DISC USING WHOLE ORGAN BIOREACTORS

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In recent years, novel therapies for intervertebral disc (IVD) regeneration have been developed that are based on the delivery of cells, biomaterials or bioactive molecules. The efficacy of these biological therapies depends on the type and degree of IVD degeneration. Whole organ culture bioreactors provide an attractive platform for pre-clinical testing of IVD therapeutics, since the cells are maintained within their native extracellular matrix, and the endplate remains intact to fulfil its function. Moreover, defined regimes of mechanical stress are applied to the IVD, representing either physiological or degenerative, detrimental loading. Different degrees of degeneration can be induced by high load, low nutrition, enzyme injection, and/or mechanical damage; while recent organ culture models also implement an inflammatory component. Using whole organ culture models, we found that mesenchymal stem cell injection into nucleotomized IVDs had an anabolic effect on the IVD cells. Furthermore, hyaluronan hydrogels were beneficial for cell delivery and mechanical support. We also found that anti-inflammatory treatment could partially prevent the induction of cytokines in an inflammatory model. However, chemokine delivery did not induce a significant repair response in an annulus fibrosus defect. In line with 3R principles, relevant ex-vivo models are essential to reliably test biological IVD treatments.

S63.3 WEIGHT-ADAPTIVE COLLAGEN-POLYLACTIDE SCAFFOLD IN CARTILAGE REPAIR IN A PORCINE MODEL

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Articular cartilage repair is assumed to improve by covering the cartilage lesion with a biomaterial scaffold tailored to the specific requirements of the weight-bearing joint surface. We have tested the feasibility of a novel composite collagen-polylactide scaffold rhCo-PLA in cartilage repair. To confirm these results and further challenge the scaffold, we tested it in a large porcine cartilage defect. A critical-sized full-thickness chondral defect was made in the medial femoral condyle of 18 domestic pigs. This technically widest possible
defect size of 11×17 mm was determined in a pilot test. Five weeks later, the defect was either treated with the novel rhCo-PLA scaffold or left untreated to heal spontaneously. After four months, the medial condyles were evaluated macroscopically using Goebel’s score, in which the worst possible result receives a total of 20 points and imaged with μCT to evaluate subchondral bone. Macroscopic score and subchondral bone microstructure were similar in both study groups. The total Goebel score was higher in spontaneous group (9.75±3.9 for spontaneous and 9.1±3.7 for rhCo-PLA, respectively) but differences between individual animals were large. Subchondral bone volume fraction was 48.2±3.6% for rhCo-PLA and 44.2±3.4% for spontaneous. Trabecular thickness was greater in operated joints (207.9±18.8 μm for spontaneous and 242.9±32.9 μm for rhCo-PLA) than in contralateral non-operated joints (193.3±15.1 μm and 213.4±33.2 μm, respectively). These preliminary data demonstrate that individual differences in the macroscopic appearance were large but there were no significant differences between the two study groups in the score or subchondral bone structure.

S63.4 THE EFFECT OF PRIMING ON MATRIX ACCUMULATION AND METABOLISM OF STEM CELLS AND CHONDOCYTES IN ALTERED INTERVERTEBRAL DISC-LIKE PH CONDITIONS

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Intervertebral disc (IVD) degeneration presents a harsh microenvironment characterised by low glucose, low oxygen and matrix acidity posing a significant challenge for cell-based therapies. The objective of this work was to assess the effect of primed bone marrow derived stem cells (BMSC) and articular chondrocytes (AC) in different pH (7.1, 6.8 and 6.5) conditions and assess metabolic activity in terms of oxygen (O2) and glucose consumption as well as lactate production. Secondly, we investigated pH effects on cell viability and matrix accumulation capacity. Primary cells were encapsulated in alginate beads and cultured in disc-like conditions (5% O2, 5mM glucose, pH 7.1, 6.8 and 6.5). For growth factor priming, cells were cultured with 10ng/ml TGF-β3 at a pH of 7.4 for 14 days prior to being subjected to acidic pH conditions. AC exhibited superior cell viability and sGAG deposition compared to BMSC at all pH levels which was further enhanced after priming. Priming also reduced O2 consumption of AC for all pH conditions while lactate production profiles of both cell types were altered with decreasing extracellular pH. This work demonstrates the importance of cell type selection to sustain disc-like microenvironmental conditions. Results show that BMSCs that have not been primed may need additional factors to sustain the harsh acidic microenvironment. In contrast, AC were capable of sustaining the low pH conditions better than BMSC and accumulated more similar disc-like matrix in all conditions. Overall this study highlights that AC may be advantageous for disc regeneration and warrant further investigation for disc repair.

S63.5 MICRORNA-29ª ALLEVIATES OSTEOPHYTE DEPOSITION AND SUBCHONDRAL DAMAGE IN KNEE OSTEOARTHRITIS BY REDUCING MINERALIZATION OF CHONDOCYTES

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Osteophyte deposition and subchondral bone damage are notable features of osteoarthritis (OA). Deregulated mineralization contributes to osteophyte and subchondral irregularity. The microRNA-29 (miR-29) family is
associated with arthritic disorders. This study is aimed to investigate miR-29a function to OA osteophyte formation and subchondral integrity. Intact and damaged articular cartilage in patients with end-stage knee OA who required total knee arthroplasty were harvested to probe miR-29a, cartilage, and mineralized matrix expression using RT-PCR and in situ hybridization. Osteophyte volume and subchondral morphology of collagenase-induced OA knees in mice were quantified using μCT and histomorphometry. Increased bone matrix expression (collagen I and bone alkaline phosphatase) and reduced cartilage matrix (collagen II and aggrecan) along with low miR-29a expression existed in human OA specimens. Aged miR-29a knockout mice showed spontaneous osteophyte formation and articular cartilage erosion. In primary articular chondrocytes, miR-29a deficiency significantly reduced cartilage matrix synthesis, whereas von Kossa staining-positive mineralized matrix production was increased. Of interest, the severity of collagenase-induced osteophyte accumulation and subchondral damage along with serum cartilage breakdown products CTX-II and COMP levels were significantly compromised in mice overexpressing miR-29a. Intra-articularly injecting miR-29a significantly reduced osteophyte volume and subchondral integrity and retained cartilage morphology in collagenase-injured knees. Reduced miR-29a signalling worsens osteophyte and subchondral destruction in OA through increasing mineralized matrix formation of chondrocytes. Restoring miR-29a shields joints from cartilage degradation, osteophyte and subchondral destruction. This study conveys new mechanistic underlying OA osteophyte pathogenesis and shines light on the remedial potential of miR-29a to OA.

S63.6 THE GLYCOMIC PROFILE OF THE INTERVERTEBRAL DISC IN HEALTH AND DEGENERATION FOR BIOMATERIAL FUNCTIONALISATION

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Discogenic low back pain affects 42% of patients suffering low back pain. Degenerative disc disease is described as failure in cellular response to external stresses leading to physiologic dysfunction. Glycosylation patterns of tissues give insights into the spatially and temporally regulated inflammatory and degenerative processes. These glycoconjugates participate in many key biological processes including molecular trafficking and clearance, receptor activation, signal transduction, and immunomodulation. We hypothesise that glycoprofile of the the intervertebral disc(IVD) is temporally and spatially distinct in health and degeneration. The glycoprofile of the IVD has been studied in murine, bovine and ovine models for injury and aging. In this study, healthy(n=2) and degenerated(n=2) human IVD samples received from Utrecht(UU, ND) with ethical approval(NUIG), were compared using lectin histochemistry. The N-glycan profile of human degenerated IVD samples was characterised by high resolution quantitative UPLC-MS. Healthy and degenerated human discs present distinct glycosylation trends intracellularly/extracellularly in annulus fibrosus(AF) and nucleus pulposus(NP) tissue. There are quantitative and spatial differences in glycosylation in healthy and degenerated tissue. These findings are consistent with previous studies of IVD in murine, bovine and ovine models. The human N-glycan profile of degenerated surgical tissues is distinct from other cited tissue profiles such as human plasma. These studies offer validation of previous animal models of IVD injury and degeneration, demonstrating similar changes in the glycoprofile in both animals and humans. Glycoprofiling may offer insight into disease progression, offering new realms of disease classification in patient specific manner while also elucidating potentials therapeutic targets, inhibiting disease progression.

S63.7 OSMOREGULATION OF AQUAPORIN 1 AND 5 IN NUCLEUS PULPOSUS CELLS

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The IVD is a highly hydrated, hyperosmolar tissue that allows the correct biomechanical function of the spine. When degenerated, water and ions are lost from the disc, especially within the central nucleus pulposus (NP), producing a hypoosmotic environment in which the resident cells can no longer function correctly, exacerbating the degenerative cascade. One potential way that IVD cells may adapt to their environment is through the expression and regulation of aquaporin (AQP) channels that control the movement of water in and out of cells. During human IVD degeneration AQP1 and 5 expression is decreased, highlighting AQPs may be of importance for the correct function of NP cells. The regulation of AQPs in NP cells by healthy and degenerate conditions, and the potential underlying molecular mechanisms, were investigated in both human and rat IVD cells. The gene and protein expression of AQP1 and AQP5 was upregulated by hyperosmotic conditions (425mOsm/kg H2O) in rat and human NP cells. Lentiviral knockdown of tonicity enhancer binding protein (TonEBP), a transcription factor responsible for maintaining the function of NP cells, resulted in the loss of AQP1 and 5 gene expression under hyperosmotic conditions. The maintenance of the IVD environment and adaptation of cells is vital for the function of the IVD. The regulation of AQPs by physiological conditions and TonEBP suggests a role for these water channels related to the adaptation of disc cells to their environment, which is dysregulated during degeneration.

S63.8 A HYALURONAN-BASED HYDROGEL SYSTEM FOR ANNULUS FIBROSUS REPAIR

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The degeneration of the intervertebral disc (IVD) is the primary cause for low back pain, which is treated with surgical interventions such as spinal fusion. A strategy to develop a regenerative and non-invasive treatment requires an injectable cell carrier system. Our efforts have focussed on developing a hyaluronan (HA)-based hydrogel system that can be used as a carrier for therapeutic agents in annulus fibrosus (AF) repair. High molecular weight HA at 20mM is chemically crosslinked with varying concentrations of 4-arm PEG. Hydrogels were optimised for degree of crosslinking, stability and rheological properties. Subsequently, the morphology and viability of the human AF cells encapsulated in the hydrogels were studied. The highest crosslinking was seen with 4-arm PEG at 1:1 HA:PEG molar ratio. This was the most stable against enzymatic and hydrolytic degradation, and had greater swelling property, which is desired as the degeneration decreases the water retention capability of the IVD. The gelation time, important for in situ injectability, was under five minutes for all formulations. Storage modulus was between 0.4-1.1 kPa. Compared to 2D cultures, cells were rounder after encapsulation, mimicking the native microenvironment, and had the similar metabolic activity for seven days. AF cells encapsulated in HA/4-arm PEG hydrogel were stiffer compared to the nucleus pulposus (NP) cells encapsulated similarly as measured with Brillouin microscopy. The 4-arm PEG crosslinked HA-based hydrogel system promises to be a candidate for an injectable carrier for cells for AF repair and regeneration.

S63.9 DIRECTING STEM CELL DIFFERENTIATION INTO THE CHONDROGENIC LINEAGE BY SERUM-FREE CONDITIONS AND MACROMOLECULAR CROWDING

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Phenotypic drift of stem cells and insufficient production of extracellular matrix (ECM) are frequently observed in tissue-engineered cartilage substitutes, posing major weaknesses of clinically relevant therapies targeting cartilage repair. Microenvironment plays an important role for stem cell maintenance and differentiation and therefore an optimal chondrogenic differentiation protocol is highly desirable. Macromolecular crowding (MMC) is a biophysical phenomenon that accelerates biological processes by several orders of magnitude. MMC was recently shown to significantly increase ECM deposition and to promote chondrogenic differentiation of stem cells. We hypothesise that the addition of sulphated high-molecular weight polysaccharides (carrageenan) to the media positively affects stem cell maintenance and chondrogenic differentiation. Herein, we venture to assess the impact of MMC on the maintenance of stem cell phenotype and multipotency, and ECM deposition in xeno-free human bone marrow mesenchymal stem cell (BMSCs) cultures. We investigate different xeno- and serum-free stem cell media with MMC for expansion of BMSCs, assessing multipotency maintenance (FACS analysis), cell viability, metabolic activity, proliferative capacity and matrix deposition (SDS-PAGE, ICC) at day 4 and day 10. Experiments will be conducted at 2 different passages (p3, p7). Medium without MMC will be used as control. Based on these results, cells expanded with the best protocol will be subsequently investigated for chondrogenic differentiation comparing different xeno-/serum-free and serum containing differentiation media. Chondrogenic differentiation will be assessed via Alcian blue and Safranin O stainings, gene expression for chondrogenic marker genes and quantification of GAG content. Finally, these findings will pave the way for developing more effective strategies for cartilage tissue engineering.

S64.1 THE IMPORTANCE OF MATRIX STRUCTURE AND STIFFNESS FOR THE MECHANOBIOLOGICAL BEHAVIOUR OF CELLS IN THE HEALTH AND DISEASE OF TENDONS

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All cells exist within a 3D microenvironment where they are exposed to a multitude of mechanobiological cues, from nano-level cell/matrix interactions, to tissue-level mechanical strain. These cues are fundamental to maintaining tissue homeostasis, but when disrupted during disease, can promote pathological outcomes and impair healing. This is particularly true in tendons; 3D load bearing connective tissue structures composed of a complex arrangement of matrix proteins, organised in a highly aligned manner and maintained by tendon cells (tenocytes). When diseased or injured (termed tendinopathy), the tendon begins a journey of poor healing, characterised by mechanically inferior disorganised scar tissue which ultimately results in compromised or total loss of function. In both health and disease, the mechanobiological stimuli experienced by tenocytes will directly affect their behaviour, yet this is a poorly studied area of research. We have used decellularised tendon slices to mimic the structure of healthy tendon, and induced degradation to mimic tendinopathic tendon. We have re-seeded these slices with tenocytes or immune cells and are building a greater picture of the role that the structure and stiffness of the matrix has on cell behaviour in health and disease.

S64.2 LIGAMENT STRUCTURE AND FUNCTION: HOW DOES IT RELATE TO DISEASE?

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Ligaments and tendons are vital musculoskeletal soft tissues, which are commonly injured due to overuse and trauma. Their distinct functions are well known however their unique structure and biochemical composition and how they change with disease is poorly described. The most commonly injured ligament in the dog and man is the cranial cruciate (CCL) and anterior cruciate ligament (ACL) respectively. Therefore, the structure, function and pathophysiology of disease of this ligament has been most commonly studied in both species. Canine cranial cruciate ligament rupture (CCLR) most commonly occurs following gradual ligament degeneration or disease (CCLD) followed by a non-contact injury or a minor trauma. Several studies have described marked degenerative histological changes in ligament structure prior to and following rupture which consist of loss of the collagen fascicular structure, areas of poor collagen fibril staining, a marked increase in “chondroid” type cells and mineralisation. The ECM protein profile is also altered with increased sulphated glycosaminoglycans content, increased immature collagen cross-links as well as enzymes involved in collagen remodelling. In man, similar findings have been described in the ACL with age and in osteoarthritis (OA). Previously it had been thought that ligament degeneration occurred following OA but these more recent studies suggest that ligament degeneration can lead to joint destabilisation and OA. Being able to determine early degenerative ligament changes in spontaneous clinical cohorts and the mechanisms which cause them are ideal starting points to determine targets for future therapies in the prevention of ligament degradation and rupture. Further identification of ligament cell types in terms of degenerative, responsive and regenerative (stem) types is essential to try and alter ligament cellular and extracellular matrices harnessing their therapeutic potential.

S64.3 USING MECHANICALLY LOADED PIEZOELECTRIC BIOMATERIALS TO DEFINE THE CELL MICROENVIRONMENT

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Electromechanical coupling (piezoelectricity) is present in all living beings and provides basis for sense, thoughts and mechanisms of tissue regeneration. Herein, we ventured to assess the influence of MMC in mesenchymal stem cell culture. In this study, we fabricated piezoelectric regenerative scaffolds to assess the role of electromechanical stimulation on tendon regeneration. Tendon cells were selectively stimulated in vitro by mechanical or electromechanical cues using non-piezoelectric or piezoelectric scaffolds and optimal mechanical loading (4% deformation at 0.5 Hz). This was followed up with an in vivo study to assess tendon regeneration in a rat Achilles tendon injury model. P(VDF-TrFE), scaffolds were observed to mimic the fibrous structure of tendon tissue (figure 1) and were capable of producing electrical charges up to 17 pC/N when mechanically loaded (figure 1. Genes associated with tendon specific markers (Col.I/Col III, Scx and Mnx) and mechanosensitive ion channels such as PIEZO1, TRAAK and TRPV1 were significantly upregulated (figure 2). The upregulated genes were validated with individual real time Q-PCR and bioinformatics revealed a possible regulated function. Those results were further validated in vivo. Protein expression of repaired tendons showed a correlation between increase in expression of tendon related proteins SCX, TNMD, Decorin and expression of ion channels KCNK2, TRAAK and TRPV1. Collectively, these data clearly illustrate that scaffolds made of PVDF-TrFE can produce electrical charges when mechanically loaded. Moreover, gene and protein analyses showed a positive regulation of tendon specific markers through activation mechanosensitive voltage-gated genes.

S64.4 PLATELET LYSATE CELL-LADEN HYDROGEL COATED SUTURE THREADS FOR TENDON REPAIR

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Tendon injuries constitute a major healthcare burden owing to the limited healing ability of these tissues and the poor clinical outcomes of surgical repair treatments. Recent advances in tendon tissue engineering (TTE) strategies, particularly through the use of biotextile technologies, hold great promise toward the generation of artificial living tendon constructs. We have previously developed a braided construct based on suture threads coated with gelMA:alginate hydrogel encapsulating human tendon cells. These cell-laden composite fibers enabled the replication of cell and tissue-level properties simultaneously. Based on this concept, in this study we explored the use of platelet lysate (PL), a pool of supra-physiological concentrations of growth factors (GFs), to generate a hydrogel layer, which is envisioned to act as a depot of therapeutic factors to induce tenogenic differentiation of encapsulated human adipose stem cells (hASCs). For this purpose, commercially available suture threads were first embedded in a thrombin solution and then incubated in PL containing hASCs. Herein, thrombin induces the gelation of PL and consequent hydrogel formation. After coating suture threads with the mixture of PL-ASCs, cells were found to be viable and homogeneously distributed along the fibers. Strikingly, hASCs encapsulated within the PL hydrogel layer around the suture thread were able to sense chemotactic factors present in PL and to establish connections between adjacent independent fibers, suggesting a tremendous potential of PL cell-laden hydrogel fibers as building blocks in the development of living constructs aimed at tendon repair applications.

**S64.5 EXTENSOR TENDON PROXIMAL PHALANX DORSAL SHAFT ATTACHMENT CONTRIBUTION TO FINGER EXTENSION**

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Extensor tendon attachment to the dorsum of the proximal phalanx may fully extend the finger metacarpal phalangeal joint (MPJ). 15 fresh-frozen cadaveric hands were axially loaded in the line of pull to the extensor digitorum communis of the index, middle, ring and small finger at the level just proximal to the MPJ. We measured force of extension at the MP joint in 3 groups: 1) native specimen, 2) extensor tendon release at the proximal interphalangeal (PIP) joint with release of lumbricals/lateral bands, 3) extensor tendon release at the PIP joint and dorsal proximal phalanx and lumbrical/lateral band release. Degree change of extension was calculated using arctan function with height change of the distal aspect of the proximal phalanx, and the length of the proximal phalanx. We used Student T-test to determine significant decrease in the extension of the phalanges. Extension of all fingers decreased slightly when the extensor tendon were severed at the PIP joint with release of the lateral bands/lumbricals (8deg+/−2deg). After this release, the finger no longer extended. Slight loss of extension was not statistically significant (p >.05) between group 1 and group 2. Groups 1 and 2 were significantly different compared to group 3. In summary, distal extensor tendon transection and release of lateral bands/lumbricals resulted in little change in force and degree of finger extension. The distal insertion of the extensor, released when exposing the PIP joint dorsally, may not need to be repaired to the base of the middle phalanx.

**S64.6 EXPLORATION OF THE LYMPHOCYTE POPULATION IN MIDPORTION ACHILLES TENDINOPATHY**

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Achilles tendinopathy is classically defined as a tendinosis devoid of an inflammatory cell population. However, recent literature suggests inflammation as a mediator in the pathogenesis. These findings were mainly based on semi-quantitative immunohistochemistry. We therefore used flow cytometry to obtain a more accurate identification and quantification of the different cell types involved. Thirty-two samples were obtained from twelve patients with chronic tendinopathic lesions undergoing Achilles tendon surgery. Samples obtained from three patients with hemiplegia requiring surgical release due to spastic Achilles tendons served as control. We used two panels to identify the myeloid and lymphoid population targeting the following markers: CD45, CD3, CD4, CD19, CD11b, CD56, CD14, CD16, Va7.2, 6b11, CD161, TCRγδ. To assess the presence of fibroblasts CD90 was targeted. The mean count of CD45+ hematopoietic cells in the tendinopathic samples was significantly higher than in the control samples, respectively 13.27% and 3.24% of the total cell count (P<0.001). The mean fraction of CD3+ cells present in the complete cell population was significantly higher in pathological samples than in control samples, respectively 1.70% and 0.37% (P<0.05). Presence of CD19+ B cells was not reported. The mean fraction of γδ T cells was significantly higher in tendinopathic samples compared to blood samples of the same patient and consisted of 12.9% and 5.8% γδ T cells respectively (P<0.05). These findings support an inflammatory cell infiltration in midportion Achilles tendinopathy that show similarities to enthesitis in SpA. This implies a potential target to investigate in novel treatment modalities.

S64.7 CELLULAR CHANGES IN IMMORTALIZED HUMAN ANTERIOR CRUCIATE LIGAMENT-DERIVED FIBROBLASTS AND ITS APPLICABILITY FOR TISSUE ENGINEERING

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Cultured primary cells have a limited life span and undergo dedifferentiation. Tissue engineering (TE) approaches require high cell numbers, but availability of human derived cells is limited and animal cells show inter-species differences. The advantages of immortalized cells are delayed senescence and phenotypic stability. The present study was undertaken to validate key properties of immortalized human anterior cruciate ligament (ACL) fibroblasts in direct comparison with non-immortalized cells from the same donor to assess their applicability as TE model. Human ACL ligamentocytes (40 years old female donor) were either immortalized using repeated transient transfection with a simian virus SV40 plasmid or remained untreated. Both cell populations were analyzed for cell survival, DNA content, tendon marker, extracellular matrix (ECM) and cytoskeletal protein expression. Cell spheroids of both populations were seeded on scaffolds embossed either from polylactic acid (PLA) threads alone or combined PLA- and PLA-co-caprolacton-(P(LA-CL)) threads, functionalized with fluor treatment and collagen foams. Cell survival on the scaffolds was monitored for up to 5 weeks. In contrast to non-immortalized ligamentocytes, immortalized cells reflected some chaotic and incomplete cell divisions, higher DNA content, numbers of dying cells and nucleoli, reduced vimentin and vinculin-associated focal adhesions. Analysed markers, other cytoskeletal and ECM components were similarly expressed. Compared to the non-immortalized ligamentocytes immortalized formed instable spheroids and died on the scaffolds after 21 d. Both cell populations reflected superior growth on the PLA-P(LA-CL) compared with PLA scaffolds. Immortalized cells share crucial properties with their non-immortalized counterparts, but TE is only possible for limited culturing periods.

S64.8 ULTRASONOGRAPHY OF THE LATERAL LIGAMENT IN ANKLE SPAINS
Acute lateral ankle sprain accounts for 85% of sprains. The lateral sprain is associated with other ligament injuries e.g. medial and syndesmosis sprain. Long-term, approximately 20% of acute lateral sprains develop into chronic lateral ankle instability (CLAI) which includes persistent pain, and recurring ankle sprains. This study evaluated the grade of an ankle ligament injury by ultrasonography (US) and compared the findings to the outcome of patient-reported questionnaires. 48 subjects (18-40 years) diagnosed with an ankle sprain attended a clinical and US examination of ankle ligaments within two weeks after the sprain. Evaluation was done by US of acute lateral ligament injuries (ATFL, CFL), syndesmosis injury (AiTFL), and medial injury (dPT, TCt) only in participants with the positive clinical signs of medial injury. Participants were then mailed a questionnaire (PROMQ) every third month for a year. 29 women and 19 men participated with a mean age at 26.50 years. One-year follow-ups need to be analyzed further for final results. Temporary results include data based on the initial 26 patients: Two clinical signs statistically correlated. Multiple logistic regression analysis confirmed the results. Positive palpated tenderness AiTFL predicted with partial ruptured ATFL and reported pain during active plantar flexion of ankle predicted with normal CFL confirmed by the US. Patients with partial rupture of ATFL presented with tenderness at AiTFL point. Patients presenting with intact CFL reported pain during active plantar flexion. Compared to the US findings, the overall examinations were inconclusive in predicting ATFL, CFL, AiTFL, and medial ligament injuries.

S64.9 THE SYNERGISTIC EFFECT OF TOPOGRAPHY AND SUBSTRATE RIGIDITY IN THE DEVELOPMENT OF A COLLAGEN SCAFFOLD FOR TENDON TISSUE ENGINEERING

I. Sallent, D.I. Zeugolis

Collagen scaffolds are generally characterized by their random fibre distribution and weak mechanical properties, which makes them unsuitable as substitutes for highly anisotropic tissues such as cornea or tendon. Recently, we developed a technique to create collagen type I scaffolds with well-defined anisotropic micro-patterns. Porcine collagen was mixed with PBS10X, NaOH and one of the following cross-linkers: glutaraldehyde (GTA), genipin and 4-arm polyethylene glycol (4SP). The resulting mixture was casted on micro-grooved (2x2x2 μm) polydimethylsiloxane (PDMS) moulds and allowed to dry in a laminar flow hood to obtain 5mg/ml collagen films. Different pH, temperatures (T°), and cross-linker concentrations were tested in the process. Collagen gelation kinetics was analysed with rheometry and surface topography was assessed with scanning electron microscopy (SEM). Human bone marrow stem cells (HBMSCs) were seeded on the films and cell alignment was analysed by rhodamine/phalloidin staining and imaged with fluorescence microscopy. From all three cross-linkers tested, only 4SP cross-linked scaffolds showed a well-defined micro-grooved pattern. Increasing pH and T° on 4SP-treated collagen decreased gelation time, which resulted in complete inhibition of the pattern, suggesting that an initial low viscous solution is required for a correct PDMS pattern infiltration. A wide range of 4SP concentrations (0.5, 1, 1.5 mM) maintained the well-defined topography on the films, opening the door to future fine-tuning of the stiffness sensed by cells. hBMSCs seeded on top of the scaffolds aligned along the pattern for 14 days in culture. Collectively, this data highlights the potential of these collagen scaffolds as tendon substitutes.

S65.1 PROMS AND CHANGE MANAGEMENT
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Patient reported outcomes have become validated objective measures of success in research studies. They take time and effort to develop and administer. However, to remain relevant and universal PROMS should be gathered routinely and used to manage evidence-based change in healthcare systems. To ensure that they are adopted individual clinician involvement is key however a framework for comparison and relevance promotes engagement. Several examples will be presented of system change using PROMS and PREMS as well as using routine data to defend patient selection. How and what we present depends on whom we are expecting to influence.

S65.2 HOW TO SELECT AND INTERPRET PATIENT-REPORTED OUTCOME MEASURES?
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Patient-reported outcomes (PROs) are widely used in the orthopaedic field to assess the impact of conservative and surgical interventions from a patient’s perspective. Available instruments cover a range of outcome parameters, such as pain, function, stiffness, quality of life or joint awareness. Choice of instrument for a specific study for clinical practice should include the appraisal of the psychometric characteristics of the measure. The presentation will focus on the assessment of the psychometric characteristics of PRO instruments and provide criteria for evaluating those. The concepts of objectivity, reliability and validity will be explained in the context of PRO instruments and the interpretation of score points derived from PRO instruments will be discussed detailing concepts such as minimal important change/difference, norm data, and thresholds based on external criteria. Finally, international guidelines that define standards for the various procedures on development, validation and translation of PRO instruments will be summarised.

S65.3 BOOSTING PERFORMANCE WITH ePRO (ELECTRONIC PATIENT-REPORTED OUTCOME)
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Patient-reported outcome measures are a cornerstone of outcome assessment in orthopaedics. However, completing the pencil and paper questionnaires in clinic is something of a burden to the individual patient and the health care institution. We do not provide much in the way of incentives to collect PRO data. Lengthy questionnaires and hidden data analysis offer no direct benefit to the individual patient nor the clinician. Employing ePRO, utilising tablet PCs for questionnaire completion, can improve this situation considerably. Swift and cost-effective data management and instant availability of results using intuitive graphical display make questionnaire completion more rewarding. Direct feedback of PRO data during the consultation can inform the individual’s care. Completing electronic questionnaires also makes computer-adaptive testing (CAT) possible. CAT creates dynamic questionnaires, adapting to the individual symptom burden of the
individual patient. CAT both increases measurement precision and reduces the number of questions required. As such, ePRO assessment may help to maximise the efficiency and the utilisation of PRO data.

**S65.4 INSURANCE CLAIMS: WHIPLASH-RELATED INJURIES AND HOW TO AVOID THEM**

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Over the past two decades much has been written regarding pain and disability following whiplash injury. Several authors have reported on the relationship between insurance claims and whiplash-associated disorders. Our own experience of over 10-years suggests that fracture may be protective of whiplash injury following road traffic accident (RTA). We exported all ‘medical legal’ cases due to RTA from our EMR system and combined this with patient-reported outcome measures. 1,482 (57%) of all medicolegal cases are due to RTA: 26% ‘head-on’, 34% ‘side-impact’ and 40% ‘rear-ended’. Over half of the vehicles involved are subsequently written-off. While the mean BMI is 27.1, ¼ of this cohort has a BMI over 30 (obese). 163 (11%) patients report a fracture occurring as a result of RTA. Type of impact is significant for fracture (p < 0.05). 47% of RTA which result in fracture are due to ‘head-on’ collision; conversely only 21% are due to ‘rear-ended’ impacts. In 1,324 (89%) of RTA without fracture, patients are twice as likely to report whiplash injury as one of their top-3 sources of pain (p < 0.01). Gender is statistically significant for age (M 44.4, F 38.6, p < 0.05). While the BMI of this cohort is alarming, it is consistent with Irish obesity statistics. Type of impact, in particular ‘head-on’ collision (high kinetic energy event), is significant for fracture. Finally, we report that fracture is significantly protective (p < 0.01) of whiplash injury following RTA.

**S65.5 TOURNIQUET USE DOES NOT AFFECT FUNCTIONAL OUTCOMES OR PAIN AFTER TOTAL KNEE ARTHROPLASTY: A PROSPECTIVE, DOUBLE-BLINDED, RANDOMISED TRIAL**

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The use of a tourniquet during total knee arthroplasty (TKA) is controversial. Return to function and pain are believed to be affected by the use of a tourniquet. The hypothesis of this study was that use of a tourniquet (T) would delay postoperative functional recovery and increase pain as compared to no tourniquet use (NT). 200 patients were recruited for this prospective, double-blinded, randomized controlled trial. All surgeries were performed by one of two fellowship trained arthroplasty surgeons at our institution. Patients were randomized to either undergo TKA with T or NT and blinded to group allocation. An otherwise standardized perioperative protocol was followed. The primary outcome measures were functional assessment testing using the timed up-and-go (TUG) and stair-climb (SC) tests and visual analog scale pain (VAS-P) scores. Secondary outcome measures included blood loss and range-of-motion (ROM). Patients completed outcomes measures preoperatively, in hospital, and postoperatively at 4-6 weeks and 6-8 months. Minimal detectable change (MDC) and Student’s T-test, alpha of p < 0.05, were used to determine significance. No significant differences were seen in postoperative TUG, SC, VAS-P, or ROM at any time point. NT patients were seen to have significantly more calculated blood loss (means: T 1,370.04mL, NT 1,743.85mL; p < 0.001), without a significant increase in transfusion events. Tourniquet use during TKA significantly decreases blood loss and
does not adversely affect early postoperative outcomes. Tourniquet use during routine TKA is safe and effective and concerns over deleterious effects on function and pain may not be justified.

**S65.6 ARE WE EVOLVING TO A SUPERIOR SURGICAL TECHNIQUE FOR IRREPARABLE CUFF TEARS? A COHORT COMPARISON OF INTERPOSITION GRAFTING VERSUS SUPERIOR CAPSULAR RECONSTRUCTION**

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Various arthroscopic techniques using differing graft materials have been described and present a potential alternative to arthroplasty for rotator cuff arthropathy. We describe the short-term outcomes of allograft reconstruction, having evolved of our surgical technique from graft interposition to superior capsule reconstruction (SCR). All patients with an irreparable tear, in the absence of clinical and radiograph evidence of osteoarthritis, who underwent an allograft (Graft JacketTM) reconstruction with either an arthroscopic interposition or SCR technique within our institution were included. A retrospective case note analysis was performed to ascertain perioperative details including total operating and consumable implant costs. 15 patients were in the interposition group, mean age 66 years (48–77). Mean postoperative follow-up time was 17 months (1.9 –27.8). The mean OSS improved from 30.6 to 35.7 (p<0.05). Additionally, mean pain scores out of 10 improved from 7.7 to 1.5 (p<0.01). Mean satisfaction for the surgery was 7.8 out of 10. Complications included 2 re-ruptures (13.3%), 1 infection (6.7%) and 1 case of no improvement (6.7%). In the SCR group, there were 10 patients, mean age 64.5 (56– 68 years). Half of these patients had previous rotator cuff surgery. Mean postoperative follow-up time was 8.7 months (1.9 – 16.3). The mean OSS improved from 24 to 32.9 (p<0.01). Similarly, pain scores decreased from 7.9 to 3.5 (p<0.01). Mean satisfaction was 7.2. Complications included 1 case of no improvement (10%) resulting in a reverse TSR and 1 re-rupture (10%). A formal, prospective comparison trial is advocated to determine if SCR is superior.

**S65.7 INTRODUCTION OF A NEW PREDICTOR FOR SUCCESS OF TREATMENT OF NEGLECTED CASES OF DDH PRESENTED AFTER THE WALKING AGE**


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Late presentation of DDH continues to remain a major problem particularly in the developing countries. Femoro-Acetabular Zones (FAZ) system is created to find a relation between acetabular maturity and severity of dislocation, in one hand, and the success of closed reduction, on the other hand. We hypothesis that the lower the acetabular index and the closer the femoral head to the acetabulum, the more likely the success of treatment. Thus, a retrospective study was performed on late diagnosed DDH hips that underwent closed treatment at a particular hospital in the Middle East. FAZ are drawn on the AP view of the pelvic x-ray and is based on a perpendicular from the acetabular index at the lateral margin of the superior acetabular rim then another perpendicular to Perkin’s line is drawn. This gives three zones, graded I-III. The center of femoral metaphysis is identified denoting the position of the femoral head in relation to the zone classification. FAZ system was applied on 65 pelvic radiographs; mean patient age was 24 months (range: 12 to 36 months) with a minimum follow up of 3 years. Overall, 37 of 65 hips (57%) achieved a satisfactory outcome (Severin I&II), while 22 hips (33%) were found to be unsatisfactory (Severin III). 6 hips (10%) needed an open reduction (p-value 0.001). FAZ could perfectly predict the successful cases. FAZ system is a simple and novel classification and if employed, could reasonably predict the outcome of non-surgical treatment of DDH after walking age.
POSTER ABSTRACTS

THE EFFECT OF THE HAND DOMINANCE ON POST OPERATION REHABILITATION AFTER KNEE ARTHROPLASTY

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There are many factors impact on rehabilitation of the patient post knee arthroplasty. To evaluate the effect of hand dominance on patient's rehabilitation following knee Arthroplasty both short and long-term evaluation. We evaluated a cohort of TKR patients operated on within 2 years period, we assessed the effect of hand dominance on post-operative recovery, length of hospital stay, Range of movement and patients reported outcomes using reintegration to normal living index. A total of 130 patient had total knee replacement during the period, 98 of them participate 65 females and 33 males. Two groups: Dominant side operated they are 52 patient’s and non-dominant side operated are 46 patients. The average length of stay in the TKR group were 5.3 days in non-dominance side group and 7.6 days in the dominance side surgery. The average RNLI (reintegration to normal living activity) after TKR performed in the dominant side was 86.6 while in the non-dominant side was 91. Statistical testing performed using regression analysis with STATA software. Hand dominance showed an effect on length of stay and post-operative rehabilitation following TKR with significant difference in LOS and RNLI index post operatively.

COMPARISON OF DERMAL AND TENDON FIBROBLASTS FOR SCAFFOLD-FREE TENDON TISSUE ENGINEERING APPLICATIONS

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Healthcare expenditure for human tendon injuries exceeds €145 billion per year. Actual repair procedures of critically injured tendons usually rely on the use of tendon tissue grafts, which have a limited regenerative efficacy. Scaffold-free tissue engineering (SFTE) is a promising approach to produce tissue equivalents in vitro but is limited by the generally slow rate of extracellular matrix deposition which occurs under standard cell culture conditions. Macromolecular crowding (MMC) is a biophysical phenomenon that accelerates in vitro matrix deposition by several orders of magnitude and has been proved to decrease the culture times needed for the production of tissue equivalents in vitro, thereby showing promise for helping the translation of SFTE techniques to the clinic. Both tendon (TFs) and dermal fibroblasts (DFs) have shown good performance in tendon regenerative settings, which makes them suitable cell sources for tendon tissue engineering. Herein, we sought to compare the performance of DFs and TFs in SFTE to evaluate its further application in tendon regenerative procedures. Cells were seeded at 25,000 cells / cm² and treated with MMC during 3, 6, and 9 days. Non-MMC treated cells served as controls. Media was changed every 3 days. Significant increase in collagen deposition in the MMC-treated groups was appreciated by SDS-PAGE. Immunocytochemistry showed significant increases of different extracellular matrix proteins in the presence of MMC. Cell viability was not affected by MMC. These data show the potential of the application of MMC in the development of SFTE constructs with TFs and DFs for tendon tissue engineering.

PULSED ELECTROMAGNETIC FIELD ACTUATED BIOMATERIALS FOR INFLAMMATION REGULATION IN TENDONS

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Inflammation is an important process of tendon healing. However, excessive, persistent or unresolved inflammation may result in impaired healing, ultimately leading to degeneration and loss of functionality. Pulsed electromagnetic field (PEMF) has shown potential to reduce inflammation and increase tissue healing rates, being a FDA approved therapy for orthopaedics. Previously, we reported that PEMF actuated membranes holding magnetic responsiveness have potential to modulate inflammation in vivo. However, the cellular mechanisms involved were not properly understood. Thus, in the present study, we propose to investigate the influence of PEMF actuation provided by a mageto therapy device (Globus) on the behaviour of human tendon derived cells (hTDCs) cultured onto magnetic membranes, exploring their modulatory role under an inflammatory environment induced by IL-1β. Magnetic membranes were produced by solvent casting, incorporating iron oxide magnetic nanoparticles into a blend of starch/polycaprolactone (SPCL). Under a PEMF actuation, magnetic membranes moderated cell mediated inflammatory reactions in vivo while in vitro hTDCs treated with IL-1β and cultured on magnetic actuated membranes showed an attenuated protein and gene expression of inflammatory associated markers, such as IL-6, TNF-α, and MMPs, assessed by ELISA assays and real time RT-PCR analysis. Collectively, these results illustrate the beneficial synergistic effect of magnetic biomaterials and PEMF in modulating cell responses to inflammatory cues, contributing for the resolution of inflammation in tendon healing.

EFFECT OF PRO-INFLAMMATORY CYTOKINE COMBINATIONS ON EXPLORATORY BIOMARKERS OF CARTILAGE DEGRADATION

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Proteomic analysis of osteoarthritic synovial fluid, cartilage secretome and the membranome of chondrocytes has identified lumican (LUM), chondroadherin (CHAD) and low-density lipoprotein 1 (LRP-1) as potential biomarkers of joint disease. Our aim was to confirm the presence of these exploratory biomarkers, in an explant model of cartilage degradation, and isolated chondrocytes, and quantify levels in response to different combinations of pro-inflammatory cytokines. Bovine articular cartilage explants and isolated bovine chondrocytes, seeded at high density, were cultured in serum-free DMEM with or without 10, 2.5 or 0.5 ng/ml bovine interleukin-1β (IL-1β) and 50, 10 or 2.5 ng/ml oncostatin M (OSM), or 10ng/ml tumour necrosis factor-a (TNF-α), for 14, and 7 days, respectively. Biomarker levels in explant and chondrocyte secretome, or chondrocyte lysate, were determined by western blotting. Gene expression was determined by qPCR. Intracellular LRP-1 expression decreased 4-fold with OSM/IL-1β and TNF-α/IL-1β treatment (p<0.001, n=3), and the effect was observed with lower concentrations of cytokines. LRP-1 was released into explant secretome with OSM/IL-1β, but not TNF-α/IL-1β. LUM in chondrocyte secretome was elevated 4-fold with OSM/IL-1β (p<0.05, n=3), and the effect was observed with lower concentrations of cytokine. LUM and CHAD release from explants was induced by OSM/IL-1β (p<0.001, n=6), but not TNF-α/IL-1β. Both cytokine combinations decreased CHAD gene expression by 5-fold (p<0.0001, n=4). Individuals (41 re-infections) with available data on comorbidities and infecting organism type, HR 1·71 (0·39, 7·50; p=0.479). We have confirmed the presence of these exploratory biomarkers in both cartilage explant and chondrocyte models. Differences in expression, and release from the matrix, with different cytokine combinations, suggests these biomarkers may have differing roles in disease progression.

SHORT TERM TREATMENT OF CHONDROCYTES WITH IL-1B INHIBITS MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

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The avascular nature of articular cartilage, with limitations in the rate of nutrient and oxygen diffusion from the synovial fluid, has led to the concept that chondrocytes are reliant on glycolysis to meet their energy needs. It has been suggested that a minimal flux of oxygen through the mitochondrial respiratory chain may be required to stabilise glycolytic enzymes, with mitochondrial reactive oxygen species helping to maintain cellular redox balance in favour of glycolysis. The aim of this study was to assess the impact of short-term pro-inflammatory cytokine exposure on chondrocyte bioenergetics. Primary bovine articular chondrocytes were seeded at 160,000 cells per well of a Seahorse XF24 cell culture microplate and cultured in serum free DMEM with ITS media supplement for 18 hours in the presence or absence of either IL-1β or TNF-α or control media. Following 18 hours chondrocytes were subject to the Mito Stress Test using a Seahorse XFe24 Analyzer, to measure key parameters of mitochondrial function. Only chondrocytes cultured in the presence of IL-1β but not TNF-α exhibited significant differences in mitochondrial function. Chondrocytes exposed to IL-1β displayed a significant reduction in basal respiration, proton leak, spare respiratory capacity, ATP production and maximal respiration.

These results indicate that chondrocytes exposed to TNF-α still utilise mitochondrial oxidative phosphorylation, while chondrocytes exposed to IL-1β oxidative phosphorylation is significantly reduced with a predominant switch to glycolysis. These preliminary findings demonstrate the short-term effect of pro-inflammatory cytokines on mitochondrial function and further studies will reveal more about mitochondrial dysfunction associated with osteoarthritis.

**3D PRINTING AND ITS ROLE IN COMPLEX REVISION HIP ARTHROPLASTY**

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Revision hip arthroplasty requires a comprehensive appreciation of areas of bony insufficiency, deficiency and discontinuity in order to conceptualise complex corrective reconstructions. Advances in radiology combined with advances in computer and manufacturing technology have made the three-dimensional (3D) representation of anatomic structures obtainable. Life size three dimensional models were manufactured from CT scans of two patients with complex acetabular defects waiting for second stage hip revisions. The models were constructed from 3D images, using MIMCs software, and manufactured using the rapid prototyping process, Selective Laser Sintering. The models allowed accurate templating using the actual prosthesis weeks prior to surgery. Acetabular cup size, augment and buttress sizes, as well as cage dimensions were selected in advance. The malleable cage template was adjusted according to the contours of the model and were then re-sterilised. Screw trajectory simulation was carried out on the models, thus reducing the chance of neurovascular injury and allowing best use of available bone stock to ensure best construct stability. With 3D printing technology, complex pelvic deformities can be better evaluated and can be treated with improved precision. The life size models allow accurate surgical simulation, enabling preoperative simulation. The accuracy and cost-effectiveness of the technique were impressive, and its use could be incorporated as a tool to aid clinical practice.

**THE EFFECTIVENESS OF PERI-OPERATIVE INTERVENTIONS IN PREVENTING CHRONIC PAIN IN PATIENTS RECEIVING PRIMARY TOTAL KNEE REPLACEMENT: A SYSTEMATIC REVIEW**

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For patients receiving total knee replacement (TKR), features of peri-operative care may be associated with chronic pain after surgery. Effects may be direct, e.g. through nerve damage or complications, or indirect by limiting mobilisation and rehabilitation. This systematic review evaluated whether peri-operative treatments can prevent chronic pain after TKR. PICOS criteria were: adults receiving primary TKR for osteoarthritis; peri-operative non-surgical intervention; control receiving no intervention or alternative; pain assessed at ≥6 months post-operative, and adverse events; with evaluation in a randomised controlled trial (RCT). We searched major bibliographic databases up to February 2018. After screening, two reviewers evaluated relevant
articles. Studies at low risk of bias according to the Cochrane tool were included in our analysis. Searches identified 1514 RCTs of which 43 assessed pain and were at low risk of bias. Intervention heterogeneity precluded meta-analysis and definitive statements on effectiveness. There was encouragement for further research into local infiltration analgesia, ketamine infusion, pregabalin, and electric muscle stimulation. Surgery without a tourniquet was associated with fewer early complications but not chronic pain and merits further research. There was no evidence that prevention of blood loss with tranexamic acid was associated with chronic pain. Extensively researched interventions including venous thromboembolism prevention have not been evaluated in relation to chronic pain. Our review summarises evidence on peri-operative treatments for the prevention of chronic pain after TKR and highlights aspects of care for further evaluation in well-conducted RCTs. Long-term consequences of many widely researched treatments have not been reported.

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Revision hip arthroplasty requires a comprehensive appreciation of areas of bony insufficiency, deficiency and discontinuity in order to conceptualise complex corrective reconstructions. Advances in radiology combined with advances in computer and manufacturing technology have made the three-dimensional (3D) representation of anatomic structures obtainable. Life size three dimensional models were manufactured from CT scans of two patients with complex acetabular defects waiting for second stage hip revisions. The models were constructed from 3D images, using MIMCs software, and manufactured using the rapid prototyping process, Selective Laser Sintering. The models allowed accurate templating using the actual prosthesis weeks prior to surgery. Acetabular cup size, augment and buttress sizes, as well as cage dimensions were selected in advance. The malleable cage template was adjusted according to the contours of the model and were then re-sterilised. Screw trajectory simulation was carried out on the models, thus reducing the chance of neurovascular injury and allowing best use of available bone stock to ensure best construct stability. With 3D printing technology, complex pelvic deformities can be better evaluated and can be treated with improved precision. The life size models allow accurate surgical simulation, enabling preoperative simulation. The accuracy and cost-effectiveness of the technique were impressive and its use could be incorporated as a tool to aid clinical practice.

INFLUENCE OF CROSS-LINKING CONCENTRATION ON THE SHEAR DEFORMATION OF COLLAGEN FIBRILS AS DETERMINED USING PIEZORESPONSE FORCE MICROSCOPY

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During aging, collagen undergoes a series of post-translational modifications that lead to an accumulation of collagen cross-links, which impacts mechanical strength and thus cellular function. Collagen is naturally piezoelectric and such electromechanical coupling may play a role in bone remodeling. As such, it is important to assess whether cross-linking also influences collagen piezoelectricity. In this study, we report on the influence of glutaraldehyde (GTA) cross-linking on the measured piezoelectric properties of collagen fibrils as determined by piezoresponse force microscopy (PFM). Fourier-transform infrared spectroscopy has been used to evaluate the chemical structure of collagen before and after cross-linking. Amide A and OH bands (from water bonded to collagen) are in the same range in FTIR spectrum (3700-3100 cm⁻¹), hence, the width between the area of the band was measured. It was found that increasing the crosslinking concentration will result in a smaller band area, which suggests that during crosslinking collagen might lose the intramolecular water bridges and intermolecular bridges between fibrils are formed instead. Besides, cross-linked fibrils with 15% GTA showed greater resistance to collagenase, where only 13% was degraded in 7 hours compared to the control sample, which dissolved within 3 hours. PFM results show a reduction in the piezoresponse for cross-linked samples (GTA 15%), in comparison to the control sample (0.6 ± 0.4 pm/V and 1.2 ± 0.1 pm/V, respectively). We conclude that increases in cross-linking restricts the fibril’s capacity to shear. Reduced electromechanical coupling could interfere with the ability of cells to sense and adapt to mechanical stress.
Prevalence of MRSA Among Health Care Workers

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The major challenges faced by the health care system are the hospital acquired infections and the resistance to the antibiotics. Most of the time the organism involved in hospital acquired infection is staphylococcus Aureus. It also frequently colonizes the humans and it is found that approximately one third of population carry staphylococcus Aureus in nose. The organism is broadly categorized into methicillin sensitive (MSSA) and methicillin resistant (MRSA). MRSA, also known as “Super Bug” is resistant to commonly prescribed antibiotics and thus difficult to treat. Screening can be done with swabs taken from nose, axilla and groin. According to American Society of Microbiology, nasal swabs have higher MRSA detection rate when compared to axilla and groin swabs. The nasal carriage rate of MRSA increases with increasing incidence of infection and the transmission can be halted by treating the carriers. The rate of MRSA colonization varies widely as different studies show different rates. At present, no study is published to described MRSA colonization rate among health care workers in Pakistan. The objective of our study is to determine the prevalence of MRSA among health care workers and the study was carried out in Shaheed Mohtarma Benazir Bhutto Trauma Centre, Karachi, Pakistan. The nasal swabs taken from 237 health care workers taking part in this screening were processed using standard laboratory techniques. Out of 237, MSSA was found in nasal cavity of 9.7% (23) and MRSA was detected in 8.8% (21). This data revealed significantly higher rate of MRSA prevalence among health care workers.

FISH SCALE AS A NOVEL REINFORCEMENT IN POLYMERIC SCAFFOLDS FOR BONE ENGINEERING APPLICATIONS

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Fish scales are used as collagen sources in biomaterial applications due to their Type I collagen and hydroxyapatite (HAp) content and similarity to bone tissue with distinctive collagen arrangement mimicking the bone matrix. Besides, these structures are bioactive biocompatible, low antigenic and biodegradable. In our previous studies, fish scales were decellularized with combination of physical, chemical, enzymatic methods and used as templates for MG-63 attachment and proliferation. Results indicated that, cells were attached and proliferated on fish scale surface due to its collagen-Hap based composition and patterned surface morphology (microchannels) inducing suitable surface with high roughness. However, there has been no study concerning the use of fish scale as reinforcement in polymeric biomaterials. Therefore, in this study, it is aimed to design a new composite scaffold with fish scale microparticle incorporation to chitosan matrix for hard tissue regeneration. Fish scales were disrupted physically and Fish scale microparticles (100 μm) were dispersed in chitosan matrix with ultrasonic homogenization. Chitosan/fish scale (FS/CS) scaffolds were fabricated by lyophilisation technique and characterized in terms of mechanical, morphological and physical properties with FT-IR, degradation and swelling test. SEM and porosity results showed that FS/CS scaffolds have uniform pore structure showing high porosity with increasing fish scale content. In vitro cytotoxicity, proliferation and osteogenic activity of fish scale reinforced scaffold were obtained with Saos-2 cell line. Results indicated that fish scale reinforcement did not cause any cytotoxic effect and found suitable for cell proliferation. In conclusion FS microparticles show promising effects as reinforcement agents for bone regeneration.

CAN WE REDUCE THE NUMBER OF MRSA SCREENING SITE SWABS IN ELECTIVE ORTHOPAEDIC PATIENTS?

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This study evaluates the possibility of reducing the number of MRSA swab sites as part of a quality improvement project. Patients on the waiting list for elective orthopaedic procedure in our trust who had a MRSA positive swab from either four sites were analysed over the time period from January 2012 to December 2014. Positive swabs of different regions were recorded and compared. There were 138 MRSA positive patients, giving an incidence of 31 per 10000 screen/year over that time period. Some patients (n=31, 22.5%) had a positive swab in more than one site. The positive sites were as follows: nose (69.60%, n=96), groin (26.10%, n=36), throat (25.30%, n=35) and axilla (8.70%, n=12). In our cohort, we would miss a significant proportion of positive patients if we change it to a two-swab screening policy. However, we would only miss 2.2% of cases for a nose, groin and throat three-swab policy. A three-swab combination of nasal, throat, and groin swabs improves pick up rate of MRSA significantly compared to a two-swab policy and misses only 2.2% compared to a four-swab policy. Axilla swabbing does not make a significant difference to the results. Based on this study, the policy has now been changed from a four-swab to a three-swab screening in our trust. This has been audited 4 times and they were all negative. This has helped reduce cost in terms of staff time and resources.

DOES INTRAMEDULLARY GRAFTING INCREASE STABILITY OF PLATED PROXIMAL HUMERUS FRACTURES?

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Treatment of complex distal radius fractures, commonly managed by volar locking plates, is challenging. Combined volar and dorsal plate fixation is valuable option, however, current biomechanical investigation on competency of supplemental dorsal plating is scant. This study's aim is to investigate biomechanically double-plated distal radius fractures in comparison to volar locking plating. Complex intra-articular distal radius fractures AO/OTA 23-C2.1&C3.1 were created in 30 artificial radii assigned to 3 groups. Lunate facet was divided in 3 equally-sized fragments in group 1, split in smaller dorsal and larger volar fragment in group 2, and divided in 2 equal fragments in group 3. Following fracture reduction, each specimen was first instrumented with volar locking plate and tested non-destructively in 40° flexion, 40° extension and 0° neutral position. Mediolateral radiographs were taken under 100N loads in flexion and extension, and under 150N loads in neutral position. Subsequently, all biomechanical tests were repeated after supplemental dorsal locking plating. Stiffness in neutral position increased after supplemental dorsal plating in groups 2 and 3, but not in group 1. In addition, stiffness in extension remained after dorsal plating without increase, whereas in flexion it increased in each group. Angular displacement between shaft and lunate facet decreased after dorsal plating in neutral position and flexion in each group, whereas in extension it decreased in groups 1 and 2, but not in group 3. In conclusion, supplemental dorsal locked plating increases fixation stability of unstable distal radius fractures after volar locked plating. However, its effect depends on fracture pattern.

DOES INTRAMEDULLARY GRAFTING INCREASE STABILITY OF PLATED PROXIMAL HUMERUS FRACTURES?

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Locked plating of proximal humerus fractures is still associated with high complication rates. The aim of this study was to investigate the biomechanical competence of PHILOS plating augmented with supplemental intramedullary graft in comparison to conventional PHILOS fixation. Complex four-part proximal humerus fractures were set in thirty artificial humeri assigned to three study groups (n=10). Group 1 was characterized by loss of medial support, group 2 – by aggravated bone quality, and group 3 – by combination of the two features. Following PHILOS plating, each specimen underwent non-destructive quasi-static biomechanical testing in 25° lateral angulation under axial loading between 150N and 400N in 50N increments, accompanied by consecutive anteroposterior x-ray imaging. Subsequently, an additional 3D-printed intramedullary graft was inserted into each specimen and all tests were repeated. Grafting resulted in significantly higher axial
stiffness in groups 1 and 3 (p<0.01), but not in group 2 (p=0.12). Non-grafted specimens represented significantly higher stiffness in group 2 compared to groups 1 and 3 (p<0.01), whereas no significant differences were detected among the three groups in grafted state (p>0.99). Varus deformation decreased significantly in each group after graft insertion (p≤0.04). Non-grafted specimens in group 2 showed significantly lower varus deformation compared to groups 1 and 3 (p≤0.04). No significant differences were registered among the three groups post grafting (p≥0.65). From biomechanical perspective, PHILOS plating augmented with intramedullary graft has the potential to increase significantly the stability against varus collapse in unstable proximal humerus fractures, when compared to conventional PHILOS fixation.

3D GEOMETRY OF FEMORAL REAMING

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The Reamer-Irrigator-Aspirator (RIA) is a minimally invasive technique used for bone graft harvesting by reaming the medullary canal of the femur. In contrast to bone graft harvesting from the iliac crest it allows the extraction of large amounts of bone graft. However, postoperative femur fractures have been observed after RIA. The reaming diameter is one of the most discussed reasons for these fractures. Therefore, the 3D morphology of the reaming (the decrease of femoral wall thickness) was analysed considering different reaming diameters with regard to the fracture morphology. Forty-five human cadaveric femora were randomized to three groups (G1-G3) and reamed with a +1.5mm (G1), +2.5mm (G2) and +4mm (G3) larger diameter than the isthmus. For morphological examination CT scans were made in intact bones, after reaming and after fracture creation in internal rotation. The relative reduction of the wall thickness was calculated and visualized for the complete femoral shaft. The region with the strongest relative decrease in wall thickness (Max-Dec) was determined and checked for an overlap with the fracture. It was found that regardless of the reaming diameter, the strongest relative decrease (G1=24.9%±9.6%, G2=28.4%±14.9%, G3=39.7%±14.3%) in femoral shaft wall thickness occurred medially (73%) in the second (10.8%), third (62.2%), and fourth (18.9%) eighth of the femoral shaft. As the diameter of the reaming increases, however, an overlap of the fracture line with the Max-Dec becomes more frequent (G1=33.3%, G2=50% and G3=61.5%). This suggests that a reaming-associated fracture is most likely to occur in this region.

THE FIXATION STRENGTH AND CUT-OUT RESISTANCE OF TFNA HELICAL BLADES AND SCREWS CAN BE INCREASED BY BONE CEMENT AUGMENTATION

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The effect of cement augmentation on the fixation strength and cut-out resistance of TFN-ADVANCED Proximal Femoral Nailing System (TFNA) blades and screws within the femoral head has so far not been evaluated biomechanically. Therefore, ten pairs fresh-frozen osteoporotic and osteopenic human cadaveric femoral heads were randomized to 2 equally sized paired groups for instrumentation with either TFNA blade or screw. One side of each pair was augmented with PMMA-based bone cement, whereas the contralateral side was left without augmentation. All specimens were biomechanically tested under progressively increasing cyclic loading until failure in a setup simulating unstable intertrochanteric fracture with lack of posteromedial support and load sharing at the fracture gap. An inclinometer mounted on the femoral head was used to monitor varus tilting. A Varus collapse of 5° was defined as clinically relevant failure and number of cycles to failure and load at failure were determined for each specimen. Statistical evaluation was performed at level of significance p=0.05. Cycles to failure for augmented/non-augmented TFNA blades and screws were 30492±8715 / 19131±11160 and 19307±802 / 12612±9138, respectively. The corresponding loads at failure were 4049.2±871.5N / 2913.1±1116.0N and 2930.7±802.1N / 2261.2±913.8N. Both cycles to failure and loads at failure were significantly higher for augmented versus non-augmented TFNA blades (p=0.003) and TFNA screws (p=0.032). Implant augmentation with PMMA-based bone cement significantly increases the fixation strength and cut-out resistance of TFNA blades and screws within the femoral head. From a biomechanical perspective it is a valid supplementary treatment option in osteoporotic bone.
ELECTROSPUN PVA NANOFIBERS FOR BONE DISEASE THERAPY USING MESENCHYMAL STEM CELL

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Mesenchymal stem cells (MSCs) constitute multipotent stromal cells that can differentiate into a variety of cell types. This characteristic provides a great potential as a cell source for tissue engineering (i.e. bone formation) and cell-based therapy for many diseases (i.e. bone diseases). It has been reported that electrospun nanofibers developed using hydroxyapatite exhibit osteoconductivity and significantly stimulate the bone forming ability, however it is difficult to produce on a large scale with controlled strength, degradation, and nanostructure with great flexibility. Previous reports of our research group have demonstrated that polyvinyl alcohol (PVA) has great potential for biomedical applications owing to its biocompatibility. Given that the interaction between MSCs and the scaffold surface is crucial to achieve directed differentiation of MSCs in tissue regeneration, the purpose of this study is to develop a biocompatible hydroxyapatite/PVA scaffold. To accomplish that, PVA nanofibers will be fabricated using electrospinning technique and will be characterized using scanning electron microscopy (SEM), swelling studies, differential scanning calorimetry (DSC), fourier transform infrared spectroscopy (FT-IR) and rheometry. After that, the MSCs will be seeded into the scaffold, after a period of culture in vitro, the cell viability, capacity of infiltration in the nanofibers and morphology will be analysed. The results of this study will help us understand how polymeric fibers can enhance the formation of new bone tissue with increased of cell adhesion and may help us develop better scaffolds to use in the therapy of bone diseases.

PREPARATION OF POLYVINYL(ALCOHOL) ALIGNED POROUS CRYOGELS USING UNIDIRECTIONAL FREEZING TECHNIQUE FOR BONE TISSUE HEALING APPLICATIONS

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The technique of unidirectional freeze polyvinyl alcohol (PVA) hydrogels can mimic the structure of bone tissues and it leads the chains in the freezing-thawing technique to produce uniform structures that can also improve the mechanical properties. However, no studies have been performed in terms of investigating how the freezing affects the chain reaction. Therefore, in this work PVA solution with a known concentration was transferred to a polytetrafluorethylene (PTFE) mould and insulated with heat insulation tapes which has a range of working from -40°C to +100°C. The mould was immersed into liquid nitrogen at a speed of 100 mm min⁻¹ until it was fully immersed. The frozen sample was then thawed at ambient temperature for 1 h. This cycle was repeated three times. The PVA freeze thawed by unidirectional freezing was evaluated using a thermal camera during the whole freezing experiment. Trough thermal Camera it was possible to observe that as the mould decreases towards the liquid nitrogen, the temperature decreased, and a linear upward freezing was obtained; unidirectional freezing occurs in the middle region and it induces an oriented distribution along the freezing direction. Through SEM it was possible to observe that the sample presented different structures in different parts and for all samples analysed, the middle region presented a fibrous structure. Conversely, the external parts of the sample presented the normal structure of hydrogels. The easy method developed and processed was confirmed to produce an unidirectional freezing which can be used as a potential apparatus.

ANTIBIOTIC LOADED COLLAGEN FLEECE, DISADVANTAGES AND THE LACK OF EVIDENCE FOR CLINICAL TREATMENT OF CHRONIC OSTEOGYELITIS

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Chronic osteomyelitis treatment requires surgical debridement accompanied by local and systemic antibiotics administration. Local antibiotics administration through biodegradable carriers has interest to obtain one-stage
This systematic review according to PRISMA studied clinical evidence and results of antibiotic loaded collagen fleeces in osteomyelitis treatment based on level of evidence, methodological quality and risks of bias. Clinical efficacy was defined as eradication of infection and distorted bone and wound healing. In addition, \textit{in vivo} pharmacokinetics of the different collagen fleeces were evaluated. Ten studies were included detailing 2 types of antibiotic-loaded fleeces. 413 patients were treated with either of the two gentamicin loaded fleeces with a success rate of 91%. Rough estimation of wound exudate concentrations of the gentamicin-sulphate fleeces shows that local antibiotic concentrations dropped below the minimal inhibitory concentration (MIC) at a maximum of 5-7 days. Adverse events reported were fistulas, prolonged wound leakage and wound healing problems. In addition, the general quality of all included studies was weak to moderate and there was a moderate to high risk for bias. Quality and level of evidence of the included studies are low and the risk of bias in these studies is high. This makes evidence regarding these fleeces inconclusive and no clinical decision-making can be based on these studies. This systematic review emphasizes that evidence is lacking; that these collagen fleeces have several disadvantages, and that there are better alternatives such as S53P4 bioactive glass with established level of evidence for one stage treatment chronic osteomyelitis.

**OSTEOCLASTIC RESORPTION OF THE PRECIPITATED CALCIUM PHOSPHATE LAYER ON BIOACTIVE GLASS SURFACES**

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\textit{In vivo} degradation of S53P4 bioactive glass (BAG), clinically used to treat osteomyelitis, is very slow. The role of cells in the degradation process remains unclear. Therefore, this study aimed to determine the capacity of human osteoclasts to resorb the calcium phosphate layer formed on BAG, when in contact with (body) fluids. Human monocytes were seeded on BAG discs that were pre-soaked in PBS for three days, to ensure calcium phosphate precipitation. Osteoclastic differentiation was induced with MCS-F and RANKL supplemented to the culture medium. Cultures on hydroxyapatite discs served as controls. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) were performed to detect resorption pits, as an indication for osteoclast resorption. Comparable numbers of resorption pits were observed on both disc types. The resorption pit surfaces on BAG were very smooth and uniform, while the pits on hydroxyapatite had an irregular appearance. EDX confirmed the initial calcium phosphate precipitation on the BAG discs, due to pre-soaking. It also indicated an incomplete removal of this layer by osteoclasts, since calcium and phosphate were the main detected elements on the surface in the resorption pits on BAG. The smooth and uniform appearance of these resorption pits suggests that the osteoclastic resorption was initiated but later hindered. The silica layer underneath the precipitated calcium phosphate might be the reason for the hindrance. In conclusion, osteoclasts were at least partially able to resorb the precipitated calcium phosphate on BAG surfaces.

Balancing Bone Resorption and Overzealous Bone Growth in Lumbar Interbody Fusion with RhBMP-2 in the Sheep Model - A Question of Dose and/or Concentration

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Thirty-six Merino sheep underwent right-anterior lumbar interbody fusion at L1-L2 and L3-L4 with the addition of a polyetheretherketone (PEEK) cage either filled with one of four different concentrations/doses of rhBMP-2 (interventional groups:0,4mg/ml, total dose of 4,0mg; 2,0mg/ml, total dose of 2,0mg; 1,0mg/ml; total dose of 1,0mg; 0,5mg/ml, total dose of 0,5mg) or the control group filled with an absorbable collagen sponge (ACS) or left empty. A pedicle screw system was implanted in all surgical levels. Thin cut CT image were taken directly postoperatively, after 3 months, 6 months and 12 months to assess bone resorption, cage subsidence and migration (indirect marker of bone resorption) and overzealous bone growth. In comparison with the control group, rh-BMP-2 groups showed a higher fusion rate at 3 (72% vs.13%), 6 (90%vs30%) and 12 (95%vs70%) months CT scans. Overzealous bone growth was detected at the right ventral circumference.
of the vertebral body as sign of the direct operative access. No ectope ossification was detected in all groups. The incidence of bone resorption as well as cage migration and cage subsidence as indirect marker of bone resorption were higher in the BMP2 groups. In this animal model the application of rhBMP-2 in the different concentrations/ doses showed much better fusion rates compared with the control group. These results could be shown in the 0,5mg BMP-2 group with clear reduction of adverse effects. Higher doses of BMP-2 doesn’t cause a benefit in fusion rate but an increase in side effects like cage migration and subsidence as marker of higher bone resorption. No inflammation reaction or systemic side effects were detected in the BMP-2 group.

MODELLING THE DEGENERATE NICHE TO INVESTIGATE EFFICACY OF MESENCHYMAL STEM CELL DELIVERY WITHIN A THERMALLY TRIGGERED HYDROGEL TO REGENERATE THE NUCLEUS PULPOSUS

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We have previously published the development of an injectable hydrogel (NPgel) which promotes differentiation of mesenchymal stem cells to nucleus pulposus (NP) like cells, integrates with IVD tissue following injection and restores mechanical properties. However the degenerate disc is a harsh environment with low glucose, pH and O2 and catabolic cytokines. Thus this study investigates the behaviour of MSCs within NPgel in environments mimicking the degenerate niche. NPgel containing MSCs were cultured under low glucose, low pH and 5% O2 ± IL-1. Whilst expression of collagen type II was decreased initially in degenerate culture media + IL-1, levels increased following 4 week culture. Similarly MMP3 expression was initially induced in MSCs cultured in NPgel in the degenerate niche conditions but expression decreased with culture duration. When NPgel containing MSCs was injected into tissue explants, MSCs increased expression of NP matrix proteins. In addition in human degenerate NP explants where NPgel+MSCs was injected a significant increase in anabolic and decrease in catabolic factors was observed in comparison to controls. Suggesting that viability and differentiation of MSCs in NPgel is maintained even under degenerate conditions. Thus NPgel + MSCs has the potential to regenerate the NP and provide mechanical support, whilst reducing the catabolic phenotype of degenerate NP cells, as a treatment strategy for IVD degeneration.

BIOCOMPATABILITY AND OSTEOCONDUCTIVE CAPACITY OF DEVITALIZED CORAL

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The gold standard in bone trauma repair is autograft, a procedure largely in decline due to the requirement for a secondary invasive procedure required to harvest bone from the patient. Coral skeletons are excellent bone grafting materials due to their biocompatibility and osteoconductive properties. The coral’s native architecture is designed to host life, influence cellular behaviour and encourage cellular adhesion, qualities retained after scaffold devitalization and preparation as a biomaterial. With newly developed controlled, environmentally responsible cultivation techniques, the GMP production of coral scaffolds for orthopaedic, dental and veterinary use is possible. Here the composition, structure and biocompatibility of cultivated coral were investigated. Using scaffolds derived from four species of devitalized coral samples, the structure and composition were characterized. The scaffolds were composed primarily of calcium carbonate ranging in density from 1,753-1,903mg/cm3. The interconnected porosity changed with species, ranging from 3.7%-41.5%. LIVE/DEAD staining viability, MTS and proliferation assays demonstrated maintained, high levels of MSC viability, metabolism and cellular proliferation indicating biocompatibility. MSCs were directly seeded onto coral scaffolds and cultured for 14 days before SEM visualization. A thick, confluent, organized layer of fibroblast-shaped MSCs was observed on the external surface of the coral skeleton and both covering and lining the calice (external pore-like) structures. The four coral species evaluated in this study were deemed biocompatible, supporting bone marrow derived MSC viability, metabolism, adhesion and growth. They are therefore prime candidates for further investigation as a replacement for auto- or allograft.
IMPACT OF DIABETES MELLITUS ON BONE MARROW PROGENITOR CELL NUMBER AND PROLIFERATIVE CAPACITY AND OSTEOCONDUCTIVE CAPACITY

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Abnormal bone mineral density and/or distribution patterns and delayed bone fracture repair are complication of type 2 diabetes mellitus (DM). Bone marrow isolated from pre-clinical models of DM contains significantly fewer multi-potential progenitor cells (MSCs) with reduced differentiation capacity as compared to non-diabetic isolates. A return to glucose homeostasis does not restore the capacity of previously diabetic MSCs, indicating the environment selectively depletes the population of MSCs. This study proposes that alterations in human bone marrow MSC number and capacity in individuals living with DM contributes to the pathology underlying DM-associated osteopathy through MSC inability to support organ homeostasis. Although all donor groups displayed comparable mononuclear cell (MNC) counts and CFU-F numbers indicating an analogous baseline for MSC-maintenance of bone homeostasis, differences were observed in the capacity of MSCs residing within a DM environment. Marrow donations from women living with DM contained ~10 times fewer CFU-Fs as compared to their male counterparts, indicating a gender disparity in the impact of DM. Further, MSCs isolated from individuals living with DM contained nearly half of the number of osteogenic clones as non-DM samples, indicating an impact of the DM environment on MSC potential to maintain bone homeostasis. Finally, MSCs isolated from the DM environment exhibited a 66% increase in doubling time, indicating a lesser capacity to respond to an injury stimulus. Together, these data indicate a biologically significant impact of the DM environment on MSCs residing within the bone marrow that may underpin DM-associated osteopathy.

SILK FIBROIN / HYDROXYAPATITE SCAFFOLDS COMBINING LYOPHILISED SPONGE AND NANOFIBERS FOR BONE REGENERATION

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Silk fibroin is a natural fibrous protein derived from Bombyx mori, which is well-studied for tissue engineering and regenerative applications. Silk can be fabricated to different forms including hydrogels, films or sponges. Combination of both lyophilized sponge 3D structure and nanofibers mimics conditions inside the bone and provides mechanical support for the bone cells. Biocompatibility of silk fibroin and hydroxyapatite provides possibility for osteogenic cells to proliferate and gradually repair bone defect without scar formation, vascularization or significant effect to patient immune system. Influence of different amounts of hydroxyapatite addition to biocompatibility to human cell lines was tested under in vitro conditions. We also studied mechanical properties, degradation rate and morphological changes in dependence on the different conditions of material stabilization and sterilization. In conclusion, the bicomponent scaffolds prepared in this study show very promising properties for bone tissue engineering.

CAN WE DEVELOP A BIOMECHANICAL FUNCTIONAL SCORE TO QUANTIFY THE JOINT MECHANICS OF THA PATIENTS?

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THA is a reliable methods to improve the quality of life in osteoarthritis patients. However, it is still unclear whether it would lead to improved functional mobility. The purpose was to develop a biomechanical functional score to quantify the joint mechanics of THA patients compared to healthy participants (CTRL). Twenty-four THA patients and 12 CTRL (age-, sex-, and BMI-matched) participants were recruited and underwent motion analysis for different ADLs tasks prior and nine months after THA. Three-dimensional joint kinematics and
ground reaction forces were collected and five kinematic and six kinetic variables were included in the analysis. The normalized root-mean-square-deviation (nRMSD) was calculated between the THA and the CTRL groups for both pre- and post-op conditions: 

\[ nRMSD = \sqrt{\frac{\sum_{i=1}^{n}(x_{i}-y_{i})^2}{n}} \]

\[ x_{\text{max}} - x_{\text{min}} \]

Kinematics and kinetics improvement scores (KMIS and KNIS) were calculated to estimate pre/post-op differences:

\[ KMIS = \sum_{i=1}^{n}(K_{\text{pre/ctrl}} - K_{\text{post/ctrl}}) \]

\[ KNIS = \sum_{i=1}^{n}(K_{\text{pre/ctrl}} - K_{\text{post/ctrl}}) \]

THA patients experienced post-op improvements, with kinetics variables closely resembling the CTRLs, especially on hip and knee power production. Total improvement scores showed that THA experienced greater improvements during squat task and this can be a practical approach to evaluate the change in biomechanical function and highlight small improvements that may go unnoticed with traditional statistical analysis.

**EARLIER USE OF JOINT REPLACEMENT SURGERY: WORSENING ADHERENCE TO GUIDELINES FOR THE NON-OPERATIVE MANAGEMENT OF HIP AND KNEE OSTEOARTHRITIS**


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Evidence based consensus guidelines for the management of hip and knee OA were published in 2008. This cross-sectional study benchmarked adherence with these recommendations in 2009 and re-evaluated this 5 years later. Patients listed to undergo hip or knee replacement at a single NHS teaching hospital were interviewed to review clinical management prior to surgery. Data is reported as percentage adherence to guidelines, analysis was by t-test or Mann-Whitney U-test. 195 patients were reviewed in 2008 (90 TKA, 105 THA) and 215 in 2014 (110 TKA, 105 THA). In the TKA cohort (compared to 2008) symptom duration prior to surgery reduced (p= 0.01). Age, gender and BMI were consistent. Weight reduction was discussed less frequently (p<0.01). Walking aids and insoles were issued less frequently (p<0.02). Physiotherapy exercises reduced and activity advice increased (p<0.05). Pharmaceutical management was unchanged in usage of paracetamol and NSAIDS (p=0.35) Prescription of opioid and narcotic medication reduced (p=0.02). In the THA cohort, symptom duration prior to surgery reduced (p<0.01). Age, gender and BMI were consistent. Weight reduction was discussed less frequently (p<0.01). There was no change in the provision of walking aids (p=0.16) or insoles (p=1.0). Physiotherapy exercises reduced (p<0.01), advice to increase activity was unchanged (p=1.0). Paracetamol usage increased (p=0.02). Oral NSAIDS usage was unchanged (p=0.12), however topical NSAIDS reduced (p<0.01). Opioid and narcotic medication was unchanged (p=1.0). Patients are undergoing joint arthroplasty earlier with a corresponding reduction in community management. Pharmaceutical management meets minimum treatment guideline in approximately 70% of cases, however it would seem that ‘hands on’ physiotherapy is being replaced with activity advice.

**TARGETING PHYSIOTHERAPY TO PATIENTS AT RISK OF POOR OUTCOMES FOLLOWING TOTAL KNEE ARTHROPLASTY: THE TRIO RCT**


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Physiotherapy is typically employed following TKA however evidence for improving patient outcomes is lacking. TRIO is the largest ever trial of physiotherapy following TKA, performed at 15 UK centres to evaluate whether therapy could be targeted to those deemed at risk of poor outcome and determine if outpatient-led therapy offered superior results to home-based rehabilitation. Patients were screened 6-weeks post-op based on a classification of the Oxford Knee Score (OKS) and randomised to rehabilitation arms comprising 18 sessions over 6-weeks. The outpatient-led group undertook a progressive functional rehabilitation protocol in contrast to a static home-exercise protocol. Primary outcome was comparative OKS at 52-weeks. Secondary outcomes evaluated pain VAS and a battery of satisfaction questions post-intervention, at 26 and 52-weeks. 334 patients were randomised, 8 were lost to follow-up, compliance was >85%. Clinically meaningful improvement in OKS was seen in both intervention arms (p<0.001). Between group difference in 52-week
OKS was 2.25 (95%CI, 0.61-3.90) points favouring the outpatient-led therapy (p=0.008). Secondary outcomes demonstrated non-significant 5% reductions in pain VAS, enhanced satisfaction with pain-relief (OR 1.66, p<0.02), ability to perform functional tasks (OR 1.6, p<0.02), and heavy functional tasks (OR 1.6, p=0.04) in the outpatient-led group. Therapy was successfully targeted to patients deemed at risk of poor recovery post-TKA and both intervention arms made clinically meaningful improvements in OKS at 52-weeks. The confidence intervals around the 52-week OKS suggest that any difference which might exist is too small to be clinically relevant. Future work could investigate which patients are most likely to respond which may enhance the overall effectiveness of a stratified approach.

VIRTUAL METHODOLOGY FOR PLANING FEMORAL OSTEOCHONDRoplastY FOR CAM-TYPE IMPINGEMENT OF THE hip

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Surgical bone resection is the most effective treatment to alleviate the abnormal contact between femur and acetabulum on patients with cam-type femoroacetabular impingement (FAI). This is a phatomechanical pathology resulting from the excess of bone presented on the femoral head-neck region. The surgical procedure known as osteochondroplasty, focuses on reshaping the femur to its ideal morphology. The virtual methodology consisted of developing finite element models from CT scans of patients diagnosed with FAI to determine the range of motion (RoM) and impingement areas of the joint. Models predicted the RoM and localised the impingement areas during internal rotation of the femur at different flexion angles. Five models were developed, four from patients exhibiting cam-type impingement and one of normal morphology. Internal rotation RoM was measured when the anteroposterior region of the femur came into collision with the acetabular rim. Then a virtual osteochondroplasty was performed based on the impingement areas in order to obtain the normal RoM but keeping the volume of bone removal to a minimum.

The virtual technique was validated by comparing results with CT scan based models of an FAI hip obtained from prior and post actual osteochondroplasty. The virtual osteochondroplasty performed on the hip resulted in increased RoM to values closer to the normal RoM than the actual surgery for a smaller femoral head volume resection demonstrating that the tool can be used to optimize the amount of bone removed during femoral osteochondroplasty to enable normal hip RoM to be restored with a minimum resection volume.

QUANTIFYING RADIAL HEAD INSTABILITY AND LIMITATIONS IN HAND ROTATION AFTER IOM AND ANNULAR LIGAMENT INJURY

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The interosseous membrane (IOM) is a key stabilizer of the forearm that is often injured in conjunction with disruption of the annular ligament. In the present study, motion of the radial head is quantified after simulated injury to the IOM and annual ligament to demonstrate how forearm stability changes as the arm is actively pronated and supinated. Fourteen fresh-frozen cadaveric arms, mid-humerus to fingertips, were used. Soft tissue was removed proximal to the carpals leaving the main stabilizing structures intact. A custom fixture supported the arm and hand with the elbow flexed 90 degrees and simulated active muscle loading of the biceps and pronator teres to rotate from a neutral forearm position to full supination and full pronation, respectively. A 10-camera Optitrack motion capture system tracked the motion of the radius for several stages of sequential sectioning: intact, annular ligament sectioned, proximal band sectioned, central band sectioned, and distal band sectioned. The position and orientation data of each sectioning stage was compared to the neutral forearm position (0 degrees) of the intact stage. In pronation, the radial head relative translations were not significantly different along each axis for any sectioning stage. In supination, the radial head moved postero-medially then anterolaterally, increasing with all sequential sectionings. After sectioning the central band, little radial rotation occurred, decreasing maximum hand supination from a mean of 36 degrees (intact) to 6 degrees. Central band
sectioning had the greatest effect on radial head translations, changing the biceps’ role from flexor/supinator to exclusively a flexor.

**PHOTOPOLYMERIZATION FOR FILLING POROUS CERAMIC MATRIX: IMPROVEMENT OF MECHANICAL PROPERTIES AND DRUG DELIVERING BEHAVIOUR**

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The present work presents the photopolymerisation of composite scaffolds where a prefabricated ceramic scaffold was impregnated with Poly(ethylene) glycol dimethacrylate (PEGDMA) macromer solution. The PEGDMA solution penetrates the porosity of ceramic scaffold where it cures in situ thereby enhancing their mechanical properties. The mechanical properties of the obtained composite approximates the mechanical properties of cancellous bone. The Young’s Modulus of the composite developed was 106±5 MPa. PEGDMA exhibited excellent gel fraction (96%), which was preserved when introduced into the ceramic scaffold as was swelling volume (134%). Thus indicating that the photoinitiation and the polymerization processes were carried out successfully despite of the ceramic scaffold tortuosity. Furthermore, thanks to their swelling properties, the composite was able to control the release vancomycin and dexamethasone. The efficacy of in situ delivery of vancomycin was verified by the inhibition of bacteria colony proliferation. Dexamethasone released is recorded for up to ten days using UV Vis spectroscopy. These excellent results make photopolymerization and PEGDMA strong candidates for the manufacture of different loaded bone void fillers.

**AGE-RELATED DECLINE OF OSTEOGENESIS DEPENDS ON INHIBITION OF PROTEIN KINASE A (PKA) BY PROTEIN KINASE INHIBITOR GAMMA (PKIγ)**

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Aging bone is characterized by decreased osteogenesis and increased adipogenesis resulting in bone loss and diminished repair capacity. We previously showed that PKIγ terminates PTH-induced activity of the cAMP/PKA pathway in mesenchymal precursor cells and that the termination of anabolic primary PKA-response genes (c-fos, IL-6, and LIF) reduces osteogenesis and enhances adipogenesis *in vitro*. We generated and characterized PKIγ−/− mice, which were indistinguishable from wild-type littermates by body weight and fertility. μCT analysis of aging mice revealed that vertebrae of female PKIγ−/− mice exhibit protection from age-related trabecular bone loss (BV/TV, 12-month: 13.9±0.5% vs 10.5±0.4%, p=0.0006 and 24-month: 13.4±1.0% vs 8.0±0.5%, p<0.0001) - resulting from increased Trabecular Number, not Trabecular Thickness. Accordingly, Trabecular Spacing was decreased and Connectivity Density increased with more plate-like geometry (SMI). Substantially smaller increases were seen in female femora, vertebrae and femora of males, and younger mice. Based on the age dependence of the μCT results, we speculated that the decline in osteogenesis with aging would be lessened in PKIγ−/− mice. We investigated intramembranous bone formation 7 days after placing a unicortical titanium implant in the distal femoral metaphysis. Biomechanical pull-out testing showed the expected age-related decline in osteogenesis in wild-type but not in PKIγ−/− mice. As a result, osseointegration in 12-month old PKIγ−/− males was significantly greater than in 12-month old wild-type males: Ultimate Force (7.6±1.7 vs 3.8±2.7N, p=0.02) and Average Stiffness (6.7±1.6 vs 3.4±2.4N/m, p=0.005). These results suggest that disrupting PKIγ regulation of PKA in aging bone can enhance endogenous activators of bone formation.

**BONE APATITE NANOSTRUCTURED COATINGS TO PROMOTE OSSEOINTEGRATION: ANALYSIS OF DIFFERENT APATITE PRECURSORS**

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To boost integration of bone implants, highly adhesive coatings having a composition as similar as possible to the host bone have been proposed. To achieve this goal, both synthetic apatites with an increasing number of ionic substitutions and deposition from biogenic apatite have been pursued. The Authors have proved the possibility to deposit bone-apatite like thin films directly by ablation of deproteinized bovine bone, by Ionized Jet Deposition. Highly adhesive nanostructured coatings were obtained, capable of promoting host cells attachment, proliferation and differentiation. Because the technique allows a precise control over the coating composition, here, several bone apatite precursors (bovine, equine, ovine and porcine bone) were compared in terms of composition and morphology (grazing incidence XRD, FT-IR, SEM/EDS, MIP), to be possibly used as deposition targets. Interestingly, the differences can be of interest for xenografts, bone cements, granulates and other biomaterials composed of biological apatite. Then, several post treatment annealing treatments were compared to optimize crystallinity. Despite all biological samples being essentially composed by hydroxyapatite and carbonated hydroxyapatite, some differences were evidenced in their composition, especially regarding the content of carbonates and magnesium, while essentially no differences were assessed in terms of crystallinity. Significant differences, instead, were assessed in terms of microstructure. A crystallinity very similar to that of bone can be obtained by annealing the coating at temperatures between 350°C and 425°C (formation of cracks is detected at 450°C), that do not cause alterations in the coatings composition.

BIRMINGHAM HIP RESURFACING AND THE ASR: REGISTRY DATA FOR METAL-ON-METAL HIP RESURFACING AT A MINIMUM OF 10 YEARS

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This was a retrospective study of registry data from a National Orthopaedic Hospital. Only ASR and BHR resurfacing systems with a minimum of 10 year data were included in the analysis. Univariate and multivariate analyses controlling for confounding variables were performed to compare outcomes between the BHR and ASR. 392 (119 ASR and 273 BHR) systems were implanted into 364 patients with 10 year follow-up data. Twenty-Seven patients had died on review. There were 38 revisions and 5 re-revisions recorded for all causes of revision. Mean time to revision was 46 months. Mean patient age was 55 and 72% were male. WOMAC scores at 10 years compared to preoperative scores (mean 19.1 at 10 years vs 53.9 preoperative) were much improved. For all-cause revision, the ASR had a 21.8% 10 year revision rate versus a 4.3% 10 year revision rate in the BHR. Other negative predictors of revision included the use of the posterior approach (p<0.01). Causes of revision in order of decreasing frequency included periprosthetic fracture (n=7), aseptic loosening (n=7), adverse reaction to metal debris (ARMD) (n=7), infection (n=5), dislocation (n=3) and pain accounting for the remainder. ASR has a significantly higher rate of revision at 10 years. The BHR has significantly better implant survival at 10 years when compared to the ASR using multivariate analysis. Acceptable 10 year outcomes can be achieved with the BHR only based on our experience. This is the first paper to directly compare these two implants using the Irish NOH joint registry.

DAY CASE PELVIC OSTEOTOMY FOR DEVELOPMENT DYSPLASIA OF THE HIP (DDH)

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We report the results of a pilot study analysing the implications of performing pelvic osteotomies for DDH as a day-case. The financial ramifications and reduction in overnight bed consumption is reported. This was a prospective study analysing Salter and Pemberton pelvic osteotomies performed for DDH for the year 2017. Economic costings were sourced from the official ‘HSE specialty costing report 2016’ which was released in February 2018. 28 Salter and Pemberton osteotomies were performed in 2017. 11 of these cases were performed as day-case procedures. A total reduction in 22 in-patient bed-days were reported. For 11 day case patients, the total cost to treat amounts to €29,370. Calculating the difference, a total saving of €75,174 was made by the hospital. Day case pelvic osteotomies can significantly reduce the number of inpatient bed days in an elective paediatric orthopaedic setting. Economic gains made by treating only 11 patients in this manner
amounted to €70,422. This figure accounts for a single readmission. There was no adverse impact on the standard of clinical care. We propose that the introduction of day case pelvic osteotomy procedures can significantly improve the cost-effectiveness of managing DDH in a paediatric population. Provided there is a stringent analgesic protocol provided on discharge with close clinical follow-up via telephone the following day, this is a safe and very effective innovation in this field which has not yet been described in the literature.

A VIRTUAL CLINIC FOR DEVELOPMENT DYSPLASIA OF THE HIP (DDH)

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This was a retrospective analysis of the activity seen in the virtual DDH clinic for OLCHC during 2017. Dedicated clinical nurse specialists collected the information prospectively and recorded this in the DDH database. Written referrals were discussed at the weekly consultant-lead virtual DDH clinic and radiographs were reviewed in conjunction with the DDH CNS and administrative staff. 350 patients were reviewed in the virtual DDH clinic during 2017. The majority (67%) were referred from area medical officers, while 29% were referred from GP’s. On consultant orthopaedic review, 99 patients were referred on to the physical DDH clinic in OLCHC. The discharge rate from the virtual clinic was 72% which in turn prevented 251 unnecessary patient visits to the physical clinic in 2017. The time saved amounted to between 33 hours and 49.5 hours of clinical time. With the average cost of an out-patient appointment estimated at €129, the overall hospital savings amount to an estimated €32,379. Waiting lists were significantly reduced through the introduction of this virtual clinic also. No patients that were discharged subsequently re-attended the physical clinic, implying that this is a safe means of review. We demonstrate here that the principle of virtual patient care in the setting of elective paediatric DDH can have many significant positive implications for hospital finances, personal patient costs, waiting lists and clinic overcrowding. We recommend the usage of virtual DDH clinics on a national scale for the safe, effective and economic provision of paediatric DDH care.

THE ROLE OF CIRCULAR FRAMES IN THE MANAGEMENT OF LOWER LIMB TRAUMA

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To assess the clinical course and outcomes of patients with tibial fractures managed in circular frames. A retrospective review of all patients requiring circular frame application for tibial fracture management was performed using clinical records, radiographic imaging and patient-reported clinical outcome scores (SF-12 form). 46 patients fulfilled the inclusion criteria. Mean patient age was 45 (10-82). 77% were male. Each had a mean number of 3.8 interventions (2-6, σ=1.179), 3.5 admissions (1-7, σ=1.73) and a total mean length of stay of 33.4 days (3-156, σ=32.5). Mean time to union was 8 months with a 17% rate of non-union. Factors significantly increasing time to union included ‘number of interventions’ (p<0.01), ‘use of bone graft’ (p<0.01) and ‘graft type’ (p<0.05) where the addition of synthetic graft to either allograft or autograft was found to increase the time to union. 25 patients (53%) experienced a delayed union. Deep tissue infection significantly increased the risk of delayed union (p<0.05) as did the use of bone graft (p<0.01). The rate of deep tissue infection was 43%. 11% of patients underwent ankle fusion at a later date. The duration of frame application ranged from 1 to 30 months (mean 6.3, σ=5.37). SF-12 scores ranged from 18-32 (mean 26.9, σ=3.52). Circular frame management incurs a significant burden on both the patient and the health service providing resources to this cohort. Deep infection rates are high and have a significant impact on delayed union. Graft types may also impact on the time to union.

EXTENDED TROCHANTERIC OSTEOTOMY (ETO) FIXATION FOR FEMORAL STEM REVISION IN PERIPROSTHETIC FRACTURES: DALL-MILES PLATE VERSUS CABLES

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Extended trochanteric osteotomy (ETO) is a well established surgical technique used for femoral stem retrieval in revision hip arthroplasty procedures. Fixation of ETO is commonly achieved through wire, cable or cable-plate fixation. No evidence exists to date to suggest which method is superior when used in an acute traumatic setting. 30 cases of acute periprosthetic fracture requiring femoral stem revision with an ETO were identified over a 10 year period. Each case had a loose femoral prosthesis which was revised using an ETO approach. 19 of these were fixed using cables only and 11 were fixed using a cable-plate construct. Radiographic outcomes measured included subsidence, osteolysis, union, time to union and overall success using the Beals and Tower classification. Clinical outcomes were assessed using the modified Harris Hip Score. 23 Vancouver B/C type fractures were identified. The remaining 7 consisted of other fracture types with a loose femoral stem requiring revision through ETO. Mean followup was 32 months in the cable group and 12 months in the cable-plate group. The cable-plate construct performed better than cables alone. Mean subsidence rates were 1.7cm lower in the cable-plate group (p<0.05). Beals and Tower classification of radiographic outcomes was significantly better in the cable-plate group (p<0.01). Modified Harris Hip scores were better in this group also (p<0.05). When utilising an ETO approach for femoral stem revision in acute periprosthetic fractures, superior clinical and radiographic outcomes can be achieved if fixation involves a cable-plate system instead of cables only.

THE PRIMARY CILIUM AS A POTENTIAL CAMP RESPONSIVE MECHANOSENSOR IN MESENCHYMAL STEM CELLS

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Bone adapts to its mechanical environment by sensing fluid flow-induced shear stress within the tissue. However, it’s unclear how mesenchymal stem cells (MSCs) sense and convert this biophysical signal into a biochemical bone forming response. A potential candidate in MSC mechanotransduction is the primary cilium which may act through the 2nd messenger, cyclic adenosine monophosphate (cAMP), which is regulated by Adenylyl Cyclases (AC). Primary cilia (IFT88) and Adenylyl Cyclase6 (AC6) expression was inhibited using siRNA technology. Transfected cells were placed in custom bioreactors where oscillatory fluid shear (OFS) at 1Pa, 1Hz was applied for 15min for cAMP activity analysis or for 2 hours for osteogenic gene expression analysis. cAMP levels peaked after 15mins of shear before returning to basal levels. Following siRNA treatment targeting IFT88 and AC6, expression was significantly knocked down. OFS-induced increases in cAMP were lost when the primary cilium was abrogated. Furthermore, AC6 was found to be expressed in MSCs, where it localises to the primary cilium. As with primary cilia knock down, increases in cAMP following OFS were lost in AC6 siRNA treated cells. MSCs displayed an increase in osteogenic gene expression in response to a mechanical stimulus, however, this response was lost in AC6 knockdown samples like that seen with IFT88 knockdown in previous studies. This study demonstrated a novel molecular mechanism of cilia-mediated mechanotransduction in MSCs, where the primary cilium and AC6 are required for flow-mediated increases in cAMP in MSCs, demonstrating that the primary cilium may act as a cAMP responsive mechanosensor, acting via AC6.

PROFILING EXTRACELLULAR VESICLES DERIVED FROM EQUINE MESENCHYMAL STEM CELLS AND TENDON DERIVED CELLS FOR TENDON REGENERATION

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Tendon injuries represent a clinical challenge for treatment in humans and horses. Extracellular vesicles (EVs) secreted by mesenchymal stem cells (MSCs) are known to be involved in repair and inflammation resolution processes in different tissues and animal species. The main aim of this study is to investigate the role of EVs derived from MSCs and tendon derived cells (TDCs) in promoting tendon regeneration and inflammation resolution pathways via paracrine mediated cellular communication. An equine in vitro model of tendon...
inflammation was used to characterise EVs released by IL-1β stimulated equine MSCs and TDCs at 24 and 48 hours. EVs were quantified in the media by flow cytometry (FCM). The chosen parameters were optimal to detect microspheres from 0.1 to 1 μm diameter simultaneously on the FSC-PMT and Annexin V conjugated with PE was used to count PS positive events. EVs were acquired at medium flow rate for 1 minute. Aliquots of fresh media were tested in the same conditions to establish EV’s background presence. FACS analysis conducted on media (n=3 horses) showed a basal expression of EVs in control conditions. There is no significant difference in EVs numbers produced by either cell types under IL-1β stimulation vs control conditions (no IL-1β) at 24 hours (p = .089) and 48 hours (p = .768). Although, the IL-1β stimulus does not induce a change in the quantity of EVs, it may trigger a qualitative change in the EV cargo. We are currently investigating the potential effect of IL-1β activated EVs to modulate the expression of inflammation pro-resolution markers.

HEME OXYGENASE-1 PREVENTS GLUCOCORTICOID AND HYPOXIA-INDUCED APOPTOSIS AND NECROSIS OF OSTEOCYTE-LIKE CELLS

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Glucocorticoid and hypoxia induce apoptosis and necrosis of osteocyte-like cells. These cell deaths are possibly involved in the development of glucocorticoid-related osteonecrosis (ON). Heme oxygenase-1 (HO-1) has antioxidant and anti-inflammatory effect. We evaluated the cytoprotective effect of HO-1 on glucocorticoid and hypoxia-induced cell deaths in osteocyte-like cells. Murine osteocyte-like cell (MLO-Y4) was cultured. Hemin was used as a HO-1 inducer. After MLO-Y4 was cultured with 10 μM hemin for 18 hours under normoxia (20 % O2), the gene and protein expression of HO-1 in hemin (+) group were compared with hemin (-) group by real-time RT-PCR and Western blotting respectively. In hemin (+) group, HO-1 gene expression significantly increased compared with that in hemin (-) group (p <0.01), and protein expression also increased. Cells were divided into the three groups as follows: control group: cultured under normoxia; DH group: cultured under hypoxia (1 % O2) with 1 μM Dexamethasone (Dex) for 24 hours; DH-h group: cultured with 10 μM hemin for 18 hours under normoxia, and then cultured under hypoxia and 1 μM Dex for 24 hours. The ratios of apoptosis and necrosis were measured by flow cytometry and compared between the three groups. The proportion of apoptosis and necrosis was measured by flow cytometry and compared between the three groups. The proportion of apoptosis and necrosis (11.93%, 3.02%) in DH-hemin was significantly lower than those (14.95%, 7.56%) in DH (p <0.01). Hemin reduced glucocorticoid / hypoxia-induced cell deaths in osteocyte-like cells. The up-regulation of HO-1 may cause the cytoprotective effect of hemin on osteocyte-like cells. The HO-1 induction could be the effective treatment of glucocorticoid-related ON.

EVALUATION OF ACCURACY AND A LEARNING CURVE OF ACCELEROMETER-BASED COMPUTER NAVIGATION IN TOTAL KNEE ARTHROPLASTY

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The KneeAlign2 (OrthAlign,Inc., Aliso Viejo, CA) is a portable accelerometer-based navigation device for use in performing the distal femoral resection in total knee arthroplasty (TKA). The aim of this study was to investigate the accuracy in positioning the femoral component and the presence of a learning curve in conducting TKA using this device. 60 knees underwent a primary TKA using the KneeAlign2. These TKAs were divided in two groups. Group1: operated by surgeon of experience using the KneeAlign2 more than 30 cases. Group2: operated by surgeons of experience using the KneeAlign2 less than 30 cases. Standing AP hip-to-ankle radiographs were obtained postoperatively. Positioning of the femoral component was measured by the radiographs. Outlier in coronal alignment were defined as >3°. The radiographic results and operation time were compared between the groups. Students t-test was performed to assess the statistical analysis (p<0.05). There was no outlier and all patients had an alignment within 90±3°to the femoral mechanical axis in the coronal plane in both groups. The mean deviation (absolute values) from the neutral alignment of the femoral component were 1.5±0.5 in group1 and1.2±0.7 in group2. There was no statistical significance between the groups. Average operation time was 106.2 minutes in group1 and 108.5 minutes in group2. There was no statistical significance between the groups. The KeeAlign2 is highly accurate in positioning the femoral
component. As the learning curve does not be observed, this portable navigation is easy to handle even for beginner users.

**A REGISTRATION METHOD TO ASSESS TIBIAL BONE MINERAL DENSITY IN THREE DIMENSION USING CT SCAN**

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Alterations of bone mineral density (BMD) are a common hallmark to both osteoarthritis and osteoporosis. BMD is usually measured using DXA. This imaging technique is however two-dimensional, prohibiting comprehensive spatial analysis of BMD. Three-dimensional imaging techniques, such as computed tomography (CT), exist, however they require statistical methods to derive anatomical correspondences among samples and thus allow analyses despite variations in bone shapes. More recently, successful computational anatomy methods were proposed to establish anatomical correspondences among bones. Nevertheless, there is a paucity of such methods for the proximal tibia, specifically when only a portion of the tibia is imaged. The objective of this study was to develop a method for three-dimensional analysis of BMD in the proximal tibia. The anatomical correspondence between tibia models, obtained by segmentation of the CT images, was established following a three-step mapping procedure. Specifically, the mapping was determined based on a rigid alignment of the tibias, followed by a non-rigid registration of the transverse bone section areas and a non-rigid registration of the models. This method was used to map 23 healthy knees onto a reference tibia and to calculate the three-dimensional distribution of BMD inside the tibia. This procedure reported excellent reproducibility (ICC of 0.964±0.01), and identified a common pattern of BMD in the tibias. In the future, it could be used to quantify BMD alterations inside the tibia in three-dimension notably in case of osteoarthritis or osteoporosis. The presented method could also be used with other three-dimensional imaging techniques and applied to other pathologies.

**STANDARDISED HISTOPATHOLOGIC SCORING SYSTEM TO ASSESS TENDON HEALING**

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Superficial digital flexor tendinopathy is a common and potentially devastating injury in all equine athletes. Regenerative medicine and cell-based therapy have shown promising results in the treatment of tendinopathy. The aim of this project was to develop and validate a specific histological scoring system dedicated to the assessment of equine tendon lesions treated by regenerative medicine. We have developed a semi-quantitative histopathologic scoring system based on commonly used scoring methods reported in the literature, which aims to improve over previous systems to focus on key parameters related to tendon regeneration that is easily assessable on longitudinal H&E sections by both inexperienced and specialized pathologists. The parameters include tenocyte density and morphology, leukocytes, neovascularization, interfascicular matrix density and collagen fibre organisation and crimp. Histological slides were blindly scored with bright field microscopy to determine morphology and cellularity and with polarized light microscopy to evaluate the collagen crimp. The scoring system was compared with the previously published systems using tendon samples from unaffected horses and horses with surgically induced core lesion of the superficial digital flexor tendon treated by regenerative medicine. The new system better reflected re-establishment of normal morphology.

**TOPOGRAPHY-INDUCED MECHANOSENSATION IS A CONTEXT-DEPENDENT REGULATOR OF STEM CELL DIFFERENTIATION TOWARDS THE TENOCYTIC LINEAGE**

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Tenocytes, the main cell type of the tendon, require mechanical stimulation for their proper function. *In vitro*, it is known that micro-topographies provide biomechanical cues and guide cell fate. In light of this, we
previously found that micro-topographies promote the expression of mechanosensitive transcription factors and tendon related matrix proteins on tenocytes. In this study, we investigated if micro-topographies can elicit a similar effect on human adipose-derived mesenchymal stem cells (AD-hMSCs) and can guide differentiation towards the tenogenic lineage. By utilizing the TopoChip, a platform containing 2176 unique micro-topographies, we identified an optimal in vitro niche by screening for Scleraxis (SCX) expression, a tenogenic differentiation marker. Through machine learning algorithms, we associated SCX levels with morphological characteristics and topographical design parameters. Large surface fabrication of micro-topographies inducing significant higher SCX levels allowed studying the dynamics of tenogenic marker expression and the pathways involved in their regulation. Through gene expression studies, we found that micro-topographies elicited an early activation of the mechanosensitive transcription factors EGR-1, SCX, and MKX, followed by a late upregulation of the tendon related matrix proteins COL-I, COL-III, BGN, and DEC. Of interest, we found a synergistic effect on SCX levels when combining micro-topographies with TGF-β2, indicating a topography-induced sensitization towards this signaling pathway. Further investigation found an upregulation of the TGF-β receptor and inhibitor experiments confirmed the importance of TGF-β signaling in topography-induced mechanotransduction. The knowledge gained from these studies will be essential for creating a new generation of culture platforms where surface architectures guide cell fate.

GRADED IMPLANTS FOR ROTATOR CUFF REPAIR – SPECIFIC ANIMAL MODELS IN THE SELECTION PROCESS

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Chronic rotator cuff tears are a well-known problem in shoulder surgery. Due to tendon contraction and fatty muscle degeneration, implant material is necessary for surgical treatment. Due to different tissue requirements (tendon, cartilage, bone) in the enthesis region, graded implant materials with special adaption to the respective demand are developed and first tested in vitro. Prior to clinical use, preclinical testing in animal models is necessary. Therefore, different small and large animal models are used in the selection process of new materials. For first angiogenesis and biocompatibility testing dorsal skin and femur chamber models in mice and rats are used. With these models, capillary density and leucocyte infiltration can be observed in vivo in the postoperative time period of up to 21 days at different selected time points additional to histological evaluations. With these models a first selection of promising materials is possible. A chronic rat tendon defect model is the next step, where a defect of the M. infraspinatus is created and refixed four weeks later with selected materials. After eight weeks, biomechanical testing of the defect area is performed. Differences in biomechanical stability can further specify suitable materials. At the end of the selection process, the material is implemented in a chronic tendon defect in a sheep, with a situation most similar to humans. These selection steps lead to a fast feedback to the production processes and offer the opportunities for specified adaptions in the material composition and therewith improvement prior to expensive and time-consuming large animal studies.

GLYCOSAMINGLYCAN DISSACHARIDES CHANGES DURING CHONDROGENIC DIFFERENTIATION OF HUMAN BONE MARROW/SYNOVIAL-DERIVED MESENCHYMAL STEM CELLS UNDER DIFFERENT OXYGEN TENSIONS

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Glycosaminoglycans (GAGs) are major components of cartilage extracellular matrix, which play an important role in tissue homeostasis not only in providing mechanical resistance to compressive loads, but also as signalling mediators of cell adhesion, migration, proliferation and differentiation. GAGs are linear, highly charged, acidic carbohydrates with a repeating disaccharide unit. Specific GAG types as well as their disaccharide sulfation patterns can be predictive of the tissue maturation level but also of disease and degeneration states. In this work, we used a highly sensitive liquid chromatography-mass spectrometry (LC-
MS) method to perform a comparative study in terms of GAG type, amount, disaccharide composition and sulfation patterns between chondrogenic micromass tissues generated from human bone marrow- and synovial membrane-derived mesenchymal stem/stromal cells (hBMSC/hSMSC). The effect of oxygen tension on GAG composition and disaccharide patterns was also evaluated by the analysis of chondrogenic cultures performed under normoxia (21%O2) and hypoxia (5%O2). Prior to LC-MS analysis, the chondrogenic differentiation of hBMSC/hSMSC cultured under different oxygen tensions was assessed by micromass average size, total collagen and GAG content as well as by histological and immunofluorescence stainings. Afterwards, chondrogenic micromasses were lysed using a commercial surfactant reagent, sonicated and digested with GAG lyases. The resulting disaccharides were recovered by centrifugal filtration, labelled and analysed by LC-MS. The GAG profiles obtained were compared with undifferentiated cells and human articular chondrocytes. In overall, the obtained GAG profiles may provide new insights to characterize the quality of MSC-generated chondrocytes from different cell sources and obtained under distinct culture conditions.

EXTRACELLULAR MATRIX DECORATED POROUS POLYCAPROLACTONE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Synthetic polymer-based materials, such as polycaprolactone (PCL), have been widely applied as scaffolds and used commercially in bone tissue engineering (TE). However, their ability to promote osteogenesis is generally limited by sub-optimal cell-material interactions resulting from the lack of biological active sites. The extracellular matrix (ECM) is a three-dimensional (3D) network of structural and functional molecules, which provide an appropriate microenvironment for cell adhesion, migration, proliferation and differentiation. Therefore, the decoration of synthetic scaffolds with decellularized ECM has received considerable interest as a strategy to enhance their biological performance. Herein, we developed an extrusion-based 3D porous PCL scaffold with controlled architecture, porosity and high interconnectivity which was decorated with human mesenchymal stem/stromal cell (hMSC)-derived ECM by culturing hMSC in PCL scaffolds for 14 days, allowing them to adhere, proliferate and secrete ECM. Afterwards, the resulting constructs were decellularized and hMSC-derived ECM decorated PCL scaffolds were obtained. Scaffold morphology and ECM presence were confirmed by scanning electron microscopy (SEM)/Energy dispersive X-ray (EDS) analysis and immunofluorescence stainings. The ability of hMSC-derived ECM-decorated PCL scaffolds to promote MSC proliferation and osteogenic differentiation was assessed and compared to the untreated scaffolds. After 21 days under osteogenic induction, ALP activity levels, mineralization deposition and mRNA levels of osteogenic markers (COL1 / Runx2 / ALP / OPN / OC) were evaluated. In overall, we described a method to fabricate hMSC-derived ECM-decorated PCL scaffolds with high interconnectivity and enhanced biological performance, which are promising for bone TE applications, as demonstrated by the beneficial effect on hMSC proliferation and osteogenic differentiation.

TRAPEZIOMETACARPAL JOINT STABILITY: LATERAL PINCH VS POWER GRIP

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Cases of trapeziometacarpal (TM) joint arthritis present clinically with some degree of dorsal subluxation. Therefore, a dorsally directed force may have a role in disease development. Herein, we endeavored to understand forces inducing dorsal subluxation and determine if they result from key pinch or power grip. Five non-arthritic cadaveric specimens were prepared by stabilizing the wrist, fixing the 2nd through 5th metacarpal-phalangeal joints in 60° flexion, and mounting the forearm onto a frame. Extrinsic and intrinsic tendons were prepared for weight suspension. Specimens were loaded into power grip and key pinch configurations, then all TM ligaments were resected in order to record their effect on joint stability. Stability was evaluated statically and dynamically in power grip and key pinch. The TM joint was statically and dynamically stable after resection of all supporting ligaments in the key pinch configuration. Conversely, in the power grip configuration, 3 specimens dislocated and 2 subluxed prior to a dynamic challenge (statically),
and all 5 specimens dislocated when dynamically challenged. We demonstrated that the forces induced on the TM joint during pinch do not generate a shearing component requiring ligament restraint to maintain stability; the forces induced during grip do. Therefore, dorsal subluxation and resulting arthrosis may be the result of a dorsal shearing force generated during gripping in the presence of incompetent TM ligaments. Power grip generates shear forces that may promote or exacerbate TM osteoarthritis, while pinch does not. This may have relevance in conceiving rehabilitation protocols, surgical techniques and designing prosthetic joints.

THE PARALLELOGRAM EFFECT: HOW CENTRAL BAND FAILURE CAN CAUSE ULNAR IMPACTION SYNDROME

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Ulnar impaction syndrome is a degenerative wrist condition caused by increased loads of the ulnar head onto the carpal bones (carpal abutment). We hypothesize that elongation of the central band of the forearm interosseous ligaments may change longitudinal radial-ulnar relationships resulting in an ulnar positive wrist and therefore carpal abutment. Six cadaveric, human forearms were used to measure relative displacement of the ulna during axial loading of the radius. The IOL complex, TFCC, radio-ulnar joints and the elbow collateral ligaments were preserved. The ulnar shaft was oriented vertically and rigidly fixed to an adjacent vertical beam. We applied axial loads onto the lunate facet of the distal radius. Radial heights were measured in supination and pronation under a 5lbF preload. Gradual axial loads were applied up to 50lbF and the resultant axial displacement was measured. All measurements were evaluated with the IOL intact and repeated with the central band cut. Due to a parallelogram effect, the radius shifted proximally under a 5lbF preload, creating an ulnar positive wrist relationship. Dynamic loading of the forearm after ligament excision resulted in increased ulnar variance, suggesting dynamic impaction often observed clinically. In supination, the radius displaced 2.1x further after the central band was cut (3.00mm). In pronation, the radius displaced 1.8x further when the central band was cut (2.84mm). In summary, our study demonstrates that the central band plays a role in maintaining longitudinal stability and therefore ulnar variance.

ALTERED WALKING AND MUSCLE PATTERNS REDUCE HIP CONTACT FORCES IN INDIVIDUALS WITH SYMPTOMATIC CAM FAI

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Cam-type femoroacetabular impingement (FAI) is a causative factor for hip pain and osteoarthritis. It is still unclear what role muscle forces play and how they affect the hip joint loading, therefore the purpose was to examine muscle contributions and hip contact forces in individuals with symptomatic cam FAI during level walking. Gait kinematics and kinetics of 18 symptomatic patients (FAI) and 18 control participants (CON) were recorded using a motion capture system (Vicon MX-13) and force plates (Bertec FP4060-08). Muscle and hip contact forces were subsequently computed using a musculoskeletal modelling program (OpenSim 3.1). The FAI group walked slower and with shorter steps, showing reduced psoas major (Median = 1.1, IQR = 1.0–1.5 N/BW) and iliacus forces (Median = 1.2, IQR = 1.0–1.6 N/BW), compared to the CON group (Median = 1.6, IQR = 1.3–1.6 N/BW, p = 0.004; and Median = 1.5, IQR = 1.3–1.6 N/BW, p = 0.03, respectively). The FAI group altered their walking mechanics to reduce their psoas major and iliacus muscle forces. This can be interpreted as a protective mechanism, which ultimately resulted in lower hip contact forces to the anterosuperior acetabulum (p < 0.05). Limited hip mobility is not only attributed to bone-on-bone impingement, caused by the cam morphology, but could be attributed to musculature as well. Athletic conditioning could further strengthen core muscles for improved hip mobility and pelvic balance. Thus, these findings can help guide nonsurgical management as well as postoperative protocols for the treatment of symptomatic FAI.

TOTAL COST OF TREATMENT: ASSESSING THE KEY FACTORS IN HIP FRACTURE

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Several authors have reported on time to surgery (TTS), length of stay (LOS) and total cost. Anecdotal evidence from our trauma service suggests that all hip fractures are not created equal. We decided to investigate this suspicion and captured data on a retrospective cohort of 400 neck of femur (NOF) performed at Mayo University Hospital. We analysed this hip fracture population and show how patient demographics and operative features may directly impact LOS and cost of treatment. While 8 patients required major optimisation pre-op, 229 (89%) reached theatre within 24 hrs of admission. 55% underwent hemiarthroplasty while 45% received fixation. Mean LOO varied significantly from 1:22 [0:23 to 5:08] depending on surgery performed. Hemiarthroplasty took 1:19 while fixation took 0:05 longer. LOS did not correlate to age or to operation performed. LOS was significantly shorter for patients discharged Home (12 days, same hemiarthroplasty/fixation ratio). Mean total cost is €22,155 with implant, theatre and ward costs €1,045, €2,555 and €18,555 respectively. LOS is significantly less for patients discharged Home (p < 0.001): they are 5-years younger than the ⅓ discharged to Nursing Home or District Hospital. Total cost of DHS (€21,758) is 23% less than that of IM Nailing (€28,385); PCCP is ½ the cost of DHS or IM Nail (p < 0.05). The cost of an orthopaedic bed (€750/day) is still the most critical factor; €18,555, or 84% of €22,155.

ARTHROSCOPIC PARTIAL MENISCECTOMY: SYSTEMATIC REVIEW AND META-ANALYSIS

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25% of adults aged over 50 experience frequent knee pain from degenerative knee disease. Today it is common practice to treat concomitant meniscal tears with arthroscopic partial meniscectomy (APM). Due to the sheer number of procedures involved annually (100 to 225 per 100,000 patients), we review the published evidence base. Literature search identified 10 RCTs (level 1 evidence) which compare APM to conservative treatment for degenerative meniscal tears in middle-aged patients. Of the 10 trials included in our meta-analysis, 90% do not report any long term benefit in terms of reduced knee pain over conservative management. When we analyse the 8 RCTs which report knee pain at 3-months, we find a statistically significant reduction in pain of -0.24 (95% CI -0.37 to -0.10, p < 0.001, I2 = 0%) equivalent to an improvement of only 0.5 points (on a 0 to 10 VAS). 8 RCTs report knee pain at 1-year follow-up: meta-analysis shows that there is absolutely no benefit for knee arthroscopy compared to conservative management (SMD = 0.00, p = 0.96). In 2017 the Canadian AAC wrote that ‘arthroscopic debridement is not indicated as primary treatment in the management of osteoarthritis of the knee. This position echoes guidelines published by the Australian AKS, American AAOS, German DGOU, French HadS and British BOA. Meta-analysis shows a benefit at 3-months corresponding to a reduction in pain of 0.5 points (on a 0 to 10 VAS). At 1-year there is no benefit for arthroscopy compared to conservative management.

ENHANCED BONE REGENERATION USING INJECTABLE HYDROGELS CONJUGATED BY OSTEOGENIC PEPTIDES

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Hydrogels have been widely used as potential biomaterials to incorporate bioactive molecules, such as proteins and peptides to improve the bone regeneration. However, the commonly physical mixing and adsorption methods limited their loading efficiency and controlled release. In this study, we developed a hydrogel that can chemically conjugate peptides and crosslinked in situ, using horseradish peroxidase (HRP)/hydrogen peroxide (H2O2)-catalyzed the reaction between phenol or aniline derivatives. A bioactive calcium accumulating peptide (CAP) containing a collagen binding motif, which can induce osteogenic differentiation, was synthesized. The tyrosine residues in CAP enable the in situ conjugation of peptide into the gelatin-hydroxyphenyl propionic hydrogels through tyrosine-tyrosine conjugation under HRP/H2O2 conditions. The human periodontal ligament stem cells (hDPLSCs) was loaded into the in situ forming gelatin hydrogel conjugated CAP, to evaluate the acceleration of bone formation. In vitro study demonstrated the bone mineralization and the increase in osteogenic marker expression of CPA/gelatin hydrogels. The remarkable recovery effect after 4 weeks implantation of hydrogels was observed in vivo. These results suggest that
injectable hydrogels conjugated CAP are potential as engineered microenvironment to enhance bone regeneration and deliver stem cells in tissue regeneration.

**QUANTITATIVE ANALYSIS OF THE OSTEOCYTE SECRETOME FOLLOWING OSCILLATORY FLUID SHEAR STIMULATION**


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The osteocyte is the most abundant cell in bone and is an important component of loading-induced bone formation, regulating its secretion in response to mechanical loading to mediate stem cell recruitment and osteogenic differentiation. Although numerous osteocyte secreted factors have been identified, the full secretome of the osteocyte and thus its therapeutic potential remain poorly explored. Therefore, the objective of this study was to identify the secretome of the osteocyte and determine how this is altered in response to mechanical loading, with the aim of identifying novel targets to enhance stem cell contributions to bone formation. Osteocytes were either cultured statically, or subjected to 2 hours of fluid flow induced shear (1Pa, 1Hz), with migration of human skeletal stem cells (hSSCs) being enhanced over 2-fold towards medium from dynamically cultured cells, as well as displaying increased COX2, OCN, OSX and BMP2 gene expression. A proteomic analysis of osteocyte conditioned medium (CM) identified over 300 proteins, with 97 of these being significantly upregulated compared to control medium. Further analysis revealed significant enrichment of several “extracellular” and “binding” related gene ontology terms, indicating a key role of the osteocyte secretome in mechanosignaling. Furthermore, 34 proteins were found to be differentially expressed in the secretome of mechanically loaded osteocytes, indicating potential roles in mechanically mediated signaling. This study highlights the key role of the osteocyte in signaling within bone, and reveals for the first time a complete map of proteins released by the osteocyte which may represent novel therapeutic targets for bone regeneration.

**CALCIUM PHOSPHATE, CHITOSAN AND HYALURONIC ACID - BIOMIMETIC SUBSTRATE MODULATES MONOCYTE/MACROPHAGE INFLAMMATION**

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The use of inorganic calcium phosphate supplemented with biopolymers has drawn lots of attention in bone regenerative medicine. While inflammation is required for bone healing, its exacerbation can alter successful tissue regeneration. Owing to their critical role in bone dynamics, investigating the interaction between monocytes/macrophages and bioactive/ osteoinductive calcium phosphate/chitosan/ hyaluronic acid (CaP-CHI-HA) will provide essential information for the rational design of new biomaterials. CaP-CHI-HA cytocompatibility was monitored by WST-1 assay, DNA quantification and intracellular accumulation of reactive oxygen species (ROS). The low metabolic activities and DNA content of THP-1 on CaP-CHI-HA, in addition to the absence of an increase in ROS intracellular accumulation compared to LPS positive control, confirmed its biocompatibility. On CaP-CHI-HA, THP-1 exhibited a sub-membranous F-actin localization with a prominent distribution of vinculin throughout the cytoplasm and the membrane. In contrast, with LPS stimulation, F-actin was mostly arranged as spike-like protrusions of the cell membrane with vinculin evenly localized at peri-nuclear region. Exposing monocytes to CaP-CHI-HA resulted in a secretion of pro-healing VEGF and TGF-β growth factors, TNF-α, MCP-1, IL-6 and IL-8 pro-inflammatory mediators, but also IL-10 anti-inflammatory cytokine along with an inflammatory index below 1.5 versus 7.5 following monocytes LPS stimulation. Although CD44 receptor seems not to be involved in the inflammatory index regulation, results suggest a potential role of the chemical composition and calcium release from CaP-CHI-HA, in affecting the intracellular expression of calcium sensing receptors. Herein, our findings indicate a great potential of CaP-CHI-HA in providing a moderate inflammatory response, suitable for bone regeneration.

**LIMITED PENETRATION OF COBALT AND CHROMIUM IONS INTO THE CEREBROSPINAL FLUID FOLLOWING METAL ON METAL ARTHROPLASTY: A CASE-CONTROL CROSS SECTIONAL ANALYSIS**
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The aims of this study were to determine whether cobalt (Co) and chromium (Cr) ions were transferred from joint fluid to cerebrospinal fluid (CSF) in patients undergoing metal-on-metal (MoM) hip and knee arthroplasty procedures, and to assess the contribution of implant history and patient factors. An observational, non-randomised cross-sectional study was conducted with 461 patients presenting to a single-surgeon private clinic for treatment of degenerative conditions of the hip and knee. Blood and fluid samples were collected intraoperatively and analysed for proteins and trace elements. The presence of an implant was associated with significantly higher Co and Cr concentrations in plasma, but not CSF. In absolute terms, <1% of joint fluid metals, and <15% of plasma metals were found in CSF. An association between the ratio of CSF and plasma concentrations, and plasma levels was observed. Partial least squares regression models revealed different mechanisms of diffusion between Co and Cr to the CSF, with the presence of an implant not associated with ion levels. The presence of MoM implants is associated with significantly higher plasma concentrations of Co and Cr but not CSF, with an apparent influence of plasma metal load on the CSF/plasma ratio. Co and Cr appear to be transferred to the CSF by different mechanisms, and their concentrations appear dependent on other factors yet to be identified. Although higher levels of plasma ions are associated with above average CSF metal concentrations, the thresholds for neurotoxicity remain unclear and require further study.

EFFICACY OF SHORT-STEM, BONE-PRESERVING HIP RESURFACING FOR OSTEONECROSIS OF THE FEMORAL HEAD: A PROSPECTIVE OBSERVATIONAL COHORT STUDY WITH MINIMUM FIVE-YEAR FOLLOW-UP
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Published medium-term outcomes of bone conserving metal-on-metal resurfacing as a surgical option for osteonecrosis of the femoral head (OFNH) remain sparse. This study reports the medium-term survival and patient-reported outcomes of bone-preserving hip resurfacing in patients presenting with avascular osteonecrosis treated in an independent clinic. A series of patients (N=28, 24 males) presenting with hip osteoarthritis associated with OFNH (Ficat-Acet stage >2) underwent hip resurfacing with a mid-head resection device. Patients were followed up with serial radiographs and patient-reported outcomes (Veteran’s Rand-36, Harris Hip Score, Western Ontario and McMaster Universities Osteoarthritis Index, Tegner Activity Scale). A survivorship analysis was performed with the Australian National Joint Replacement Registry. Latest follow-up was a median of 6.5 years (IQR 5.5 - 8.0), with one revision detected (96.7% cumulative survival) and significant (P<0.001) improvements overall, exceeding minimally clinically important improvements in patient-reported outcomes. A high incidence of lucency was observed around the cup (85.7%) and stem (74.1%), with neck narrowing exceeding 10% of initial neck width in 27.8% of the sample. Hip resurfacing with a mid-head resection device provided excellent outcomes in younger patients with ONFH at medium-term follow up. Future studies should compare its performance against alternative options (hip resurfacing, total hip arthroplasty) for this indication in a larger sample.

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM A PATIENT WITH AUTOSOMAL RECESSIVE OSTEOPETROSIS
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Autosomal recessive osteopetrosis (ARO) is a rare heterogeneous disorder, diagnosed by increased bone density in early infancy associated with dysfunctional osteoclasts in bone resorption. Genetic association analysis revealed the relationship between mutations in the SNX10 gene and ARO pathogenesis. It has been shown that this mutation results in dysfunctional osteoclasts by disturbing the formation of ruffled borders in the cells. In order to further understand the molecular mechanisms and consequences of the SNX10 mutation in ARO development, we generated iPSCs from a 41-year-old female ARO patient, who carries the homozygous c.212 + 1G > T (g.72742G > T) mutation in SXN10. In total, 18 iPSC-like colonies were
generated from the patient's dermal fibroblasts using retroviral plasmids containing hOCT4,OX2,C-MYC and hKLF4. G-banding analysis proved that the selected ARO-iPSC1-11 line carried a normal karyotype. Furthermore, genomic DNA sequencing verified that the disease related mutation (g.72742G N T) was retained in the generated iPSCs. To evaluate the pluripotent state of ARO-iPSC1-11, gene expression analysis confirmed the positive expression of endogenous reprogramming factors (OCT4, SOX2 and NANOG), but silencing of reprogramming factors expressed by the retroviral transgenes. Immunofluorescent staining demonstrated that ARO-iPSC1-11 also expressed pluripotency markers including TRA1-60 at the protein level. This ARO patient-specific iPSC line containing the SNX10 mutation provides opportunities to study the pathobiology of SNX10-dependent ARO, and provide a testing platform for screening therapeutic agents in osteopetrosis and metabolic bone diseases.

APPLICATION OF CALCIUM PHOSPHATE-BASED BONE SUBSTITUTE ISOLATED AND ASSOCIATED WITH COLLAGEN MEMBRANE IN BONE DEFECTS

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The aim of this study was to evaluate the process of bone regeneration in defects of rabbit tibias using bone cement isolated or associated with collagen membrane. The study was approved by the ethics committee of the Federal University of Campina Grande under number 097/2016. Twelve adult rabbits of both sexes, divided into two experimental groups bone cement group (GBC) and bone cement group plus collagen membrane (GBCM), were studied. Each group was subdivided into two subgroups, according to the period that these animals were euthanized, 30 and 60 postoperative days. Two bone defects with 2 mm of diameter were performed, one at the proximal tibiae diaphysis and another one on the distal tibiae diaphysis of each pelvic limb. In the right limb (GBC) the defects were filled with bone cement; in the left limb (GBCM) the defects were filled with bone cement plus collagen membrane of bovine origin. Radiographic, histological and Scanning Electron Microscopy (SEM) analysis were performed. Osseointegration of the implants was shown through radiographic evaluation. Histological results indicated an intense bone neoformation but a progressive degradation of the implants was noticed by SEM. However, no significant differences were observed in relation to the bone regeneration between the experimental groups; nonetheless, it was verified that at 60 postoperative days the bone repair was more pronounced and the bone neoformation was intensified when compared to the 30 days. According to the results obtained, all the samples were gradually absorbed during the evaluated periods, being replaced by neoformed bone tissue.

PRODUCTION AND CHARACTERIZATION OF UV-CURABLE MATERIALS FOR ENHANCED BONE REGENERATION

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In this work, materials for enhanced bone regeneration after multiple fractures were investigated. In a conventional treatment of a multiple bone fracture, gathering bone fragments can take long time for healing, followed by the fixation of the bone using bone screws and plates. In case of the removal of a larger amount of bone, the metal objects have to stay in the body. Therefore, it would be interesting to be able to apply a material to fix the multiple bone fragments during a surgery, so they can be incorporated in the regrowing bone. For this purpose, a UV-curing material would be interesting, as portable UV-curing devices are already applied for example in dental treatments. Therefore, in this work, NiPAAm polymer-based composite materials with ceramic additives were synthesized via UV-initiated radical polymerization. The influence of different additives on mechanical properties of the polymer as well as the prepolymer mixture were investigated in order to analyze the suitability of the composites for bone healing applications. Further, the results were compared with the properties of the polymers in swollen state. It can be stated that it was possible to find mechanically suitable additive to increase applicability of the prepolymer mixture in surgeries. The addition of ceramics to these composites did not decrease their stability drastically and it was even possible to polymerize the monomers using hand-held UV devices. Cytotoxicity tests revealed these samples are non-toxic and slightly increases these values when ceramics additives are incorporated into the polymer matrix.
ESTIMATING JOINT LOADING USING INERTIAL MEASUREMENT UNITS AND GROUND REACTION FORCES

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To calculate joint loading, integrated 3D motion capture and ground reaction force (GRF) measurements in combination with musculoskeletal modelling is typically used, limiting its use to a lab-based environment. Inertial measurement units (IMU) can be used outside the lab to measure kinematics. Relating IMU kinematics to joint loading therefore holds the potential to estimate joint loading in activities of daily living in an ambulatory setting and even in the patients’ home environment. The goal of the present study is to determine if IMU kinematics can estimate hip and knee loading or if GRF are additionally required. Twelve healthy control subjects performed gait while synchronously measuring 3D marker trajectories (Vicon), GRF (AMTI) and IMU kinematics (Xsens). Hip and knee loading were calculated using OpenSim 3.3 using the 3D marker trajectories and GRF. A multiple regression analysis related peak hip and knee loading to peak sagittal plane IMU lower limb joint angles, linear and angular accelerations and GRF. The minimal number of variables required for a significantly good coefficient of determination (R2>0.5) were taken into account (p<0.1). Hip loading can be estimated using only IMU kinematics (with 6 variables R2 = 0.71). For knee loading the GRF is additionally needed (with 2 variables R2 = 0.52). These results suggest that a combination of multiple IMU kinematics is required to estimate hip loading reliably, whereas the estimation of knee joint loading remains inferior even when including GRF data.

EVALUATION OF CORTICAL BONE IN DIABETIC RATS USING SWIFT

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Diabetes decreases bone strength, possibly because of cortical bone changes. Sweep imaging with Fourier transform (SWIFT) has been reported to be useful for cortical bone evaluation. The Purpose of this study was to evaluate cortical bone changes in diabetic rats using SWIFT, assess the usefulness of this technique through comparisons with conventional MRI, and clarify the mechanism underlying cortical bone changes using histomorphometry. 8-week-old male Wistar/ST rats (N = 36) were divided into diabetes (induced by streptozotocin injection) and control groups. 6 animals from each group were sacrificed at 2, 4, and 8 weeks after injection. Tibial bones were extracted and evaluated using MRI. Proton density-weighted imaging (PDWI) and SWIFT were performed. The signal-to-noise ratio (SNR) was calculated for each acquisition. The bone formation rate was evaluated using histomorphometry. Findings at each time point were compared using Mann–Whitney U tests. At all time points, PDWI-SNR showed no significant differences between groups (P = 0.59, 0.70, and 0.82 at 2, 4, and 8 weeks, respectively), SWIFT-SNR was significantly lower in the diabetes group than in the control group (P < 0.05 at 2 and 4 weeks and P < 0.01 at 8 weeks), and the bone formation rate was significantly lower in the diabetes group than in the control group (P < 0.01 for all). Our results suggest that SWIFT can detect cortical bone changes in diabetic rats earlier and more sensitively than conventional MRI. Thus, it may be a useful tool for evaluation of the bone turnover and bone quality, which will aid in the diagnosis of osteoporosis, in patients with diabetes.

WHAT IS THE BENEFIT OF USING AMNIOTIC MEMBRANE IN ORAL SURGERY? AN EXHAUSTIVE REVIEW OF CLINICAL STUDIES

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Since its first use for the reconstruction of tissue defects in the oral cavity in 1985, human amniotic membrane (hAM) has been widely studied in the field of oral surgery. However, there is no systematic review concerning its clinical applications and relevance for soft and hard tissue reconstruction in the oral cavity. The aim of this review is to provide a thorough understanding of the potential use of hAM in oral surgery. A systematic
electronic and a manual literature search of the MEDLINE-PubMed database and Scopus database was realized. Patient, Intervention, Comparison and Outcomes (PICO) technique was used to select the relevant articles to meet the objective. Studies using hAM for oral reconstruction, and conducted on human subjects, were included in this survey. A total of 22 articles were analyzed, and five potential clinical applications were identified: periodontal surgery, cleft palate and tumor reconstruction, prosthodontics and peri-implant surgery. Periodontal surgery was the only area to assess the efficacy of hAM with randomized clinical trials. A wide variability of the preservation methods of hAM and the lack of objective measurements were observed. Much studies now supports the use of hAM in the field of oral surgery, but, they consisted mostly in retrospective case series. Thanks to its biological and mechanical properties, hAM is promising as a treatment for wound healing in various areas of oral reconstruction. However, further randomized clinical trials are needed to confirm these preliminary results.

TRANSLATION OF CHONDROGENESIS FROM IN VITRO TO IN VIVO: ROLE OF MATRIX MECHANICAL PROPERTIES AND DEGRADATION

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Despite the tremendous progress in the past decade in translating biomaterials in cartilage regenerative medicine from in vitro studies to in vivo applications, there is still a lack of complete appreciation of the interplay between degradation and mechanical properties of biomaterials on cell fate decisions. While engineering cartilage tissue in vitro (ex vivo) is well established, the fate of such engineered tissue upon implantation remains less investigated. Recently, tissue engineered nasal chondrocyte constructs have been successfully used in knee articular surface arthroplasty and in facial reconstruction following tumour resection. However, maintenance of chondrogenic phenotype in constructs derived from articular chondrocytes following transplantation has proven rather challenging. Using 3D architected hydrogels (ArcGels) derived by crosslinking gelatin using ethyl lysine diisocyanate (LDI) as a model system, we investigated the impact of mechanical properties and ArcGel degradation on the retention of the cartilage matrix following implantation to identify conditions that could rescue the chondrogenic phenotype in human articular chondrocytes (HAC). By comparing the fate of ex vivo engineered cartilaginous constructs with the de novo formation of cartilaginous tissue, in HAC laden ArcGels in an ectopic nude mouse model, we have identified that mechanical properties are more important in dictating fate of HAC in vitro. However, in vivo, chondrogenesis is governed by a subtle interplay between degradation and biomaterial mechanics. Our findings show that development of maintenance of cartilaginous tissue by human articular chondrocytes (HC) are governed by different variables and this highlights the importance for establishing translational correlation in cartilage tissue engineering.

MECHANOREGULATIVE COMPARISON OF CONVENTIONAL AND 3D-PRINTED TITANIUM

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There is a growing trend of use of 3D printed prostheses made of metallic materials such as titanium alloys. It is also known that the microstructure and topology of porous layers are different from similar conventional materials made by sintering or powder spraying technology. In this way local fluid permeability and respectively nutrients transport are also varied. This affects expected mechano-regulative signalling to cells when fluid mass and momentum transfer are caused by the material deformation and not by some external perfusion (alike in bioreactors). Here we are analysing mechano-regulative index variations for different titanium specimens during representative loading scenarios. We show that under three-point free bending conditions it is possible to create zones in the specimen favouring bone, cartilage or fibrous tissue formation in one specimen. However, for physiologically relevant tissue formation conditions (~1 Hz, 300-1000 microstrains) conventional materials lead to mechanoregulation highly dominated by fluid velocity rather than mechanical strain. For 3D printed titanium with the same specimen geometry it is possible to tailor porous
permeability to combine mechanical strain and local fluid flow matching the expected values in vivo (such as for 20-100 μm micromotions). The outputs of in silico modelling of these cases and their potential translation to implants are discussed.

CLINICAL AND RADIOLOGICAL OUTCOMES OF HOOK PLATE FIXATION IN THE ACROMIOCLAVICULAR JOINT DISLOCATION

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The purpose of this study was to evaluate the shapes of subacromial erosions and their effect on underlying rotator cuff after hook plate removal. In addition, we evaluate the clinical and radiological outcomes after hook plate fixation for AC dislocation. We evaluated the 20 patients with AC dislocations treated with hook plate fixations, who could follow up at least 2 years. The clinical outcomes were evaluated using the Constant-Murley score (CMS), Visual Analog Score (VAS) for pain, and Korean Shoulder score (KSS) at final follow-up. Coracoclavicular distance (CCD) was measured to evaluate the maintenance of reduction and computed tomography (3D-CT) was checked on all patients after hook plate removal. In addition, all patients performed musculoskeletal ultrasonography (US) to evaluate the presence of rotator cuff lesions which might be related with subacromial erosion at last follow up. The mean CMS, VAS for pain, and KSS were 94.56 ± 10.3, 1.5 ± 0.8, 94.6 ± 11.1, respectively. The mean CCD was not statistically different from the contralateral unaffected shoulder (8.99 mm ± 1.49 mm vs. 8.00 mm ± 1.37 mm, p=0.152). In 3D-CT, various types of depressed subacromial erosions were observed in 14 patients (70%) and did not show any marginal inferior protrusion of subacromial erosion. Also, there were no rotator cuff lesions such as partial tear in US. Hook plate fixation for the AC dislocation resulted in good clinical and radiological results. And, subacromial erosion did not influence on the underlying rotator cuff after hook plate removal.

GAIT BIOMECHANICS DURING DIFFERENT PHASES OF GAIT: IS FOOT CENTRE OF PRESSURE SUPERIOR?

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Foot centre of pressure (COP), knee adduction angles (KAA) and knee adduction moments (KAM) are important in the development, and potentially useful in the identification of knee osteoarthritis (OA). This study aimed to characterise and compare these measures in healthy and osteoarthritic groups in each phase of gait and determine whether COP is superior to KAA in identifying osteoarthritic patients. 108 subjects were recruited; 84 had no known pathology; 18 had medial and 6 lateral knee OA. Gait analysis was performed using motion capture and force plates. The normalized COP, KAA and KAM were measured during early, mid and late gait phases. The first phase of gait demonstrated significant differences between groups for all measures: KAA in all phases, COP in phase one and three and KAM in phase one only. Healthy subjects had a lateral COP for early and mid-stance and medial for late stance (mean (SD): 17 (22), 11 (19) and -1 (14) mm respectively). Medial OA subjects had lateral pressure positions similar to healthy subjects for all phases, whereas for lateral osteoarthritis subjects this shifted medially for early stance. The largest mechanical changes are seen in the first phase of gait in osteoarthritic patients. Although not a substitute for KAA, COP can be helpful in identifying osteoarthritic patients and evaluating surgical and non-surgical interventions in this phase. Measuring COP is easier than measuring other biomechanical factors. As such it is a potential simple assessment tool for orthopaedic interventions.

CHEMOPROPHYLAXIS IN LOWER LIMB IMMOBILISATION: IS THERE A ROLE FOR NOACs?

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Deep vein thrombosis (DVT) is a common complication following lower limb immobilization. A systematic review and meta-analysis were performed to review the effectiveness of low molecular weight heparin (LMWH) and novel oral anticoagulants (NOACs) for thromboprophylaxis in trauma patients treated non-operatively with lower limb immobilisation. All randomized controlled trials (RCTs) comparing thromboprophylaxis (either LMWH or novel oral anticoagulant) to no prophylaxis or standard for prevention of VTE in patients with lower limb trauma treated with immobilisation were included in the data analysis. Eight studies totaling 3190 patients were included. The overall incidence of thromboembolic events in the control group ranged from 2.3% to 40% (137/871) and from 0% to 37% (77/884) in the LMWH group (RR 0.57; 95% CI = 0.45 to 0.73), P < 0.00001. There was no significant difference in bleeding. No RCTs comparing NOACs with placebo or no treatment were found. One cohort study demonstrated equivalence of NOACs in VTE rate to LMWH with another demonstrating a significant reduction in VTE rates. Our results demonstrate that LMWH is an effective agent in reducing DVT in these patients with an acceptable safety profile. Although studies evaluating NOACs such as rivaroxaban as a thromboprophylactic agent in patients with lower-limb immobilisation are limited, there appears to be a potential for this regimen. Further randomised controlled trials are needed to assess this.

PREVENTING BACKFLOW LEAKAGE OF STEM CELLS INJECTED INTO ATROPHIC NON-UNION FRACTURE MODEL USING THE Z-TRACK METHOD

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Atrophic non-union is a major clinical problem. Treatment is usually prolonged, over years, and commonly leads to permanent disability. Replacement of biological factors by stem cells is of growing interest. A minimally invasive technique is used to deliver stem cells into the fracture site via percutaneous injection, however, this technique is significantly affected by a backflow leakage following injection, this occurs through the injection tract into the skin, subsequently the net number of injected cells might be reduced and their therapeutic privilege is affected. The Z-track method is currently being used in the clinical practice for intramuscular injections, it has been proved to be effective in sealing the injected materials and to prevent backflow leakage. Therefore, it could be used for the percutaneous injection in non-union models. Using rat’s cadaver (n=3), a mixture of toluene blue stain and contrast material was used as an injection material into the fracture site to allow visual and radiological detection of possible leaks. Ante grade nailing of tibia via tibial tuberosity was done, then tibia bone was fractured using 3-point close technique to keep intact skin and overlying soft tissue. Injection was performed into the fracture gap by pulling skin over the shin of tibia towards the ankle and injection of the stain into the fracture gap. The needle was then partially pulled, the skin was returned to its normal position and a complete extraction of the needle was followed. Observation at the injection site revealed no blue stain could be detected over the skin, the injected material initially formed a palpable mass around tibia and that was located away from the injection site. The mass looked sealed with no occurrence of leakage on mild compression. X-rays revealed a localized radiopaque area around the tibia with no material escape noticed. In summary, the Z-track method is an effective way to prevent fluid backflow escape after injection. It can ensure delivering and sealing of the injected material into the targeted area. Therefore, the therapeutic privilege of stem cells injection could be preserved and their efficacy can be optimised.

OBTAINING RELIABLE X-RAY VIEWS OF THE LEG IN A MODEL OF ATROPHIC NON-UNION

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Appropriate in vivo models can be used to understand atrophic non-union pathophysiology. In these models, X-ray assessment is essential and a reliable good quality images are vital in order to detect any hidden callus formation or deficiency. However, the radiographic results are often variable and highly dependent on rotation and positioning from the detector/film. Therefore, standardised A-P and lateral x-ray views are essential for providing a full radiological picture and for reliably assessing the degree of fracture union. We established and
evaluated a method for standardised imaging of the lower limb and for reliably obtaining two perpendicular views (e.g. true A-P and true lateral views). The normal position of fibula in murine models is posterolateral to the tibia, therefore, a proper technique must show fibula in both views. In order to obtain the correct position, the knee joint and ankle joints were flexed to 90 degrees and the foot was placed in a perpendicular direction with the x-ray film. To achieve this, a leg holder was made and used to hold the foot and the knee while the body was in the supine position. Lateral views were obtained by putting the foot parallel to the x-ray film. Adult Wister rat cadavers were used and serial x-rays were taken. A-P view in supine position showed the upper part of the fibula clearly, however, there was an unavoidable degree of external rotation in the whole lower limb, and the lower part of the fibula appeared behind the tibia. Therefore, a true A-P view whilst the body was in the supine position was difficult. To overcome this, a P-A view of the leg was performed with the body prone position, this allowed both upper and lower parts of the fibula to appear clearly in both views. This method provides two true perpendicular views (P-A and lateral) and helped to optimise radiological assessment.

**ACUTE OPEN CHARCOT FOOT**

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Charcot foot is a form of chronic progressive destructive arthropathy affecting the bones and soft tissue of the foot. It is an important entity although rare, affecting only 0.12 – 0.3% of diabetics per year. It has a high associated patient morbidity and healthcare cost the classic presentation is the patient with acute onset red, swollen foot with poorly defined pain. In this case we report a case of a lady who presented to the emergency department with an acute open charcot foot, with extrusion of the navicular bone. This was atraumatic, resulting from normal weightbearing. The patient was managed with excision of the navicular and skin closure and after prolonged wound care healed and maintains a stable foot. Charcot foot significantly alters the normal operative treatment for open fracture-dislocations. Here the mainstay of treatment is offloading and soft tissue protection to avoid ulceration, which can lead to amputation. However where surgery is required corrective procedures should focus not on restoration of the normal foot anatomy but in establishing a weightbearing plantigrade foot with soft tissue cover. Here we demonstrate that good results can be minimising surgical intervention even in apparently limb-threatening presentations.

**LONGITUDINAL ASSESSMENT OF PATELLAR TENDON MORPHOLOGY ON IMAGING (MRI AND ULTRASOUND) AND VISA-P SCORES IN COLLEGIATE BASKETBALL PLAYERS ACROSS A SINGLE SEASON**

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This study of male collegiate basketball players aims to 1) assess for change in patella tendon (PT) morphology on MRI and US across a single season; 2) to correlate patella tendinopathy grade on imaging to VISA-P scores; 3) characterize non-PT related knee pathology seen on MRI. In eleven male collegiate basketball players (mean age 19, age range 18-21), PT ultrasound and MRI of both knees were performed at pre-season and post-season time points, and players reported their VISA-P scores throughout the season. Patella tendinopathy was graded on MRI and US. Non-PT related pathology visible on knee MRI were recorded. Spearman coefficients correlated patella tendinopathy grade to VISA-P. Imaging findings: 6 of 11 players (54.5%) had baseline patella tendinopathy on MRI and US. Non-PT related pathology visible on knee MRI were recorded. Spearman coefficients correlated patella tendinopathy grade to VISA-P. Imaging findings: 6 of 11 players (54.5%) had baseline patella tendinopathy on MRI and US. At post-season, progression of patella tendinopathy was seen in 1 of 6 players on MRI and 4 of 6 players on US. Post-season change in MRI and US patella tendinopathy grades were not statistically significant. New, non-PT related pathology on post-season MRI included gastrocnemius strains, medial meniscal tear, and iliotibial band friction syndrome. VISA-P: The mean change in VISA-P score was 15.18 (+/-8.55). VISA-P scores decreased ≥10 points in 9 out of 11 players (81.8%). Neither MRI or US grades of tendinopathy correlated with VISA-P. MRI and US detected changes in patella tendinopathy grade in collegiate male basketball players across a single season, although not statistically significant. Players demonstrated varied non-patella related pathology. Neither MRI nor US grades of patella tendinopathy correlated with VISA-P.
EVALUATION OF EQUINE OSTEOARTHRITIS USING VISCOELASTIC PROPERTIES OF SYNOVIAL FLUID. VARIATION BETWEEN NORMAL AND PATHOLOGICAL METACARPOPHALANGEAL SYNOVIAL FLUID

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The viscoelastic properties of synovial fluid (SF) are crucial to the performance of joint function. Lameness due to joint injury is the most prevalent cause of diminished athletic function and wastage in athletic horses. The aim of the present research was to detect the possible variation in the viscoelastic character of equine SF from normal and diseased metacarpophalangeal joints. For this purpose, SF was aspirated by aseptic arthrocentesis from 20 Thoroughbred horses and 20 Warmblood horses and subjected to routine cytological analysis. For determining hyaluronic acid (HA) concentrations in equine SF samples, a commercially available ELISA kit was used. Moreover, full rheological sample characterization was performed in order to measure the elastic \( G' \) and viscous \( G'' \) moduli respectively, at horse’s body (37.5°C) temperature. The ANOVA findings indicated statistically significant main effects (p < 0.001) for radiographic diagnosis and breed on the mean values of HA measurements. Generally, we can observe that subjects with positive radiographic diagnosis seem to present lower HA measurements compared to the reference category (normal horses). A statistically significant main effect of radiographic osteoarthritis on the mean values of \( \log G'' \) (p < 0.001) and \( \log G' \) (p = 0.004) measurements was also noted. Horses with positive radiographic diagnosis seemed to present lower \( \log G'' \) and \( \log G' \) measurements compared to the reference category (normal horses). In conclusion, values of viscoelastic properties and HA concentration of diseased metacarpophalangeal joints of Warmblood and Thoroughbred horses seem to be significantly lower compared to the ones obtained from healthy subjects.

ANATOMIC PREDICTORS OF CARTILAGE DEGENERATION USING T1ρ MAGNETIC RESONANCE IMAGING IN ASYMPTOMATIC HIPS WITH CAM MORPHOLOGY

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This study aims to determine 1. If asymptomatic hips with cam morphology are at risk of further cartilage degeneration (as evaluated by T1ρ) over time, 2. Whether T1Rho changes are predictive of symptom-onset 3. Whether bony morphological parameters are associated with T1ρ signal changes. In a prospective, longitudinal, IRB-approved study, seventeen asymptomatic volunteers/hips [16 males; 32.9±6.0 years] with cam morphology underwent two T1ρ MRI scan and functional assessment (WOMAC) at recruitment and at 3.9 years (range: 2.2–6.3). Images were acquired and analyzed using a validated technique. The differences in T1ρ values (ΔT1ρ) and relative differences (%ΔT1ρ) were calculated as: \( \Delta T1\rho= T1\rho_{\text{Follow-Up}} - T1\rho_{\text{Initial}} \) and \( %\Delta T1\rho = \Delta T1\rho / T1\rho_{\text{Initial}} \). A \%ΔT1ρ greater than 17.6% was considered significant. Using CT data, femoral-acetabular- and spino-pelvic parameters were measured. The global T1ρ remained unchanged between initial (mean:34.8±4.7ms) and follow-up scans (mean:33.9±3.3ms) (p=0.46). Similar T1ρ values were seen initially between the anterolateral and posterolateral (33.7ms Vs. 33.0ms) regions (p=0.7); at follow-up T1 values were significantly higher posterolaterally (35.5ms Vs. 32.4ms) (p=0.03). Two volunteers reported lower WOMAC, one of which exhibited a significant increase in %ΔT1ρ (-26.2%). The degree of acetabular coverage correlated with %ΔT1ρ (rho=0.59–0.61, p=0.002). Although, signs of joint degeneration posterolaterally were detected, these were not on the whole associated with symptoms and only one of the 2 volunteers with the onset of symptoms had significant changes. Therefore, further study is required to define the use of T1ρ in clinical practice. Reduced acetabular coverage should be considered when stratifying hips at risk.

AN EVALUATION OF THE EFFECTIVENESS AND SAFETY OF TOTAL HIP ARTHROPLASTY AS AN OUTPATIENT PROCEDURE

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Outpatient hip arthroplasty is being performed more routinely, however safety remains a concern. The purpose of this study was to compare the adverse events rate of outpatient total hip arthroplasty (THA), and assess barriers to discharge. We examined 136 patients who underwent unilateral THA by one surgeon and were discharged on the same day of surgery. Using propensity matching, 136 inpatients who received the same procedure, and were discharged on post-operative day one or later, were identified. For each cohort, 90-day occurrence of adverse events, readmissions, and ER visits were recorded and compared. Adverse events were graded using the Ortho-SAVES tool. A secondary objective was to assess potential barriers to same day discharge. Within 90 days post-operatively, 12 outpatients (8.82%) and 14 inpatients (10.29%) developed an adverse event. There were no significant differences between rate or severity of adverse events between the two groups, and no serious adverse events in either group. In the outpatient group there was a correlation between the dosage of spinal anesthetic (bupivacaine) given and time required to stay in PACU post-operatively. When comparing the two groups, there were no differences in adverse events at 90 days. At our center, in the correct patient population, outpatient THA is a safe and cost effective option. A potential barrier to mobility post-operatively, and successful same day discharge is the time required to stay in PACU post-operatively, which was significantly correlated with an increased dose of spinal anesthetic given in our outpatient cohort.

**IN VIVO WEAR RATE OF HIGHLY CROSSLINKED POLYETHYLENE COMPARING REMELTED TO ANNEALED MANUFACTURING**

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The production of highly crosslinked polyethylene (HXLPE) can be done by either a re-melting or annealing thermal treatment processes with different implications in regards to performance. The aim of this study was to evaluate in vivo wear and clinical performance of HXLPE liners manufactured using these processes. Linear and volumetric wear was assessed using Martell Hip Analysis Suite, and clinical performance was assessed through patient reported outcome measures and rate of revision surgeries. Eighty re-melted and 53 annealed liners were included in the wear analysis due to radiological follow up and software-based exclusions, mean of patients was 66. At a mean follow-up of 3.3 years, there was no significant difference (p=0.10) in total wear rate between linear wear for re-melted (0.04mm/yr) and annealed liners (0.01mm/yr). There was no significant difference (p=0.30) in annual volumetric wear between re-melted (43.01mm^3/year) and annealed liners (46.95mm^3/yr). No hips were revised due to liner related complications and patient reported outcome measures were comparable between groups. Results from this study suggest that both thermal processes are valid options for improving the longevity of total hip replacements.

**THE EFFECT OF SURGICAL RESECTION OF HIP IMPINGEMENT DEFORMITIES: A WITHIN-PATIENT EXPERIENCE**

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Femoracetabular impingement (FAI) involves abnormal contact in the hip due to deformities and is associated with osteoarthritis. Bone mineral density (BMD) in the acetabulum is higher in subjects with convex femoral (cam) FAI deformities compared to control subjects. The objective of this study was to assess post-operative changes of BMD with and without surgical correction of the cam deformity. Thirteen patients with bilateral cam deformities underwent pre-operative and follow-up CT scans of both hips. The deformity was surgically removed from the symptomatic hip. BMD was measured in regions of interest around the superior acetabulum from CT scans at both time points. The contralateral untreated hip was used as a within-patient control. Changes in BMD were assessed by two-way repeated measures ANOVA (side, time) and paired t-tests. A greater BMD decrease was seen in the treated compared to the untreated hip (p<0.02). BMD within the superior acetabulum decreased by 7.1% on the treated side (p<0.0001) but only 3.2% (p=0.04) in the untreated contralateral hip. In the antero-superior rim where impingement primarily occurs, the decrease was -7.5% in the treated hip (p=0.0002) and -2.7% (p=0.1) in the untreated hip. BMD decreased in the treated hip, suggesting a positive effect of surgical correction in relieving stresses within the hip joint. Longer term follow-up is
required to assess the ultimate fate of the articular cartilage within the joint. This study showed that surgical correction of the cam deformity in patients with FAI may alter the pathological biomechanics within the joint.

ESTABLISHING ‘THE REASONABLE PATIENT’S’ EXPECTATION OF ‘MATERIAL RISKS’ TO BE DISCLOSED WHEN CONSENTING FOR TOTAL HIP ARTHROPLASTY

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‘Montgomery v Lanarkshire Health Board’ is the current landmark legal trial introducing a profound shift from a ‘reasonable doctor’ approach to an emphasis on ‘the reasonable patient’ when determining sufficient risk disclosure during the consent process’. A critical component of valid consent is disclosure of ‘material risks’ to the patient regarding the procedure. This study aims to establish ‘the reasonable patient’s’ expectation of ‘material risks’ to be disclosed when consenting for total hip arthroplasty (THA). A questionnaire conveying the risks and incidence of undergoing a THA was distributed to patients undergoing THA to complete to establish which risks they felt were ‘material’. Patients were asked their preference about how these risks should be communicated. 60 patients met inclusion criteria. The median patient age was 71(range 42-84). 82% of patients lacked third level education. No statistically significant correlation was found between educational level, religion or income and specific ‘material risks’. 87% of patients chose discussion with their surgeon as their preferred method of risk information. The remainder preferred a leaflet or to attend a day at ‘joint school’. 12% of patients would rather not be made aware of any risk prior to surgery while 55% wished to know all risks. 84% of patients determined 1 in 100 risk as the ceiling to which they would wish for risks to be disclosed. This patient cohort demonstrates gross heterogeneity in what patients determine as ‘material risk’. We recommend disclosure of all risks known to the surgeon to each patient to account for such heterogeneity.

PERIPROSTHETIC FRACTURE FOLLOWING FIRST METATARSOPHALANGEAL JOINT ARTHRODESIS IN PATIENTS WITH OSTEOPAENIA

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First metatarsophalangeal (MTPJ) arthrodesis is an effective treatment for hallux valgus with arthritis with success rates of 77-100%. The incidence of peri-prosthetic fracture following first MTPJ arthrodesis is largely unknown. These are the among the first documented cases of peri-prosthetic fracture following first MTPJ arthrodesis to the best of our knowledge. We present three patients on long term steroid therapy for systemic disease with hallux valgus and first MTPJ arthritis who underwent first MTPJ fusion using a dorsal anchorage plate and screws™, and a plantar fixos lag screw™. All patients who developed a peri-prosthetic fracture post-operatively had reduced bone quality noted intra-operatively. The fractures appear to be related to a stress riser effect given their pattern and location. The fractures healed uneventfully and both patients have had a subsequent uneventful post-operative course at a minimum of one year follow up. Ultimately we feel caution is appropriate when treating this patient cohort. Pre-operative pharmacotherapy optimization with the rheumatologist should be considered. Bone health should be assessed and optimized. It can be surmised that using a longer dorsal plate to increase the load sharing of the construct may decrease the incidence of this complication. Alternative surgical interventions such as first MTPJ resection arthroplasty could be considered. Other considerations include adjusting the weight-bearing regimen post-operatively, allowing earlier unrestricted weight-bearing or more gradual increments to full weight-bearing. More research is required to ascertain the most appropriate fixation method and weight-bearing regimen for patients with systemic disease and decreased bone quality with hallux valgus and first MTPJ arthritis.

ADDITIVELY MANUFACTURED BIODEGRADABLE POROUS IRON

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Topologically ordered, porous additively manufactured (AM), metallic biomaterials with proper biodegradation profile offer a rare combination of properties ideal for bone regeneration: an interconnected porous structure, bone-mimicking mechanical properties, and the potential to fully regenerate bony defects. While the majority of such biomaterials is based on magnesium, and thus degrading fast, we used Direct Metal Printing (DMP) to generate topologically ordered porous iron scaffolds based on a repetitive diamond unit cell. Our full-scale study comprises in vitro biodegradation behavior (up to 28 days), electrochemical performance, time-dependent mechanical properties, and biocompatibility of such scaffolds. Mechanical properties of AM porous iron (E = 1600-1800 MPa) fell within the range of values reported for trabecular bone even after 4 weeks of biodegradation. Electrochemical tests showed up to ≈12 times higher rates of biodegradation for porous iron as compared to cold-rolled (CR) iron. Only 3.1% of weight loss was measured after 4 weeks in immersion tests, while biodegradation mechanisms were found to be topology-dependent and different between periphery and central parts of the scaffolds. Cytotoxicity according to ISO 10993, was evaluated in static MG-63 culture and compared to Ti-6Al-4V, for up to 72 h. Our study shows that DMP holds potential to increase the surface area and decrease grain sizes of topologically ordered porous iron, a metal that usually is considered to degrade too slowly. Our approach thus paves the way for developing novel biodegradable biomaterials.

TREATMENT OF KNEE OSTEOARTHRITIS WITH CONCENTRATED ADIPOSE TISSUE INFUSION: CLINICAL RESULTS AND HISTOLOGICAL OBSERVATIONS


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Osteoarthritis (OA) is characterized by articular cartilage degeneration and subchondral bone sclerosis. Early OA begins as a focal damage; thus, its repair is envisioned to spare the joints from further degeneration and resume pain free movement. OA may benefit from non-surgical treatments based on articular infusions of adipose tissue derived-Stromal Vascular Fraction (SVF) or -mesenchymal stem cells (ASCs). Since both cultured-expanded ASCs and collagenase-isolated SVF need manipulation in laboratory setting, we investigated the possibility to reduce lipoaspirate manipulation using autologous concentrated adipose tissue, injected intra-articularly in the knee. The infusion of concentrated adipose tissue resulted safe, and all patients reported an improvement in term of pain reduction and function increase (VAS and WOMAC scores), even though the MRI evaluation was unable to detect augment in the thickness of cartilage. SVF and ASCs isolated from adipose tissue samples were cultured in vitro in standard conditions and plated on a composite bone scaffold, showing capabilities to differentiate into osteoblasts and chondrocytes upon stimulation. Immunohistochemistry performed both on bone scaffold and on knee joint intra-operative biopsies of patients, who underwent joint prosthesis, showed new tissue formation close to the osteochondral lesions. Overall our data indicate that concentrated adipose tissue infusion can stimulate tissue regeneration and might be considered an innovative and safe treatment for knee osteoarthritis, to place side by side to arthroscopy.

IS OSTEOARTHRITIS A VASCULAR DISEASE?

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OA pathophysiology has a vascular component consisting of venous stasis resulting in intraosseous hypertension and hypoxia. In response, osteoblasts change their cytokine expression, accelerating bone remodelling and cartilage breakdown consistent with OA. We have characterized circulatory kinetics in OA bone in animal models with dynamic contrast enhanced MRI (DCE-MRI) and 18F PET and have demonstrated venous stasis and reduced perfusion that temporally precede and spatially coincide with OA lesions. Osteoblast uptake of 18F is consistent with abnormal perfusion, bone remodelling, and severity of OA. Circulatory kinetics with DCE-MRI in humans with OA of the knee exhibit similar venous outflow obstruction. Venous stasis is associated with hypoxia in subchondral bone. As an example of the effects of hypoxia on OA osteoblasts, we have described upregulation of fibrinolytic peptides, but a deficiency in the upregulation of PAI-1, leading to the generation of plasmin by human OA osteoblasts exposed to hypoxia in vitro. Plasmin is a serine protease that has been shown to degrade cartilage in OA. Abnormal circulatory kinetics by DCE-MRI may be an
imaging biomarker of OA. Pharmacologic modulation of venous stasis would have a salutary effect on the physicochemical microcirculation of subchondral osteoblasts and the pathophysiology of OA.

**THE EFFECTS OF TREADMILL EXERCISE AT A SINGLE TIME ON KNEE ARTICULAR CARTILAGE IN VIVO**

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The purpose of this study was to investigate the metabolism of articular cartilage in knees of rat treadmill exercise at a single time and to elucidate the role of HIF-1,2α in exercise. Twelve-week-old male Wistar rats ran on a treadmill at 12 m/min or 20 m/min for 45-mins at a single time. Rats in control group were kept sedentarily. All were sacrificed after running. Total RNA was extracted from right knee cartilage. We analyzed gene expressions regarding cartilage metabolism with quantitative RT-PCR. Left knee of each group was stained with immunostaining for HIF-1,2α. Almost all gene expression in the 12 m/min group didn’t change compared with control group. Gene expressions of sox9 and ADAM-TS5 in the 20 m/min group was increased. In immunostaining, HIF-1α was more strongly stained in the 12 m/min group than in the control group. HIF-2α was more strongly stained in the 20 m/min group than in the control group, but weakly stained in the 12 m/min group. Twelve m/min running on treadmill at a single time did not adversely affect the gene expression of homeostasis on articular cartilage except for production of HIF-1α. On the other hand, the results of 20 m/min group show that cartilage metabolism was activated from the early stage with excessive exercise. Moreover, HIF-1,2α production in articular cartilage was changed according to running speed. These results indicate that HIF-1α and HIF-2α may regulate in the balance of cartilage metabolism on exercise.

**FINANCIAL IMPACT AND EFFECT ON THE OUTCOME OF PREOPERATIVE TESTS FOR AT-RISK OLDER HIP FRACTURE PATIENTS**

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Older patients with proximal femoral fractures often undergo preoperative tests due to coexisting morbidities. Our aim was to evaluate these tests and their impact on patient outcome and medical expenses. This retrospective study includes data on head computed tomography, carotid ultrasound, echocardiography and pulmonary functional tests calculated according to the type of surgery (osteosynthesis or hip arthroplasty) carried out on 2798 patients. Time-to-surgery, test repeated postoperatively, American Society of Anesthesiology Physical Status score, additional procedures, hospitalization time, 30-day mortality and associated medical expenses were evaluated. A total of 921 preoperative tests were carried out in 780 (28%) patients, and 375 postoperative tests were carried out in 329 (12%) patients (P < 0.001). A total of 23 procedures were carried out after surgery, none related to the originally carried out tests. Significant group differences were found for American Society of Anesthesiology Physical Status score, days to surgery, hospitalization time (days) and mortality rates. The medical expenses of these tests were 1.3% of the average income per case, and 0.6% of the average study group income. Non-routine preoperative tests prolong time-to-surgery, increased hospitalization time and contribute to 30-day mortality. No postoperative procedure was related to preoperative test findings. The financial cost for these tests does not burden the medical expenses per procedure.

**ELECTROSPINNING-BASED MODULAR CONSTRUCTS FOR TENDON AND CARTILAGE TISSUE ENGINEERING**

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Tissue engineering holds a great potential in tendon and cartilage regenerative medicine strategies, since the traditional methods of repair for injuries of these tissues have disadvantages that can deter their long-term effects. To achieve production of tendinous or cartilaginous tissue, scaffolds must provide a desirable 3D environment, where tenocytes and chondrocytes respectively will deposit their tissue-specific extracellular matrix. The electrospinning process has been used to produce nanofibrous scaffolds for various musculoskeletal tissues including cartilage tendon and ligament. In this work two commercially available polymer materials, a) BIOSYN®: poly (glycolide -co- dioxanone -co- trimethylene carbonate) and b) MAXON®: poly (glycolide-trimethylene carbonate) were used to fabricate electrospun scaffolds. The structural, mechanical and thermal properties were assessed with electronic microscopy, uniaxial mechanical testing and differential scanning calorimetry (DSC) respectively. Human chondrocytes and tenocytes were expanded up to passage 3 in DMEM media, supplemented with 10% fetal bovine serum and 1% penicillin / streptomycin. 50,000 cells / cm² were subsequently cultured for up to 14 days. Cell viability and metabolic activity was assessed using Live/Dead® and alamarBlue® assays respectively. Cell morphometric analysis was carried out using DAPI and Rhodamine conjugated Phalloidin and subsequent image analysis (ImageJ). Extracellular matrix deposition was assessed with immunocytochemistry.

A MINIMUM OF 10-YEARS FOLLOW-UP OF FEMORAL REVISION WITH THE WAGNER TAPERED STEM

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Reconstruction of severely deficient femoral bone stock is a critical issue in hip revision surgery. The present study evaluates retrospectively the long-term clinical and radiographic outcome of the cementless Wagner Self-Locking (SL) stem. From September 1992 to March 1998, 68 hips (66 patients) with extended femoral bone loss underwent revision using the Wagner SL stem. Twenty-six patients died of unrelated causes without additional surgery. Forty hips were available for clinical and radiographic follow-up evaluation at a mean follow-up of 13.9 years (range, 10.4-15.8) after surgery. There were 11 male and 29 female patients, with an average age of 61 years (range, 29-80). In 31 hips a transfemoral approach was performed. In 5 cases stem revision was required because of infection (2), progressive subsidence (2), and recurrent dislocation (1). Complications included dislocations (3) and subsidence ≥ 10 mm (8). The mean Harris hip score improved from 33.0 points preoperatively to 73.3 points at follow-up (p< 0.001). In 32 stems (91.4%) radiological signs of stable bone fixation were assessed. The cumulative survival rates at 15.8 years with femoral revision for any reason and for stem failure as the end points were 92.0% and 96.6%, respectively. Revision of severe proximal femoral bone loss is a technically demanding procedure because of the difficulty in obtaining the primary stability of the new prosthesis. The tapered and fluted Wagner SL stem, by means of a stable distal fixation, enables restoration of periprosthetic bone stock ensuring highly successful long-term outcomes.

A MINIMUM OF 10-YEARS FOLLOW-UP OF FEMORAL REVISION WITH THE WAGNER TAPERED STEM

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Hip resurfacing has conventionally been undertaken through a posterior approach, but recent investigations expressed concerns with the damage of capsular blood supply. To date, few papers have reported only preliminary outcomes of surface arthroplasty performed through alternative approaches. This retrospective study evaluates the mid-term clinical and radiographic results of current generation metal-on-metal resurfacing prostheses performed using an anterolateral Watson-Jones approach. Fifty-seven hips in 52 patients underwent metal-on-metal resurfacing arthroplasty. Two patients died from unrelated causes, leaving 55 hips in 35 males (3 bilateral) and 15 females (2 bilateral), with a mean age of 56 years (range, 27-70). Clinical and radiographic follow-up was carried out in all the cases. The cumulative survival rate was determined according to Kaplan-Meier. At a mean follow-up of 5.2 years (range, 2-9.2), 2 hips required revision because of early aseptic loosening of the acetabular component and were successfully converted to conventional arthroplasty. Average Harris hip score improved significantly from 59.8 points (range, 30.4-90.6) preoperatively to 93.7 points (range, 53-100) at the latest examination. Neck narrowing showed an average of 3.27%, but it never exceeded
10%. Nonprogressive acetabular radiolucencies and osteolysis were detected both in 2 hips. The cumulative survival rate at 9.2 years with revision for any reason as the end point was 93.0%. Medium-term clinical and radiographic results of modern metal-on-metal hip resurfacing performed through an anterolateral approach are promising, but longer-term evaluation is necessary. A rigorous patient selection is essential to minimize the risk of complications and prevent early failure.

**DOCUMENTED DIFFERENCES IN PRE-OPERATIVE PAIN SUBTYPES AND OUTCOME EFFECTS IN KNEE REPLACEMENT PATIENTS**

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The main indication for knee replacement surgery is pain in an arthritic knee. Various factors such as anxiety and depression have been noted as predictors of poor outcome in knee replacement surgery, and overall post-surgical pain is the most common problem affecting the knee replacement patient population. The primary focus of industry and surgeons has been centred on implant design and morphology. Our clinical observation suggested patient related pre-operative factors may be as important, and we therefore created a pain diary to identify pain sub-types and track how these patients fared post-surgery. A consecutive series of patients were counselled about the pain diary at pre-operative assessment as asked to complete it. From a patient group of 148, we obtained 85 completed pain diaries, who’s data we analysed. Within this group the mean age was 67 and there was a 2.3 female to 1 male ratio. Sixty three patients had a total knee, 22 a partial unicompartmental replacement. The somewhat startling finding was that 1/3 of patients in both the total and partial knee groups had some form of atypical neuropathic pain, anxiety, depression or some combination, pre-operatively. We found differences within these sub-groups eg more isolated neuropathic pain in the partial knee group and more combined anxiety, depression and neuropathic pain in the total knee groups. These differences produced subtle differences in 6 month knee score outcomes when compared to the typical pain group, but none in the atypical group attained the highest knee scores of over 90% of normal. The risk of having neuropathic pain post-operatively was also higher in this group. We conclude that routine mapping and qualification of pain subtypes pre-operatively is important for patients and surgeons, and patients should be counselled as to their expectations based on this. It also confirms that there is diagnostic complexity in what has been previously considered a straightforward diagnosis, such that some patients with painful knee replacements are having unnecessary or non-useful surgery.

**APPLICATION OF COMPLEXITY SCIENCE PRINCIPLES TO CLINICAL ORTHOPAEDIC PRACTICE. THE SWINDON COMPLEXITY SCORE**

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Complexity science is based around the study of the interaction of factors to predict potentially unpredictable, so-called emergent events. Other features of complexity and complex adaptive systems include non-linearity, feedbacks, self-similarity and scalability. This is in contra-distinction to Newtonian, deterministic models of behaviour often focusing on one agent or factor, and reliance on an average parameter, of which the medical model is an example. Use of fractal analysis has transformed the understanding of natural science subjects, and in medicine has thus far centred on cardiac and cancer basic science studies. Over the past 10 years we have undertaken a number of clinical assessments based on clinical complexity and the interaction of just 2 factors namely local and systemic complexity, generating a 4 part classification, in a 2x2 matrix. We have undertaken such studies for both elective and trauma conditions and found statistically significant differences between the most complex groups and straightforward patients in a variety of parameters ranging from complication and mortality rates, speed and degree of recovery and ability of more junior surgeons to undertake complex case surgery. We specifically present our results in total knee replacement surgery, which also shows a statistically significant difference between the most straightforward and most complex cases at 1 year. Additionally our observations of complexity have also highlighted differences in patient presentation, which we would describe as diagnostic complexity. The summation of our results suggests we look more closely at
the principles of complexity science, as this may provide an alternative methodology and be more accurate than present modelling of healthcare outcomes and delivery and at the same time demonstrating which additional data we need to collect. Stratification of clinical complexity of a single diagnosis or index operation using this methodology seems so far to have almost universal applicability and may help us to define and deliver optimal, efficient and tailored high-quality healthcare into the future. It is also likely to explain the present mis-match between current data and expected costs and delivery targets and is likely to be applicable to analysis of entire departments and entire organisations. Analysis based on complexity theory will lead to a paradigm shift in how we map healthcare into the future.

CONFIRMATION OF HLA-B27 TRANSGENIC RATS AS A SPONDYLOARTHRITIS MODEL

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The spondyloarthritis (SpA) are inflammatory diseases of the eye, intestinal tract, genitalia and skin. Taurog et al. developed HLA-B27-Transgenic rats mating with human β2-microglobulin as a SpA model. The final goal of our research is to develop more effective therapy for SpA. Based on the previous paper by Taurog et al., we established transgenic rats by crossbreeding between 21-3 rat transgenic line (with 20 transgene copies of HLA-B27 and 15 transgene copies of human β2-microglobulin: hemizygous) and 283-2 rat transgenic line (with 35 transgene copies of hβ2m: homozygous). We identified that 34 rats out of 54 rats has transgene successfully (with 20 transgene copies of HLA-B27 and 50 transgene copies of human β2-microglobulin), analyzed by genotyping technique. The male rat with HLA-B27/hβ2m transgenes (21-3 x 283-2) began to exhibit SpA like phenotype (Epididymoorchitis, Arthritis, or Spondylitis) from 100 days after birth. We scored and classified the phenotypic severity based on paw thickness, tail swelling and size of epididymis every 4 weeks from 100 days to 250 days. Finally, scoring evaluation, X-ray and immunohistological analysis suggested that male HLA-B27/hβ2m transgenic rats (21-3 x 283-2) had severe arthritic phenotype. In conclusion, we established the model rat exhibiting strong SpA-related phenotypes by cross-bleeding transgenic rat with transgenes HLA-B27 and hβ2m for further experimental utilization.

CLINICAL RESULTS OF AN INDIVIDUALISED MINI-METAL IMPLANT FOR FOCALCARTILAGE LESIONS IN THE KNEE

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We report prospective detailed results of patients undergoing treatment for chondral defects using a patient specific 2nd generation individualized mini-metal implant. Prospective analysis of sequential patients from 11 surgeons. Detailed specific MRI data was used to manufacture patient specific implants and guide instruments by a CAD/CAM process, to fit the unique anatomy of each individual knee. Implants were uncemented and made of chrome-cobalt, double coated with hydroxyapatite on top of Titanium. Demographic, operative and clinical scores (VAS and KOOS) were collected preop and at 6 months, 1 and 2 years postoperatively. 92 patients (46 men, 46 women) with focal cartilage lesions ICRS grade 3 or 4 underwent partial resurfacing, 75 on medial condyle, 6 lateral condyle and 11 on trochlea. Mean age 49 (27-69) years, mean BMI 29 (21-41). 30% had failed previous cartilage surgery. 2 patients (4.5%) underwent revision (at 9 months for infection and at 30 months for progression of arthritis). All mean KOOS domain scores were significantly improved at 1 and 2 years (p<0.05). Mean preoperative aggregated KOOS (38) improved to 62 at 12 months and 60 at 24 months (p<0.05). Mean VAS score improved from 62 preoperatively to 37 at 24 months. The study shows excellent early clinical results in the treatment of focal full thickness symptomatic cartilage lesions on the femoral condyles or trochlea with a second-generation patient specific metal implant and cutting guides. Adherence to strict indications has allowed for high patient reported scores and low early revision rate.

THE EVALUATION OF RELIABILITY IN QUADRANT METHOD FOR THE EVALUATION OF FEMORAL TUNNEL POSITION AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION
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The Quadrant method is frequently used for the evaluation of femoral tunnel position after ACL reconstruction. It was drawn along Blumensaat's line. But Blumensaat's line was known to be not straight. The purpose of this study was to define a line on 3DCT for each variation of Blumensaat's line and evaluate the Quadrant method for the femoral tunnel position. 40 patients underwent anatomical double bundle ACL reconstruction using outside-in technique. 3D-CT photographed within 2 weeks after surgery and we observed the intercondylar lateral wall. We classified it as straight type (ST), large hill type (LA), small hill type (SM) according to the classification of Iriuchishima. Then we defined the Blumensaat's line, which becomes the reference line, as the S-line matching the line ahead of the condyle. It was also defined as H-line matching the tangent at the apex of Hill. In ST, only S-line was used. In each case, femoral tunnel positions of anterior medial bundles (AMB) and posterior lateral bundles (PLB) were evaluated. In the shallow-deep directions, there was no significant difference between the 3 groups on the S-line and H-line. In the high-low directions, there was no significant difference between the 3 groups on the S-line. But in the high-low directions, a significant difference was observed between ST and LA and ST and SM in the H-line. Quadrant method showed a difference in the value due to the difference in the definition of the Blumensaat's line which is the reference line.

INTRAMEDULLARY PIN STABILITY AFFECTS THE PATTERN OF FRACTURE HEALING IN MICE WITH DIFFERENT SIZE MARROW CANALS

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Mouse models have been created with genetic deficiencies to imitate human inherited bone diseases and are used to examine molecular mechanisms of bone repair. Despite the well-documented importance of the mechanical environment during bone healing, hypodermic needles are currently used to stabilize fractures that do not provide appropriate mechanical stability. Although inserting one of these devices is technically easy, their mechanical stability is very poor. This is particularly important when age, genetic background, or gene alterations result in differently sized marrow canals. For instance, a hypodermic needle implanted in a mouse that has a bigger marrow canal will result in an unstable fixation and will heal with greater callus formation, whereas bones with a smaller canal will result in a more stable fixation and therefore smaller callus size. Regrettably, numerous studies can be found using various mouse models that have attributed a different pattern of fracture healing to the function of the specific gene or genetic variation, when in fact it is highly probable that the fixation stability played a more important role. The latest findings will be presented to demonstrate the effect of fixation stability on the healing of closed fractures in mice with different sized marrow canals.

EFFECTS OF ACETABULAR ABNORMALITIES ON THE FRACTURE SITE OF NON-TRAUMATIC SUBCHONDRAL FRACTURE OF THE FEMORAL HEAD

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Based on the hypothesis that mechanical stress induced by acetabular abnormalities may affect non-traumatic subchondral fracture of the femoral head (SF), we compared fracture sites in non-traumatic SF between acetabular dysplasia and retroversion. We examined 13 consecutive non-traumatic SF patients with dysplasia and 14 patients with retroversion. Dysplasia was evaluated by the presence of a lateral center-edge angle of <20° on radiographs. Retroversion was evaluated by the presence of a crossover sign or posterior wall sign on radiographs. Both mediolateral and anteroposterior location and extent of SF were evaluated by measuring each edge of low-intensity bands on all coronal-slices of T1-weighted MRI. Stress distribution on femoral head cartilage was evaluated in contralateral unaffected hips with same acetabular abnormality using finite element modeling. Medial edge of SF in retroversion was medially located compared to that in dysplasia, while there was no significant difference in locations of both lateral and anteroposterior edges of SF between the two
groups. Mediolateral extent of SF in retroversion was significantly larger than that in dysplasia, while there was no significant difference in anteroposterior extent of SF between the two groups. Contact stress in retroversion was widely distributed from lateral edge of acetabular rim to medial region, while that in dysplasia was concentrated on lateral edge of acetabular rim. This study demonstrated that both the mediolateral location and extent of SF differ between hips with acetabular dysplasia and retroversion due to different stress distribution, suggesting that acetabular morphology can affect the fracture site of non-traumatic SF.

HISTOLOGICAL EVALUATION OF TREADMILL RUNNING ON KNEE JOINT OF RAT ARTHRITIS MODEL

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Exercise therapy is widely used and effective against various diseases. However, the indication for rheumatoid arthritis (RA) is unclear because the progression of synovitis and cartilage degeneration are concerned. The purpose of this study is to evaluate the effects of treadmill running on the knee joint in the rat arthritis model histologically. Collagen-induced arthritis (CIA) rat was prepared using 8-week old male DA rat as arthritis model and divided into 4 groups: control (CIA -, treadmill -), treadmill (CIA -, treadmill +), CIA (CIA +, treadmill -), and CIA treadmill (CIA +, treadmill +) group. Each rat in treadmill and CIA treadmill group was forced running at 12m/min and 30 min/day from 10-week old and sacrificed 4 weeks later. Both knees were extracted. The right side was stained with safranin O, and the left was immunostained by connexin (Cx) 43. The safranin O staining was lower in the CIA and CIA treadmill groups and was the lowest in the CIA treadmill group. Immunohistochemical staining for Cx43 increased in the synovium of CIA group but decreased in the CIA treadmill group. Although the treadmill running at 12m/min has chondroprotective effects in normal rats, degeneration has progressed in CIA rats. The mechanism of cartilage degeneration in CIA rats may differ from in normal rats. Moreover, treadmill running may suppressed synovitis, based on the results of Cx43 immunostaining. Appropriate intensity exercise should control synovitis and cartilage degeneration simultaneously.

INFECTIONS ARE INCREASED AS THE CAUSE OF REVISION TOTAL HIP ARTHROPLASTIES IN THE SUPER-AGING AREA IN NORTHERN JAPAN

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Northern part of Japan is a head of the super-aging society, and the rate of it already reached 30.8% in 2016. Along with aging society, rapid increase of revision total hip arthroplasties (THAs) with primary THA has been predicted. The aim of this study is to estimate the trend of revision THAs in our super-aging area in Northern Japan. Trend on number and rate of primary and revision THA in one of the local area of leading super-aging society were surveyed in the last decade using the database of diagnostic procedure and surgical records from 2004 to 2015. The cause of revision THA was analysed in 2004-2009 versus in 2010-2015. Spearman’s rank-correlation coefficient and student’s t-tests were performed using the PASW 25 software. The data revealed 24,822 cases of orthopaedic surgery, including 3,905 primary and 405 revision THA from 2004 to 2015. All THA increased from 282 cases in 2004 to 450 in 2015 year by year. The revision contained 300 aseptic loosening, 69 infections and 36 dislocations. The value of infections and dislocations as cause of revision THA in the latest six years (2010-15) was larger compared to the value in the former six years (2004-09) (2.0 and 1.6 times, p<0.05). The number and rate of revision THA with primary THA increased annually, because of expansion of elderly people in the super-aging society. The number of revision THA due to infections or dislocation may be still increasing year by year.