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HUMAN TRABECULAR LACUNOCANALICULAR FLUID PRESSURE: CANCELLOUS BONE BIOLOGY

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Remodeling of the cancellous bone is more active than that of the cortical bone. It is known that the remodeling is governed by the intracancellous fluid pressure. Particularly, the lacunocanalicular pore (PLC) fluid pressure (FP) is essential for survival of the osteocyte and communication of remodeling signals between the PLC and intertrabecular pore (PIT). As a result, knowledge about the PLCFP generation of trabeculae is required to understand human cancellous bone biology. At this moment, the PLCFP measurement of human trabeculae is not reported. The purpose of this study was a direct measurement of PLCFP generation of human proximal femoral trabeculae in the direction of superior-to-fovea. Twenty one microscopic cylindrical trabecular specimens from trabeculae of five fresh human proximal femur (75 to 77 years) were fabricated using a micro-milling machine composed of the laser (Teemphotonics: 532nm), 3-dimensional PZT stage (PI Gmbh, resolution: 0.5nm), and microscope (lens: Navitar, and CCD: Hitachi) with the image processor. The fabrication resolution of the micro-milling machine was 0.4 um. Based on the trabecular trajectory of femoral head, the specimens were obtained in the direction of superior-to-fovea. The cylindrical specimen size had 120 um in diameter and 240 um in length. The test methods described in the previous study were utilized. The used undrained uniaxial strain condition could induce the maximum PLCFP within the trabecular elastic limit.

The measured trabecular PLCFP (±SD) at the strain of 0.4% was 693.7±79.1 kPa. Since this experiment is equivalent to the instantaneous response of PLCFP with free flow boundaries after application of an extremely fast loading speed such an ideal step loading, a PLCFP generation in the physiological condition will be much less than the results obtained in this study. Base on the linear isotropic poroelasticity, the obtained Skempton's coefficient is almost 0. Thus, the load bearing capability by trabecular PLC fluid is negligible. The Biot coefficient is 0.35 which is higher than that of the cortical tissue (0.14). As a result, the intraosseous fluid communication through trabecular surfaces is active compared to that through Haversian canal surfaces. This imply that mass transports from the trabecular PLC into the PIT and from the PIT into the trabecular PLC could be significantly affected by the PITFP (the physiological blood systolic and diastolic pressure: 16 and 11 kPa, respectively) that acts as the FP boundary condition for the PLC flow. It is known that the PLC flow generates the electrical charges on the trabecular surface ('+'for being spouted into the PIT and '-'for being flown into the PLC), which control differentiation and proliferation of the osteoblast and mesenchymal stem cell. Thus, significant changes in the PITFT could cause changes in the intra-trabecular PLC flow characteristics, mass transports between the PLC and PIT, and electrical charges on the trabeculae. Eventually, these could result in pathologies related to the trabecular remodeling.

KNEE ADDUCTION MOMENTS ARE NOT INCREASED IN OBESE KNEE OSTEOARTHRITIS PATIENTS DURING STAIR NEGOTIATION

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Osteoarthritis is one of the major causes of immobility. Most commonly, osteoarthritis manifests at the knee joint. Prevalence of knee osteoarthritis (KNOA) increases with age. Another important risk factor for KNOA is obesity. Research has shown that obese subjects have almost four times the risk of developing KNOA, which may be explained by both an increased knee loading. In medial compartment KNOA, the knee adduction moment (KAM) during gait is considered a marker for disease severity. KAM is dependent of the magnitude of the ground reaction force and its moment arm relative to the knee joint centre. In addition, obesity has been reported to augment KAM during gait. However, after removal of the direct contributions of body weight, KAM parameters may be different due to obesity-related gait adaptations to limit knee loading. While KAM has been thoroughly investigated during gait, little is known about KAM during stair negotiation, during which knee loads are higher compared to gait. The aim of the current study is therefore to compare normalized KAM during the stance phase of stair negotiation between lean KNOA patients, obese KNOA patients, and healthy controls. This case control study included 20 lean controls, 14 lean KNOA patients, and 16 obese KNOA patients. All subjects ascended and descended a two-step staircase at a self-selected, comfortable speed. Radiographic imaging and MRI were used to evaluate knee cartilage and KNOA status. Motion analysis was performed with a three-dimensional motion capture system. Kinetic data were obtained by one force platform. The parameters of study included: stance phase duration, toe-out angle, KAM peaks and KAM impulse. During stair ascent obese KNOA patients showed a longer stance phase than healthy controls (P 0.050). Despite high between-subject variability, KAM impulse was found 45% higher in the obese KNOA group during stair descent, when compared to healthy controls (P =0.012). The absence of a significant effect of groups on the normalized KAM during stair negotiation may be explained by a lower ambulatory speed in the obese KNOA group, that effectively lowers GRFz. Decreasing ambulatory speed may be an effective strategy to lower KAM during stair negotiation.

FEMORAL IMPACTION BONE GRAFTING IN STAGED REVISION TOTAL HIP ARTHROPLASTY FOR INFECTION: CLINICAL AND RADIOSTEREOMETRIC ANALYSIS.

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Femoral impaction bone grafting (IBG) may be used to restore bone stock in revision total hip arthroplasty (THA) and allow use of a shorter, than otherwise, length prosthesis. This is most beneficial in young patients who are more likely to require further revision surgery. This study aimed to assess the results of femoral IBG for staged revision THA for infection. A prospective cohort of 29 patients who underwent staged revision THA for infection with femoral IBG and a cemented polished double-tapered (CPDT) stem at the final reconstruction was investigated.

The minimum follow-up was two years (2 - 10 years, median 6 years). Stem subsidence was measured with radiostereometric analysis. Clinical outcomes were assessed with the Harris Hip, Harris Pain, and and Société Internationale de Chirurgie Orthopédique et de Traumatologie Activity (SICOT) Scores. The original infection was eradicated in 28 patients. One patient required a repeat staged revision due to re-infection with the same organism. At two-year follow-up, the median subsidence at the stem-bone interface was -1.70 mm (-0.31 to -4.98mm). The median Harris Hip Score improved from 51 pre-operatively to 80 at two years (p=0.000), the Harris Pain Score from 20 to 44 (p=0.000) and the SICOT Score from 2.5 to 3 (p=0.003).

As successful eradication of infection was achieved in the majority of patients and the stem migration was similar to that of a primary CPDT stem, this study supports the use of femoral IBG during the final reconstruction of the femur after staged revision THA for infection.

CAN A MEDIALLY STABILIZED TKA DESIGN APPROACH A NATURAL KNEE KINEMATICS?

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Physiological kinematics is very difficult to restore after total knee arthroplasty (TKA). A new model of medial stabilized (MS) TKA prosthesis has a high spherical congruence of the internal compartment, which guarantees anteroposterior (AP) stability associated with a flat surface of the insert in the lateral compartment, that allows a greater AP translation of the external condyle during knee flexion. The aim of our study is to evaluate, by dynamic radiostereometric analysis (RSA), the knee *in vivo* kinematics after the implantation of a MS prosthesis during sit to stand and lunge movements. To describe the *in vivo* kinematics of the knee after MS Fixed Bearing TKA (GMK Sphere (TM) Medacta International AG, Castel San Pietro, Switzerland) using Model Based dynamic RSA.

A cohort of 18 patients (72.1 ± 7.4 years old) was evaluated by dynamic RSA 9 months after TKA. The kinematic evaluation was carried out using the dynamic RSA tool (BI-STAND DRX 2), developed at our Institute, during the execution of sit to stand and lunge movements. The kinematic data were processed using the Grood and Suntay decomposition and the Low Point method. The patients performed two motor tasks: a sit-to-stand and a lunge. Data were related to the flexion angle versus internal-external, varus-valgus rotations and antero-posterior translations of the femur with respect to the tibia.

During the sit to stand, the kinematic analysis showed the presence of a medial pivot, with a significantly greater ($p=0.0216$) anterior translation of the lateral condyle (3.9 ± 0.8 mm) than the medial one (1.6 ± 0.8 mm) associated with a femoral internal rotation (4.5 ± 0.9 deg). During the lunge, in the flexion phase, the lateral condyle showed a larger posterior translation than the medial one (6.2 ± 0.8 mm vs 5.3 ± 0.8 mm) associated with a femoral external rotation (3.1 ± 0.9 deg). In the extension phase, there is a larger anterior translation of the lateral condyle than the medial one (5.8 ± 0.8 mm vs 4.6 ± 0.8 mm) associated with femoral internal rotation (6.2 ± 0.9 deg). Analysing individual kinematics, we also found a negative correlation between clinical scores and VV laxity during sit to stand ($R= -0.61$) and that the higher femoral extra-rotation, the poorer clinical scores ($R= 0.65$).

The finding of outliers in the VV and IE rotations analysis highlights the importance of a correct soft tissue balancing in order to allow the prosthetic design to manifest its innovative features.

A NOVEL TECHNIQUE OF MINIMALLY INVASIVE DYNAMIC HIP SCREW.

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Increasing incidence of osteoporosis, obesity and an aging population have led to an increase in low energy hip fractures in the elderly. Perceived lower blood loss and lower surgical time, media coverage of minimal invasive surgery and patient expectations unsurprisingly have led to a trend towards intramedullary devices for fixation of extracapsular hip fractures. This is contrary to the Cochrane review of random controlled trials of intramedullary vs extramedullary implants which continues recommends the use of a sliding hip screw (SHS) over other devices. Furthermore, despite published literature of minimally invasive surgery (MIS) of SHS citing benefits such as reduced soft tissue trauma, smaller scar, faster recovery, reduced blood loss, reduced analgesia needs; the uptake of these approaches has been poor. We describe a novel technique one which remains minimally invasive, that not only has a simple learning curve but easily reproducible results. All patients who underwent MIS SHS fixation of extracapsular fractures were included in this study. Technique is shown in Figure 1. We collated data on all intertrochanteric hip fractures that were treated by a single surgeon series during period Jan 2014 to July 2015. Data was collected from electronic patient records and radiographs from Picture Archiving and Communication System (PACS). Surgical time, fluoroscopy time, blood loss, surgical incision length, post-operative transfusion, Tip Apex Distance (TAD) were analyzed. There were 10 patients in this study. All fractures were Orthopaedic Trauma Association (OTA) type A1 or A2. Median surgical time was 36 minutes (25-54). Mean fluoroscopy time was similar to standard incision sliding hip screw fixation. Blood loss estimation with MIS SHS can be undertaken safely and expeditiously for extracapsular hip fractures.

INFLUENCE OF DIFFERENT LISFRANC LIGAMENT INJURIES ON CT FINDINGS

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Being commonly missed in the clinical practice, Lisfranc injuries can lead to arthritis and long-term complications. There are controversial opinions about the contribution of the main stabilizers of the joint. Moreover, the role of the ligament that connects the medial cuneiform (MC) and the third metatarsal (MT3) is not well investigated. The aim of this study was to investigate the influence of different Lisfranc ligament injuries on CT findings under two specified loads.

Sixteen fresh-frozen human cadaveric lower limbs were embedded in PMMA at mid-shaft of the tibia and placed in a weight-bearing radiolucent frame for CT scanning. All intact specimens were initially scanned under 7.5 kg and 70 kg loads in neutral foot position. A dorsal approach was then used for sequential ligaments cutting: first – the dorsal and the (Lisfranc) interosseous ligaments; second – the plantar ligament between the MC and MT3; third – the plantar Lisfranc ligament between the MC and the MT2. All feet were rescanned after each cutting step under the two loads.

The average distances between MT1 and MT2 in the intact feet under 7.5 kg and 70 kg loads were 0.77 mm and 0.82 mm, whereas between MC and MT2 they were 0.61 mm and 0.80 mm, without any signs of misalignment or dorsal displacement of MT2. A slight increase in the distances MT1-MT2 (0.89 mm; 0.97 mm) and MC-MT2 (0.97 mm; 1.13 mm) was observed after the first disruption of the dorsal and the interosseous ligaments under 7.5 kg and 70 kg loads. A further increase in MT1-MT2 and MC-MT2 distances was registered after the second disruption of the ligament between MC and MT3. The largest distances MT1-MT2 (1.5 mm; 1.95 mm) and MC-MT2 (1.74 mm; 2.35 mm) were measured after the final plantar Lisfranc ligament cut under the two loads. In contrast to the previous two the previous two cuts, misalignment and dorsal displacement of 1.25 mm were seen at this final disrupted stage.

The minimal pathological increase in the distances MT1-MT2 and MC-MT2 is an important indicator for ligamentous Lisfranc injury. Dorsal displacement and misalignment of the second metatarsal in the CT scans identify severe ligamentous Lisfranc injury. The plantar Lisfranc ligament between the medial cuneiform and the second metatarsal seems to be the strongest stabilizer of the Lisfranc joint. Partial lesion of the Lisfranc ligaments requires high clinical suspicion as it can be easily missed.

AUTOMATED ELECTROMECHANICAL SYSTEM DESIGNED TO INVESTIGATE THE EFFECT OF LOCAL MECHANICAL CONDITIONS ON FRACTURE HEALING PROGRESSION

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In the course of uneventful secondary bone healing, a fracture gap is progressively overgrown by callus which subsequently calcifies and remodels into new bone. It is widely accepted that callus formation is promoted by mechanical stimulation of the tissue in the fracture gap. However, the optimal levels of the interfragmentary motion's amplitude, frequency and timing remain unknown. The aim of this study was to develop an active fixation system capable of installing a well-controlled mechanical environment in the fracture gap with continuous monitoring of the bone healing progression.

The experimental model was adapted from Tufekci et al. 2018 and required creation of a critical size defect and an osteotomy in a sheep tibia. They were separated by a mobile bone fragment. The distal and proximal parts of the tibia were fixed with an external fixator, whereas the mobile fragment was connected to the proximal part with an active fixator equipped with a linear actuator to move it axially for mechanical stimulation of the tissue in the fracture gap. This configuration installed well-controlled mechanical conditions in the osteotomy, dependent only on the motion of the active fixator and shielded from the influence of the sheep's functional weightbearing. A load sensor was integrated to measure the force acting in the fracture gap during mechanical stimulation. The motion of the bone fragment was controlled by means of a custom-made controller allowing to program stimulation protocols of various profiles, amplitudes and frequencies of loading events. Following in vitro testing, the system was tested in two Swiss White Alpine Sheep. It was configured to simulate immediate weightbearing for one of the animals and delayed weightbearing for the other. The applied loading protocol consisted of 1000 loading events evenly distributed over 12 hours resulting in in a single loading event every 44 seconds.

Bench testing confirmed the ability of the system to operate effectively with frequencies up to 1Hz over a range of stimulation amplitudes from 0.1 to 1.5 mm. Continuous measurements of in vivo callus stiffness revealed progressive fracture consolidation in the course of each experiment. A delayed onset of fracture healing was observed in the sheep with simulated delayed weightbearing.

The conducted preclinical experiments demonstrated its robustness and reliability. The system can be applied for further preclinical research and comprehensive in-depth investigation of fracture healing.

THE EFFECT OF POINT OF CARE (POC) INR TESTING ON DELAY TO THEATRE IN PATIENTS ON WARFARIN WITH NECK OF FEMUR FRACTURES

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Various studies have demonstrated that the necessity for reversal of Warfarin through the use of Vitamin K (Vit K) in neck of femur fracture patients introduces increased duration of stay and poorer outcomes as measured by operative complications and mortality rate. One reason for this delay may be the time latency between admission and the clinicians decision to investigate the INR. In this study we aim to explore the different causes of latency which contribute to a delay to theatre and ascertain whether point of care testing may negate this.

We carried out an audit of a cohort of neck of femur fracture patients between 2012 and 2015. Between September 2011 and September 2013, paper notes of 25 patients who were on warfarin at the time of sustaining a Neck of femur fracture (NOF) was obtained within Addenbrookes hospital archives. An additional 80 patients records from the year 2015 were retrieved from EPIC digital records. Time intervals were recorded as follows (from time of A&E assessment by Medical doctor); Interval to orthopaedic specialist assessment, Interval to first INR order, Interval to first INR result seen by specialist, Interval to first Vit K prescribed, Interval to first Vit K given, Interval to Second INR ordered, Interval to second INR seen by specialist, Interval to operation time (as determined by time of team briefing). Analysis of the time intervals as a proportion of total time elapsed between A&E assessment and Time to theatre was performed. Point of care (POC) testing of INR on admission to A&E was introduced and a symmetrical time period was analysed for the same intervals.

The latency generated by time taken for a NOF to be assessed by an orthopaedic specialist occupied 8.60% of the total time, the interval between ordering and recording an INR value accounted for 7.96% of time to theatre, the interval between an INR being recorded and subsequently seen by a clinician accounted for 13.4% of time to theatre, the time between orthopaedic specialist assessment and prescription of Vit K took up 7.83% of the total time and the percentage time between Vit K prescription and administration was 12.3%. The time between the first dose of Vit K prescription and arriving at theatre accounted for 76.1% of latency and the time between viewing a second INR and time to theatre occupied 33% of the total time. Following introduction of POC INR testing, there was a statistically significant decrease in time taken for warfarin reversal and consequently a reduction between time of admission to time to theatres.

NOF patients who are on warfarin at time of injury introduces complexity to surgical management and planning for theatre. In our audit we demonstrate that causes of delay are distributed throughout the pathway of care and there are several stages. POC INR testing represents an effective method of reducing this latency and improves patient outcome.

THE RADIOLOGICAL ASSESSMENT OF KNEE PROTHESES AT FOLLOW UP AFTER TOTAL KNEE ARTHROPLASTY; DO WE NEED TO CHANGE OUR PRACTICE?

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The current standard of practice following knee arthroplasty is to demonstrate the appropriate alignment of knee replacements using knee radiographs. Recent studies have suggested that standard knee radiographs provide adequate accuracy for tibial prosthesis alignment assessment as compared with long knee view radiographs which are more technically demanding and carry greater radiation exposure. In this study, we aim to address whether alignment measured on standard knee radiographs are reliable and reproducible over time.
We examined a cohort of 80 patients 37 male (46%), 43 females (54%), mean age = 68 years) who underwent total knee arthroplasty (TKA). Standard knee anteroposterior radiographs performed within 2 days following surgery were compared to standard knee anteroposterior radiographs taken 1 year following the surgery in patients with well-functioning prosthesis. Tibial prosthesis alignment angles between the longitude of the tibial shaft and the tibial baseplate were calculated using Centricity Enterprise Web V3.0 software. The data was examined using R software.
In well-functioning primary knee arthroplasties, tibial prosthesis alignment angles measured in the 1-year follow-up standard view knee radiographs were found to deviate from measurements obtained with the same radiographic specifications in the immediate post-operative period. A significant mean percentage difference was found between the two radiographs.
Long knee view radiographs may be required in order to accurately assess tibial prothesis alignment following total knee arthroplasty.

ROSTHETIC KNEE COMPONENT SIZE AND PATIENT REPORTED FUNCTIONAL OUTCOMES AFTER TOTAL KNEE ARTHROPLASTY: A ONE YEAR FOLLOW-UP STUDY

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Companies manufacturing total knee arthroplasty (TKA) prostheses produce a variety of tibial and femoral components of different dimensions denoted by numbers or letters. Surgeons frequently implant components that are compatible but not of the same size on the femur and tibia. Recent studies suggest that equally sized femoral and tibial components produce better outcomes compared to size-mismatched components. In our study, we aim to explore the relationship between component size and outcome measured by oxford knee score at six weeks and one year following TKA.

A cohort of twenty-four patients who underwent TKA and had well-functioning prosthesis were studied. Thirteen (54%) had equally sized TKA components implanted, seventy-four patients (42%) had components that were mismatched by one size, and one (4%) had components that were mismatched by more than one size. The Oxford Knee Score (OKS) obtained preoperatively, at six weeks and one year postoperatively were retrieved from an electronic database. All data were analysed using R software.

A significant improvement in pre-operative and one-year postoperative OKS was observed. Patients who received one-size mismatched tibial and femoral components demonstrated a less pronounced improvement in OKS as compared with patients who received equally sized components.

When possible, it may be best to utilise equally sized prosthetic tibial and femoral components when performing total knee arthroplasty. Manufacturers may be able to produce better patient outcomes by including prostheses that are between sizes as part of their production line.

CONTINUOUS MONITORING OF FRACTURE HEALING TO ANALYZE SHORT-TERM RESPONSE OF BONE REPAIR TISSUE TO MECHANICAL STIMULATION

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The course of secondary fracture healing typically consists of four major phases including inflammation, soft and hard callus formation, and bone remodeling. Callus formation is promoted by mechanical stimulation, yet little is known about the healing tissue response to strain stimuli over shorter timeframes on hourly and daily basis. The aim of this study was to explore the hourly, daily and weekly variations in bone healing progression and to analyze the short-term response of the repair tissue to well-controlled mechanical stimulation.

A system for continuous monitoring of fracture healing was designed for implantation in sheep tibia. The experimental model was adapted from Tufekci et al. 2018 and consisted of 3 mm transverse osteotomy and 30 mm bone defect resulting in an intermediate mobile bone fragment in the tibial shaft. Whereas the distal and proximal parts of the tibia were fixed with external fixator, the mobile fragment was connected to the proximal part via a second, active fixator. A linear actuator embedded in the active fixator moved the mobile fragment axially, thus stimulating mechanically the tissue in the osteotomy gap via well-controlled displacement being independent from the sheep's functional weightbearing. A load sensor was integrated in the active fixation to measure the force acting in the osteotomy gap. During each stimulation cycle the displacement and force magnitudes were recorded to determine in vivo fracture stiffness. Following approval of the local ethics committee, experiments were conducted on four skeletally mature sheep. Starting from the first day after surgery, the daily stimulation protocols consisted of 1000 loading events equally distributed over 12 hours from 9:00 to 21:00 resulting in a single loading event every 44 seconds. No stimulation was performed overnight.

One animal had to be excluded due to inconsistencies in the load sensor data. The onset of tissue stiffening was detected around the eleventh day post-op. However, on a daily basis, the stiffness was not steadily increasing, but instead, an abrupt drop was observed in the beginning of the daily stimulations. Following this initial drop, the stiffness increased until the last stimulation cycle of the day.

The continuous measurements enabled resolving the tissue response to strain stimuli over hours and days. The presented data contributes to the understanding of the influence of patient activity on daily variations in tissue stiffness and can serve to optimize rehabilitation protocols post fractures.

EVIDENCE-BASED GENERIC ASIAN PELVIC BONE MODELS FOR RESEARCH, DEVELOPMENT AND TEACHING USING CT-BASED 3D STATISTICAL MODELING

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Artificial bone models (ABMs) are commonly used in traumatology and orthopedics for training, education, research and development purposes. The aim of this study was to develop the first evidence-based generic Asian pelvic bone model and compare it to an existing pelvic model.

A hundred clinical CT scans of intact adult pelvises (54.8±16.4 years, 161.3±8.3 cm) were acquired. They represented evenly distributed female and male patients of Malay (n=33), Chinese (n=34) and Indian (n=33) descent. The CTs were segmented and defined landmarks were placed. By this means, 100 individual three-dimensional models were calculated using thin plate spline transformation. Following, three statistical mean pelvic models (male, female, unisex) were generated. Anatomical variations were analyzed using principal component analysis (PCA). To quantify length variations, the distances between the anterior superior iliac spines (ASIS), the anterior inferior iliac spines (AIIS), the promontory and symphysis (conjugate vera) as well as the ischial spines (diameter transversa) were measured for the three mean models and the existing ABM.

PCA demonstrated large variability regarding pelvic surface and size. Principal component one (PC 1) contributed to 24% of the total anatomical variation and predominantly displayed a size variation pattern. PC 2 (17.7% of variation) mainly exhibited anatomical variations originating from differences in shape. Female and male models were similar in ASIS (225±20 mm; 227±13 mm) and AIIS (185±11 mm; 187±10 mm), whereas differed in conjugate vera (116±10 mm; 105±10 mm) and diameter transversa (105±7 mm; 88±8 mm). Comparing the Asian unisex model to the existing ABM, the external pelvic measurements ASIS (22.6 cm; 27.5 cm) and AIIS (186 mm; 209 mm) differed notably. Conjugate vera (111 mm; 105 mm) and diameter transversa (97 mm; 95 mm) were similar in both models. Low variability of mean distances (3.78±1.7 mm) was found beyond a sample number of 30 CTs.

Our analysis revealed notable anatomical variations regarding size dominating over shape and gender-specific variability. Dimensions of the generated mean models were comparatively smaller compared to the existing ABM. This highlights the necessity for generation of Asian ABMs by evidence-based modeling techniques.

LOCAL NON-VIRAL GENE DELIVERY TO IMMUNOMODULATE AND ENHANCE FRACTURE HEALING

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Although 80% of fractures typically heal without any problems, there is a small proportion (<20%) that suffer complications such as delayed healing and potential progression to non-union. In patients with healing complications, the coordinated regulation between pro- and anti-inflammatory cytokines, such as interleukin-1β (IL-1β) and interleukin-1 receptor antagonist (IL-1Ra) respectively, is often dysregulated. The aim of this study is to develop a therapeutic strategy based on the local delivery of genes to reparative mesenchymal stromal cells (MSCs) migrating into the local fracture microenvironment, thereby promoting a more favourable healing environment to enhance fracture repair. Our approach involves the local delivery of nanoparticles complexing the non-viral vector polyethyleneimine (PEI) with therapeutic plasmid DNA (pDNA) encoding for IL-1Ra.

pDNA encoding green fluorescent protein and Gaussia luciferase were used as reporter genes to determine the transfection efficiency of both rat and human MSCs using flow cytometry and to assess the transgene expression profile using a luciferase expression assay. The effect of transfection with PEI on the viability of MSCs was assessed using the metabolic assay Cell Titer Blue and dsDNA quantification. Levels of IL-1Ra produced by cells following transfection with nanoparticles encoding IL-1Ra was assessed using enzyme-linked immunosorbent assays (ELISA). HEK-Blue IL-1β reporter cells, which secrete alkaline phosphatase in response to IL-1β stimulation, were used to confirm that the IL-1Ra produced by transfected cells is functionally active, i.e. the successful antagonism of IL-1β bioactivity.

We have determined that using PEI-based nanoparticles we can achieve a transfection efficiency of 14.8 + 1.8% in rat MSCs. Transgene expression was found to be transient, with a peak in expression at 7 days post-transfection and a gradual decrease over time, which was maintained for up to 4 weeks. Using an optimized concentration of PEI, the impact of the nanoparticles on MSC viability was limited, with no significant difference in cellular metabolic activity compared to non-transfected cells at 10 days post-transfection. We have additionally demonstrated the capacity to successfully transfect both rat and human MSCs with pDNA encoding for IL-1Ra, resulting in enhanced levels of IL-1Ra, which is functionally active.

The use of non-viral gene therapy to locally deliver immunomodulatory genes, such as IL-1Ra, to MSCs presents a promising strategy to enhance bone healing. Specifically, the transgene expression levels achieved with such an approach can remain therapeutically effective and are transient in nature, presenting an advantage over other methods such as recombinant protein delivery and viral-based gene delivery methodologies.

BIPHASIC PLATING – IN VIVO STUDY ON A NOVEL FIXATION CONCEPT TO ENHANCE MECHANOBIOLOGICAL FRACTURE HEALING

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Fracture fixation has advanced significantly with the introduction of locked plating and minimally invasive surgical techniques. However, healing complications occur in up to 10% of cases, of which a significant portion may be attributed to unfavorable mechanical conditions at the fracture. Moreover, state-of-the-art plates are prone to failure from excessive loading or fatigue. A novel biphasic plating concept has been developed to create reliable mechanical conditions for timely bone healing and simultaneously improve implant strength. The goal of this study was to test the feasibility and investigate the robustness of fracture healing with a biphasic plate in a large animal experiment. Twenty-four sheep underwent a 2mm mid-diaphyseal tibia osteotomy stabilized with either the novel biphasic plate or a control locking plate.

Different fracture patterns in terms of defect location and orientation were investigated. Animals were free to fully bear weight during the post-operative period. After 12 weeks, the healing fractures were evaluated for callus formation using micro-computer tomography and strength and stiffness using biomechanical testing. No plate deformation or failures were observed under full weight bearing with the biphasic plate. Osteotomies stabilized with the biphasic plate demonstrated robust callus formation. Torsion tests after plate removal revealed no statistical difference in peak torsion to failure and stiffness for the different fracture patterns stabilized with the biphasic plate. However, the biphasic plate group specimens were 45% stronger ($p=0.002$) and 48% stiffer ($p=0.007$) than the controls.

The results of this large animal study demonstrate the clinical potential of this novel stabilization concept.

ENGINEERED EXTRACELLULAR MATRIX ENHANCES THE BONE REGENERATION POTENTIAL OF AGED HUMAN BONE MARROW STROMAL CELLS

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Regeneration of bone defects in elderly patients is limited due to the decreased function of bone forming cells and compromised tissue physiology. Previous studies suggested that the regenerative activity of stem cells from aged tissues can be enhanced by exposure to young systemic and tissue microenvironments. The aim of our project was to investigate whether extracellular matrix (ECM) engineered from human induced pluripotent stem cells (hiPSCs) can enhance the bone regeneration potential of aged human bone marrow stromal cells (hBMSCs).
ECM was engineered from hiPSC-derived mesenchymal-like progenitors (hiPSC-MPs), as well as young (70 years) hBMSCs. ECM structure and composition were characterized before and after decellularization using immunofluorescence and biochemical assays. Three hBMSCs of different ages were cultured on engineered ECMs. Growth and differentiation responses were compared to tissue culture plastic controls.

Decellularized ECMs contained collagens type I and IV, fibronectin, laminin and < 5% residual DNA. Cultivation of young and aged hBMSCs on the hiPSC-ECM in osteogenic medium significantly increased hBMSC growth and markers of osteogenesis, including collagen deposition, alkaline phosphatase activity, bone sialoprotein expression and matrix mineralization compared to plastic controls. In aged BMSCs, matrix mineralization was only detected in ECM cultures in osteogenic medium. Comparison of ECMs engineered from hiPSC-MPs and hBMSCs of different ages suggested similar structure, composition and potential to enhance osteogenic responses in aged BMSCs.

Our studies suggest that aged BMSCs regenerative activity can be enhanced by culture on hiPSC-engineered ECM.

AN OSTEOPOROTIC OSTEOARTHRITIS PHENOTYPE IS PREVALENT IN PREMATURELY AGEING MITOCHONDRAL DNA MUTATOR MICE

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Mitochondrial dysfunction has been demonstrated in aging and osteoarthritic tissues. We investigated knee joints of prematurely aging mitochondrial DNA mutator mice (PolgD275A) to evaluate a relationship between mitochondrial dysfunction and osteoarthritis.

Cartilage damage was evaluated using OARSI histopathology grading and osteoclast numbers were quantified by tartrate-resistant acid phosphatase staining in wild type, heterozygous and homozygous PolgD275A mice. Subchondral cortical plate and epiphyseal trabecular bone structures were determined by micro-computed tomography. Apoptosis in cartilage and subchondral bone tissues was studied using an indirect TUNEL method.

Homozygous mutants displayed osteopenia of the epiphyseal trabecular bone and subchondral cortical plate in comparison to wild type and heterozygous mutants. Subchondral osteopenia was associated with a strong increase of osteoclast numbers (0.88±0.30/mm bone perimeter) compared to heterozygous (0.25±0.03/mm) and wild type mice (0.12±0.04/mm). Wild type mice as well as hetero- and homozygous mutants displayed low-grade cartilage degeneration due to loss of cartilage proteoglycans. In contrast, chondrocyte hypertrophy was more abundant in the homozygous mice. There were no differences in chondrocyte apoptosis rates between groups.

Prematurely ageing mtDNA mutator mice with or without further mechanic or metabolic stimuli might serve as a valuable model for further experimental studies on aging-induced osteoporotic OA phenotype.

HIGH INCIDENCE OF PREVIOUSLY NEGLECTED MENISCUS LESIONS INFLUENCING KNEE BIOMECHANICS IN ASSOCIATION WITH ACL INJURIES.

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Recent findings have identified the importance of previously undiagnosed or neglected meniscus lesions in association with anterior cruciate ligament (ACL) injuries (e.g. medial meniscus ramp lesions and posterior root tears of the lateral meniscus). There is increasing biomechanical evidence that they bear the potential to alter both anteroposterior and rotational laxity patterns in ACL injured knees. Few data exist with respect to the presence of these specific tear entities in large series of ACL injured patients. The purpose of the study was to analyze the meniscus tear pattern in a series of ACL injured knees with a special focus on ramp lesions of the medial meniscus and posterior root lesions of the lateral meniscus. The hypothesis was that a significant number of ACL injured patients would display these types of lesions.

Data from 358 patients undergoing an ACL reconstruction (227 males /131 females, age: 28±10) were extracted from a center-based registry. The type of ACL tear (partial versus complete) as well as the presence of associated meniscus lesions were documented. Meniscus lesions were classified into the following categories: medial ramp lesions, lateral root lesions, medial ramp and lateral root lesion, other medial meniscus injuries, other lateral meniscus injuries, other bimeniscal injuries. Chi-square tests were used to determine whether the percentage of meniscal lesions differed between types of ACL tear, gender and age (below 21, 21-35, above 35). Significance was set at $p < 0.05$.

Isolated ACL tears were present in 107 (30%) of the operated knees (31 partial; 327 complete). Complete ACL lesions were more likely to present an associated meniscus injury (321 out of 327, 71%) than partial tears (13 out of 31, 42%).

The incidence of meniscus injuries which are associated with ACL tears is very high (70%). Previously undiagnosed or neglected meniscus injuries like medial ramp or lateral root tears could be identified in 35% of patients. As such, the hypothesis was confirmed that an important amount of ACL injured knees display this specific intraarticular soft tissue damage. A systematic evaluation of these lesions under arthroscopy should thus be performed and specific repair needs to be evaluated.

THE RELATIONSHIP BETWEEN TIBIAL CARTILAGE THICKNESS AND BONE MINERAL DENSITY VARIES WITH BONE DEPTH AND OSTEOARTHRITIS SEVERITY

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While knee osteoarthritis (OA) is now recognized as a complex disease affecting the whole joint, not just the cartilages, there remains a paucity of data regarding the interactions between knee components. One relationship of particular interest is between the spatial variations in cartilage thickness (CTh) and subchondral bone mineral density (BMD). Indeed, bone and cartilage are two mechanosensitive tissues that interact as a functional unit and there is evidence of a biomechanical coupling between both tissues. Particularly, a recent in vivo study has shown a positive relationship in non-OA knees with thicker cartilage where bone is denser, and an alteration of this relationship in OA knees. These observations support the concept of an osteochondral unit and warrant additional research to assess the influence of bone depth. Therefore, this study aimed to characterize the relationship between spatial variations in CTh and BMD measured at various depths below the bone surface.

CT-arthrography of 20 non-OA tibias and 20 severe medial-compartment OA tibias were segmented to build 3D mesh models of the bones and cartilages. Each individual tibia model was registered to a reference tibia, allowing to calculate BMD maps at 1, 3, 5 and 10mm below the bone-cartilage interface in the medial compartment. Pearson correlations between CTh maps and the four BMD maps were then calculated for each knee. Lastly, differences in correlation coefficients between successive bone layers were assessed using Wilcoxon signed-rank tests.

In both OA and non-OA tibias, the correlation coefficients were higher with the BMD measured in the 1mm layer, and followed a pattern of statistically significant decrease with bone layers of increasing depth ($p < 0.021$). In non-OA tibias, the median relationship was positive with a strong effect size in the 1, 3 and 5mm layers, while in OA tibias the median relationship was positive only in the 1mm layer and with a medium effect size. In the OA tibias, the median relationship was negative with a weak effect size in the 3 and 5mm layers, and it was negative with a medium effect size in the 10mm layer.

In conclusion, the results of the present study support the value of considering bone and cartilage as a unit, and more generally support OA pathophysiology models based on relationships among knee properties.

IMPROVING FIXATION TECHNIQUES OF PROXIMAL HUMERUS FRACTURES BY MEANS OF FINITE ELEMENT ANALYSIS

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To date, the fixation of proximal humeral fractures with angular stable locking plates is still insufficient with mechanical failure rates of 18% to 35%. The PHILOS plate (DePuy Synthes, Switzerland) is one of the most used implants. However, this plate has not been demonstrated to be optimal; the closely symmetric plate design and the largely heterogeneous bone mineral density (BMD) distribution of the humeral head suggest that the primary implant stability may be improved by optimizing the screw orientations. Finite element (FE) analysis allows testing of various implant configurations repeatedly to find the optimal design. The aim of this study was to evaluate whether computational optimization of the orientation of the PHILOS plate locking screws using a validated FE methodology can improve the predicted primary implant stability.

The FE models of nineteen low-density (humeral head BMD range: 73.5 - 139.5 mg/cm3) left proximal humeri of 10 male and 9 female elderly donors (mean ± SD age: 83 ± 8.8 years) were created from high-resolution peripheral computer tomography images (XtremeCT, Scanco Medical, Switzerland), using a previously developed and validated computational osteosynthesis framework. To simulate an unstable mal-reduced 3-part fracture (AO/OTA 11-B3.2), the samples were virtually osteotomized and fixed with the PHILOS plate, using six proximal screws (rows A, B and E) according to the surgical guide. Three physiological loading modes with forces taken from musculoskeletal models (AnyBody, AnyBody Technology A/S, Denmark) were applied. The FE analyses were performed with Abaqus/Standard (Simulia, USA). The average principal compressive strain was evaluated in cylindrical bone regions around the screw tips; since this parameter was shown to be correlated with the experimental number of cycles to screw cut-out failure ($R^2 = 0.90$). In a parametric analysis, the orientation of each of the six proximal screws was varied by steps of 5 in a 5x5 grid, while keeping the screw head positions constant. Unfeasible configurations were discarded. 5280 simulations were performed by repeating the procedure for each sample and loading case. The best screw configuration was defined as the one achieving the largest overall reduction in peri-screw bone strain in comparison with the PHILOS plate.

With the final optimized configuration, the angle of each screw could be improved, exhibiting significantly smaller average bone strain around the screw tips (range of reduction: 0.4% - 38.3%, mean ± SD: 18.49% ± 9.56%).

The used simulation approach may help to improve the fixation of complex proximal humerus fractures, especially for the target populations of patients at high risk of failure.

A NOVEL 3-DIMENSIONAL IN VITRO MODEL FOR OSTEOMYELITIS ASSOCIATED STAPHYLOCOCCAL ABSCESS COMMUNITIES

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Staphylococcus aureus is the main cause of osteomyelitis and forms biofilm and staphylococcal abscess communities (SACs) in humans. While S. aureus has several toxins with specificity for human targets and working with human host cells would be preferred, for SACs no in vitro models, two-dimensional (2D) or three-dimensional (3D), have been described in literature to date. Advanced 3D in vitro cell culture models enable the incorporation of human cells and resemble in vivo tissue more closely than conventional 2D cell culture. Therefore, the aim of this study was to develop an in vitro model of SACs by using a 3D system. The model should allow for studies into antibiotic tolerance and S. aureus - human host cells interactions.

With a clinical isolate (S. aureus JAR) or a lab strain (S. aureus ATCC 49230-GFP), SACs were grown in a collagen gel (1.78 mg/ml, Gibco) supplemented with 200 µl human plasma at 37 °C. Transmission and scanning electron microscopy was used to obtain a detailed overview of SACs, whereas immunofluorescent stainings were done to determine whether the pseudocapsule around SACs consist of fibrin. Antibiotic tolerance of SACs was assessed with 100x the minimal inhibitory concentration (MIC) of gentamicin (Roth). Bacterial clearance of non-establised SACs and established SACs with or without pseudocapsule was determined by exposure to differentiated PLB neutrophil-like cells (differentiation with 1.25% DMSO and 5% FBS for 5 days; dPLB) or primary neutrophils isolated with lymphoprep from fresh heparin blood. Degradation of the pseudocapsule was done with 7.5 Âµl/ml plasmin (Sigma). Colony forming unit (CFU) counts were performed as quantification method. Statistical analysis was performed with the ANOVA multiple comparison test or, when data was not normally distributed, with a Mann-Whitney U test.

We have developed a 3D in vitro model of SACs which after overnight growth were on average 200 micrometers in diameter, consisted of 8 log10 CFUs and were surrounded by an inner and outer fibrin pseudocapsule. The in vitro grown SACs tolerated 100x the MIC of gentamicin for 24h and did not significantly differ from control SACs ($p=0.1000$). dPLB neutrophil-like cells or primary neutrophils did not clear established in vitro SACs ($p=0.1102$ and $p=0.8767$, respectively). When the fibrin pseudocapsule was degraded by the enzyme plasmin, dPLB neutrophil-like cells or primary neutrophils caused for a significant decrease in total CFU compared the SACs that did had a pseudocapsule ($p=0.0333$ and $p=0.0272$, respectively).

The in vitro SACs model offers a tool for host-pathogen interaction and drug efficacy assessments and is a valuable starting point for future research.

TRESHOLD EXPRESSION OF NERVE GROWTH FACTOR IN CARTILAGINOUS TISSUES IN HUMAN SPINE OSTEOARTHRITIS

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Recent clinical studies on targeting nerve growth factor (NGF) in chronic low back pain and knee osteoarthritis have demonstrated efficient pain reduction in a short-term treatment regimen. However, the increased risk for the development of rapid progressive osteoarthritis at the required high drug dose remains a serious concern and prompts thorough analysis of the tissue distribution and role of NGF in degenerative musculoskeletal disorders. Here, we sought to investigate tissue distribution of NGF, its high affinity receptor TrkA and CD68-positive macrophages in human facet joint osteoarthritis of the lumbar spine.

Facet joint specimens (n=10) were harvested by facetectomy from patients undergoing elective lumbar intervertebral spine fusion. Facet joint osteoarthritis and presence of synovitis was graded using preoperative magnetic resonance imaging. Tissue distribution of NGF, TrkA and CD68 was determined using immunohistochemistry. Tissue degradation was graded on safranin-O-stained tissue sections. Association between imaging parameters and tissue distribution was determined using Pearson correlation analysis.

Synovitis was present in 6 cases and facet joints displayed moderate to severe radiological osteoarthritis (median Weishaupt grade; 2 [1.5-3]). NGF was expressed in 8 of 10 specimens. NGF was expressed in connective tissue, articular and fibrocartilage, but not bone tissue. Cartilaginous NGF expression was predominantly found in the extracellular matrix of superficial cartilage tissue with complete loss of proteoglycans, chondrocyte death and structural damage (fissures). Loss of cartilage proteoglycan staining alone did not display NGF immunoreactivitiy. NGF expression was not correlated with radiological osteoarthritis severity or presence of synovitis. NGF high affinity receptor TrkA was exclusively expressed in bone marrow tissues. Differential grades of bone marrow infiltration by CD68-positive macrophages were observed, yet these were not associated with NGF expression.

Targeting NGF in chronic low back pain and/or facet joint osteoarthritis might affect pathomechanisms in cartilaginous tissues and NGF signalling in the bone marrow compartment.

EXTERNALIZED LOCKED PLATING OF UNSTABLE PROXIMAL TIBIA FRACTURES CAN PROVIDE SUFFICIENT STABILITY UNDER PARTIAL WEIGHTBEARING – A FINITE ELEMENT STUDY

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Osteosynthesis of high-energy metaphyseal proximal tibia fractures is still challenging, especially in patients with severe soft tissue injuries and/or short stature. Although the use of external fixators is the traditional treatment of choice for open comminuted fractures, patients' acceptance is low due to the high profile and therefore the physical burden of the devices. Recently, clinical case reports have shown that supercutaneous locked plating used as definite external fixation could be an efficient alternative. Therefore, the aim of this study was to evaluate the effect of implant configuration on stability and interfragmentary motions of unstable proximal tibia fractures fixed by means of externalized locked plating. Based on a right tibia CT scan of a 48 years-old male donor, a finite element model of an unstable proximal tibia fracture was developed to compare the stability of one internal and two different externalized plate fixations. A 2-cm osteotomy gap, located 5 cm distally to the articular surface and replicating an AO/OTA 41-C2.2 fracture, was virtually fixed with a medial stainless steel LISS-DF plate. Three implant configurations (IC) with different plate elevations were modelled and virtually tested biomechanically: IC-1 with 2-mm elevation (internal locked plate fixation), IC-2 with 22-mm elevation (externalized locked plate fixation with thin soft tissue simulation) and IC-3 with 32-mm elevation (externalized locked plate fixation with thick soft tissue simulation). Axial loads of 25 kg (partial weightbearing) and 80 kg (full weightbearing) were applied to the proximal tibia end and distributed at a ratio of 80%/20% on the medial/lateral condyles. A hinge joint was simulated at the distal end of the tibia. Parameters of interest were construct stiffness, as well as interfragmentary motion and longitudinal strain at the most lateral aspect of the fracture. Construct stiffness was 655 N/mm (IC-1), 197 N/mm (IC-2) and 128 N/mm (IC-3). Interfragmentary motions under partial weightbearing were 0.31 mm (IC-1), 1.09 mm (IC-2) and 1.74 mm (IC-3), whereas under full weightbearing they were 0.97 mm (IC-1), 3.50 mm (IC-2) and 5.56 mm (IC-3). The corresponding longitudinal strains at the fracture site under partial weightbearing were 1.55% (IC-1), 5.45% (IC-2) and 8.70% (IC-3).

From virtual biomechanics point of view, externalized locked plating of unstable proximal tibia fractures with simulated thin and thick soft tissue environment seems to ensure favorable conditions for callus formation with longitudinal strains at the fracture site not exceeding 10%, thus providing appropriate relative stability for secondary bone healing under partial weightbearing during the early postoperative phase.

BETTER STABILITY AND MORE PREDICTIVE FIXATION OF THE FEMORAL NECK SYSTEM VERSUS TWO HANSSON PINS IN PAUWELS II FEMORAL NECK FRACTURES: A BIOMECHANICAL STUDY

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Femoral neck fractures account for half of all hip fractures and are recognized as a major public health problem associated with a high socioeconomic burden. Whilst internal fixation is preferred over arthroplasty for physiologically younger patients, no consensus exists about the optimal fixation device yet. The recently introduced implant Femoral Neck System (FNS) (DePuy Synthes, Zuchwil, Switzerland) was developed for dynamic fixation of femoral neck fractures and provides angular stability in combination with a minimally invasive surgical technique. Alternatively, the Hansson Pin System (HPS) (Swemac, Linköping, Sweden) exploits the advantages of internal buttressing. However, the obligate peripheral placement of the pins, adjacent to either the inferior or posterior cortex, renders the instrumentation more challenging. The aim of this study was to evaluate the biomechanical performance of FNS versus HPS in a Pauwels II femoral neck fracture model with simulated posterior comminution. Forty-degree Pauwels II femoral neck fractures AO 31-B2.1 with 15° posterior wedge were simulated in fourteen paired fresh-frozen human cadaveric femora, followed by instrumentation with either FNS or HPS in pair-matched fashion. Implant positioning was quantified by measuring the shortest distances between implant and inferior cortex (DI) as well as posterior cortex (DP) on anteroposterior and axial X-rays, respectively. Biomechanical testing was performed in 20Â° adduction and 10Â° flexion of the specimens in a novel setup with simulated iliopsoas muscle tension. Progressively increasing cyclic loading was applied until construct failure. Interfragmentary femoral head-to-shaft movements, namely varus deformation, dorsal tilting and rotation around the neck axis were measured by means of motion tracking and compared between the two implants. In addition, varus deformation and dorsal tilting were correlated with DI and DP. Cycles to 5/10° varus deformation were significantly higher for FNS (22490±5729/23007±5496) versus HPS (16351±4469/17289±4686), P=0.043. Cycles to 5/10° femoral head dorsal tilting (FNS: 10968±3052/12765±3425; HPS: 12244±5895/13357±6104) and cycles to 5/10° rotation around the femoral neck axis (FNS: 15727±7737/24453±5073; HPS: 15682±10414/20185±11065) were comparable between the implants, P≥0.314. For HPS, the outcomes for varus deformation and dorsal tilting correlated significantly with DI and DP, respectively (P=0.025), whereas these correlations were not significant for FNS (P≥0.148).

From a biomechanical perspective, by providing superior resistance against varus deformation and performing in a less sensitive way to variations in implant placement, the angular stable Femoral Neck System can be considered as a valid alternative to the Hansson Pin System for the treatment of Pauwels II femoral neck fractures.

IS TOURNIQUET USAGE STILL NEEDED IN LIGHT OF EVER EVOLVING BLOOD MANAGEMENT STRATEGIES?

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Blood management protocols attempt to reduce blood loss by strategies including autologous blood donation, red cell salvage, normovolaemic haemodilution and haemostatic agents such as tranexamic acid (TXA). TXA usage in particular has become increasingly commonplace with numerous studies demonstrating a significant reduction in peri-operative blood loss and proportion of patients requiring transfusion, without increasing the risk of venous thromboembolism. Tourniquet usage has now become ubiquitous in TKA operations with reported benefits of improved visualization, shorter operative time and decreased intra-operative bleeding. However, its use is not without considerable complications including wounding dehiscence, increased venous thromboembolism, superficial wound infection and skin blistering. It is therefore imperative that we review tourniquet usage in light of ever evolving blood management strategies. The aim of this study was to evaluate the effect of stopping tourniquet usage in primary TKRs, performed by an experienced surgeon, in light of new blood reduction measures, such as a TXA.

A retrospective analysis identified a total of 31 patients who underwent primary TKR without the use of a tourniquet from January 2018 to March 2019. This was compared to an earlier group of patients from the same surgeon undergoing TKR with the use of a tourniquet; dating from July 2016 to November 2017. All surgeries were performed within the same hospital (CXH). Peri-operative factors and outcome measures were collected for analysis.

There was no significant difference in post-operative haemoglobin drop (Tourniquet, 23.1 g/L; No Tourniquet, 24.4 g/: p=0.604) and fall in haematocrit (Tourniquet, 0.082; No Tourniquet, 0.087: p=0.604). Allogenic blood transfusion rates were the same in both groups at 12.9% (2 patients) and blood loss was not found to be significantly different (Tourniquet, 1067ml; No tourniquet, 1058mls). No significant difference was found in operative time (Tourniquet, 103 minutes; No Tourniquet, 111.7 minutes: p=0.152) or length of stay (Tourniquet, 5.5 days; No Tourniquet, 5.2 days: p=0.516). Tranexamic acid usage was not found to be significant (p=1.000). ROM of motion and analgesia requirement was significantly better in the no tourniquet group on one post-operative day out of five analysed (p=0.025, p=00.011). No post-operative thromboembolic events were reported in either group. There was no significant difference in readmission rates (p=0.492) or complications (p=0.238).

The increase in minor complications and potential increased VTE risk with tourniquet usage must be balanced against an improved visual field and reduced blood loss in TKR patients. Our study found no difference in post-operative blood loss and transfusion rates between tourniquet and no tourniquet groups. With ever evolving and improving blood loss management strategies, including the use of TXA, the application of tourniquet may not be needed. Further prospective RCTs are needed to assess the impact of tourniquet usage in light of this.

SHOULD WE ROUTINELY PERFORM A POST-OPERATIVE HAEMOGLOBIN CHECK FOLLOWING UNICOMPARTMENTAL KNEE ARTHROPLASTY?

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In recent years, reduction in the length of stay in patients undergoing UKA has gained considerable interest. This has led to development of 'fast-track' and even day-case protocols aimed at decreasing length of stay (LOS), enhancing post-operative recovery and decreasing post-operative morbidity. One potential barrier to faster discharge and patient recovery is the need for post-operative haemoglobin checks and allogenic blood transfusion; which has been shown to increase LOS. Allogenic blood transfusion itself is not without risk, including immunological reactions, transfusion associated lung injury, infection and transmission of disease, thus reducing blood loss and the need for transfusion is imperative. Currently there is a knowledge gap regarding post-operative transfusion need and blood loss following UKA. We aimed to investigate blood loss and transfusion rates following UKA. Our primary aim was to evaluate the extent of post-operative transfusion need following UKA and identify which patients are at higher risk of needing transfusion.

Following institutional approval, a retrospective analysis of all patients undergoing unicompartmental knee arthroplasty (UKA) at our level one academic university hospital was conducted. Operative records of all patients undergoing primary UKA were reviewed between March 2016 and March 2019. Patients' pre-operative haemoglobin and haematocrit, BMI, co-morbidities, application of tourniquet, tourniquet time, administration of Tranexamic Acid, need for post-operative blood transfusion, hospital length of stay, complications and re-admission were all recorded. Blood loss was estimated using the post-operative haematocrit.

A total number of 155 patients were included. There were 70 females (45%) and 85 males (55%). The mean age was 66±10 years. Median pre-op blood volume was 4700mls (IQR; 4200-5100). Median blood loss was 600 mls (IQR; 400-830). Mean pre-op Haemoglobin was 135±14g/L and mean post-op Haemoglobin was 122±13g/L. No patient had a post-op Haemoglobin under 80g/L (Range 93-154). No patients in our study needed transfusion. A further comparison group of high-blood loss and low-blood loss patients was included in analysis. High-blood loss patients were defined as those losing greater than 20% of their pre-operative blood volume whilst low-blood loss patients were defined as those losing ≤20% of their blood volume. Results of these groups are presented in Table 3. No significance was found between the two groups in patient's demographics and in terms of intra-operative factors including TXA usage ($p=0.68$) and tourniquet time ($p=0.99$). There was no difference in terms of post-operative complications ($p=1.0$), length of stay ($p=0.36$) or readmission rates ($p=0.59$).

The results of our study indicated that post-operative haemoglobin and haematocrit check proved unnecessary in all of our patients and could have been omitted from post-operative routines. We conclude that routine post UKA check of haemoglobin and haematocrit can be avoided and be saved for special circumstances depending on patient's physiology.

24 HOUR DISCHARGE IN UNICOMPARTMENTAL KNEE REPLACEMENT USING THE NAVIO◊ ROBOTIC SYSTEM: A RETROSPECTIVE ANALYSIS

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There has been significant interest in day-case and rapid discharge pathways for unicompartmental knee replacements (UKR). Pathways to date have shown this to be a safe and feasible option; however, no studies to date have published results of rapid-discharge pathways using the NAVIO robotic system. To date there is no published experience with rapid discharge UKR patients using the NAVIO robotic system. We report an initial experience of 11 patients who have safely been discharged within 24 hours. With the primary goal of investigating factors that led to rapid discharge and a secondary goal of evaluating the safety of doing so.

All patients were discharged within 24 hours; there were no post-operative complications and no readmissions to hospital. The mean length of stay was 16.9 hours (SD=7.3), with most patients seen once on average by physiotherapy. Active range of motion at 6 weeks was 0.7o to 130.5 o, with all patients mobilising independently. The average 6-month post-operative Oxford Knee Score was 43.5 out of 48. There were no readmission or complications in any of our patients.

This initial feasibility study identified that patients could be safely discharged within 24 hours after UKR using the NAVIO robotic system. With growing uptake of robotic procedures, with longer operative durations than traditional procedures, it is essential to ensure a rapid discharge to reduce healthcare cost whilst ensuring that patients are discharged home in a safe manner.

A STERILIZABLE MECHANICAL INCLINOMETER TO MEASURE ACETABULAR CUP INCLINATION: A PROOF-OF-CONCEPT STUDY

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The angle of acetabular inclination is an important measurement in total hip replacement (THR) procedures. Determining the acetabular component orientation intra-operatively remains a challenge. An increasing number of innovators have described techniques and devices to achieve it. This paper describes a mechanical inclinometer design to measure intra-operative acetabular cup inclination. Then, the mechanical device is tested to determine its accuracy. The aim was to design an inclinometer to measure inclination without existing instrumentation modification. The device was designed to meet the following criteria: 1. measure inclination with acceptable accuracy (+/- 5o); 2. easy to use intra-operatively (handling & visualization); 3. adaptable and useable with majority of instrumentation kits without modification; 4. sterilizable by all methods; 5. robust/reusable.

The prototype device was drafted by computer aided design (CAD) software. Then a prototype was constructed using a 3D printer to establish the final format. The final device was CNC machined from SAE 304 stainless steel. The design uses an eccentrically weighted flywheel mounted on two W16002-2RS ball bearings pressed into symmetrical housing components. The weighted wheel is engraved with calibrated markings relative to its mass centre. Device functioning is dependent on gravity maintaining the weighted wheel in a fixed orientation while the housing can adapt to the calibration allowing for determining the corresponding measurement. The prototype device accuracy was compared to a digital device. A digital protractor was used to create an angle. The mechanical inclinometer (user blinded to digital reading) was used to determine the angle and compared to the digital reading.

The accuracy of the device compared to the standard freehand technique was assessed using a saw bone pelvis fixed in a lateral decubitus position. 18 surgeons (6 expert, 6 intermediate, 6 novice) were asked to place an uncemented acetabular cup in a saw bone pelvis to a target of 40 degrees. First freehand then using the inclinometer. The inclination was determined using a custom-built inertial measurement unit with the user blinded to the result. Comparison between the mechanical and digital devices showed that the mechanical device had an average error of -0.2, a standard deviation of 1.5, and range -3.3 to 2.6. The average root mean square error was 1.1 with a standard deviation of 0.9. Comparison of the inclinometer to the freehand technique showed that with the freehand component placement 50% of the surgeons were outside the acceptable range of 35-45 degrees. The use of the inclinometer resulted all participants to achieve placement within the acceptable range. It was noted that expert surgeons were more accurate at achieving the target inclination when compared to less experienced surgeons.

This work demonstrates that the design and initial testing of a mechanical inclinometer is suitable for use in determining the acetabular cup inclination in THR.

Experimental testing showed that the device is accurate to within acceptable limits and reliably improved the accuracy of uncemented cup implantation in all surgeons.

THE EFFECT OF IL-1B IN OSTEOCHONDRAL TISSUES USING A NOVEL PATELLAR EXPLANT FOR OSTEOARTHRITIS

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Osteoarthritis (OA) is a disease that affects both bone and cartilage. Typically, this disease leads to cartilage degradation and subchondral bone sclerosis but the link between the two is unknown. Also, while OA was traditionally thought of as non-inflammatory condition, it now seems that low levels of inflammation may be involved in the link between these responses. This is particularly relevant in the case of Post-Traumatic OA (PTOA), where an initial phase of synovial inflammation occurs after injury. The inflammatory mediator interleukin 1 beta (IL-1B) is central to this response and contributes to cartilage degradation. However, whether there is a secondary effect of this mediator on subchondral bone, *via* bone-cartilage crosstalk, is not known. To address this question, we developed a novel patellar explant model, to study bone cartilage crosstalk which may be more suitable than commonly used femoral head explants. The specific aim of this study was to validate this novel patellar explant model by using IL-1B to stimulate the inflammatory response after joint injury and the subsequent development of PTOA.

Female Sprague Dawley rats (n=48) were used to obtain patellar explants, under an institutional ethical approval license. Patellae were maintained in high glucose media , under sterile culture conditions, with or without IL-1B (10ng/ml), for 7 days. Contralateral patellae served as controls. One group (n= 12) of patellae were assessed for active metabolism, using two both Live and Dead (L/D) staining and an Alamar Blue assay (AB). A second group (n=12) was used for tissue specific biochemical assays for both bone (Alkaline Phosphatase) and cartilage (sulfated proteoglycan and glycosaminoglycan (sGaG)). Finally, a third group (n=28) of explants were used for histologically analysis. Samples were decalcified, embedded in paraffin and sectioned to 7µm thickness, and then stained using H&E; and Safranin O with fast green. Additionally, toluidine blue and alkaline phosphatase staining were also performed.

Our results demonstrate that our system can maintain good explant viability for at least 7 days, but that IL-1B reduces cell viability in patellar cartilage, as measured by both L/D and AB assays after 0, 2, 4 and 7 days in culture. In contrast, sGaG content in cartilage were increased by this treatment. Additionally, ALP, a marker of osteoblastic activity, was increased in IL-1B treated group 4 and 7 days, but was also showed some increase in control groups. Histological analyses showed that IL-1B treatment resulted in reduced proteoglycan staining, demonstrating the powerful effect of this factor in injury response over time.

Thus, we conclude that IL-1B affects both bone and cartilage tissues independently in this system, which may have relevance in understanding bone-cartilage crosstalk after injury and how this is involved in PTOA development.

TOPOLOGICAL AND COMPOSITIONAL GRADIENTS TO GENERATE 3D TEXTURED LIVING MICROFIBERS RESEMBLING TENDON-TO-BONE INTERFACE

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Tendon-to-bone multi-tissue transition exhibits a hierarchical and continuous gradient of matrix composition and alignment, allowing for efficient transmission of mechanical loading between tendon and bone. Upon injury, main problems associated with tendon-to-bone regeneration include disorganized matrix deposition, with a gradual loss of mineral content resulting in poor mechanical properties, limiting tissue integration and the formation of a graded interface. Therefore, we propose to assembly two types of continuous microfibres with distinct topological and compositional features tailored to guide cell alignment and matrix deposition while matching the mechanical requirements of the native tissue.

Wet-spinning was used to produce textured composite microfibres using different flow rates and two polymer blends to replicate the anisotropic architecture of tendon (PCL/Gelatin, 22/9%, w/v) and the isotropic organization together with mineral composition of bone (PCL/Gelatin/Hydroxyapatite, 22/9% w/v and 7.7% w/w HAp). Obtained microfibres morphology, chemical and mechanical properties were evaluated. Biological performance was studied using human adipose-derived stem cells (hASCs). Cytoskeleton alignment, nuclei elongation and matrix mineralization were evaluated. Textile techniques were used to create a 3D fibrous scaffold. Morphological features were analyzed by micro-CT.

PCL/Gelatin fibers produced at 1 mL/h extrusion rate exhibited the highest anisotropic alignment, in opposition to PCL/Gelatin/HAp fibers produced under the same condition. Micro-CT analysis of PCL/Gelatin/HAp fibers demonstrated variations within pore diameter and particles size between the different flow rates. Herein, PCL/Gelatin fibers induced a higher cytoskeleton alignment and nuclei elongation ($p < 0.0001$) in seeded hASCs. In contrast, significantly higher mineralization was found in PCL/Gelatin/HAp fibres (day 7, $p < 0.04$; day 14, $p < 0.0001$) as observed by alizarin red staining and quantification, suggesting the induction of an osteogenic-like phenotype. As proof of concept, textile techniques were used to assemble the two types of fibers and create a 3D scaffold presenting a continuous gradient in HAp content, as well as topological cues. After 14 days of culture with hASCs, a gradient of collagen deposition and matrix mineralization was found ($p < 0.014$, $p < 0.0001$). Higher deposition of collagen type II was observed in the tendon and interface parts of the fibrous scaffold and collagen type X in the interface.

Overall, the wet-spinning method was efficiently used to engineer continuous textured composite microfibers. PCL/Gelatin fibers supported cell alignment mimicking tendon one, while PCL/Gelatin/HAp fibers induced mineral deposition and a possible phenotypic change without additional medium supplementation. Textile

techniques allowed fibres assemblage and 3D scaffolds fabrication envisioning tendon-to-bone applications.

LABEL-FREE PROTEOMIC ANALYSIS OF OSTEOCHONDROTIC CHONDROCYTES

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Osteochondrosis (OC) is a common joint disease that affects developing cartilage and subchondral bone in humans, and in multiple animal species including horses. It is an idiopathic localized joint disorder characterized by focal chondronecrosis and retention of growing cartilage that can lead to the formation of fissures, subchondral bone cysts or intra-articular fragments. OC is considered a complex multifactorial disease with chondrocyte biogenesis impairment mainly due to biochemical and genetic factors. Likewise, the molecular events involved in the OC are not fully understood. Moreover, the OC pathogenesis seems to be shared across species. In particular, equine OC and human juvenile OC share some symptoms, predilection sites and clinical presentation. In this study, by using the label-free mass spectrometry approach, proteome of chondrocytes isolated from equine OC fragments has been analysed in order to clarify some aspects of cell metabolism impairment occurring in OC.

Equine chondrocytes isolated from 7 healthy articular cartilages (CTRL) and from 7 osteochondritic fragments (OC) (both obtained from metacarpo/metatarsophalangeal joints) were analysed. Proteins were extracted using urea and ammonium bicarbonate buffer, reduced, alkylated and digested with Trypsin/Lys-C Mix. Peptides were analysed using Q Exactive UHMR Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific). All mass spectra of label-free samples analysed was set up to search against SwissProt human database (*Homo sapiens*) and SwissProt horse database (*Equus caballus*). One-way ANOVA was used for hypothesis testing. Proteins with a ≥1.5 fold change and with a FDR adjusted *p* value of ≤0.05 were defined as differentially expressed.

Statistical analysis evidenced 41 proteins up-regulated in OC while 18 were down-regulated with respect to the CTRL. Functional analysis showed that up-regulated proteins in OC were related to extracellular matrix degradation, lysosome, apoptotic execution phase, unfolded protein response, hyaluronan and keratan sulfate degradation, oxidative stress response and negative regulation of BMP signalling pathway. The down-regulated proteins were associated with endochondral ossification, vitamin D in inflammatory disease, Wnt signalling pathway and ECM proteoglycans. Validation assays confirmed these findings

These findings may contribute to clarify the events determining the onset and progression of both equine and human OC. Imaging MS analysis of OC and healthy cartilage to analyse lipid and metabolomic changes occurring in OC cartilage is in progress

A CLINICAL AUDIT TO INVESTIGATE THE CLINICAL UTILITY OF THE OTTAWA ANKLE RULE SCORING SYSTEM TO REDUCE IONISING RADIATION EXPOSURE TO PATIENTS WITH ACUTE ANKLE INJURIES

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The ankle radiograph is a commonly requested investigation as the ankle joint is commonly injured. Each radiograph exposes 0.01 mSv of radiation to the patient that is equivalent to 1.5 days of natural background radiation [1]. The aim of the clinical audit was to use the Ottawa Ankle Rule to attempt to reduce the number of ankle radiographs taken in patients with acute ankle injuries and hence reduce the dose of ionising radiation the patient receives.

A retrospective audit was undertaken. 123 ankle radiograph requests and radiographs taken between May and July 2018 were evaluated. Each ankle radiograph request including patient history and clinical examination was graded against the Ottawa Ankle Rule. The rule states that 1 point(s) indicates radiograph series; (1) malleolar and/or midfoot pain; (1) tenderness over the posterior 6cm or tip of the lateral or medial malleolus (ankle); (1) tenderness over the navicular or the base of the fifth metatarsal (foot); (1) unable to take four steps both immediately and in the emergency department [2]. Patients who score 0 do not need radiograph series. Each radiograph was reviewed if a fracture was present or not.

The clinical audit identified 14 true positives where the Ottawa Ankle Rule scored 1 and the patient had an ankle fracture, and 2 false negatives (sensitivity 88%). There were 81 false positives, and 23 true negatives (specificity 22%). Therefore, a total of 23/123 ankle radiographs were unnecessary which is equivalent to 34.5 days of background radiation. The negative predictive value of the Ottawa Ankle Rule in this audit was 92%.

The low rate of Ottawa rule utilisation may unnecessarily cause patient harm that should be addressed. An educational intervention with physicians combined with integration of the Ottawa rule scoring in ankle radiograph requests is planned with re-audit in 6 months.

NANOSTRUCTURED SILVER THIN FILMS FOR BIOMEDICAL DEVICES: EVALUATION OF BIOFILM INHIBITION CAPABILITY

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Infections are among the main complications connected to implantation of biomedical devices, having high incidence rate and severe outcome. Since their treatment is challenging, prevention must be preferred. For this reason, solutions capable of exerting suitable efficacy while not causing toxicity and/or development of resistant bacterial strains are needed. To address infection, inorganic antibacterial coatings, and in particular silver coatings, have been extensively studied and used in the clinical practice, but some drawbacks have been evidenced, such as scarce adhesion to the substrate, delamination, or scarce control over silver release.

Here, antibacterial nanostructured silver-based thin films are proposed, obtained by a novel plasma-assisted technique, Ionized Jet Deposition (IJD). Coatings are obtained by deposition of metallic silver targets. Films thickness is selected based on previous results aimed at measuring extent and duration of silver release and at evaluating toxicity to host cells (fibroblasts). Here, composition (grazing incidence XRD) and morphology (SEM) of the obtained coatings are characterized for deposition onto different substrates, both metallic and polymeric. For heat sensitive substrates, possible alterations caused by coatings deposition in terms of morphology (SEM) and composition (FT-IR) is assessed. Then, a proof-of-concept study of the capability of these films to inhibit microbial biofilm formation is performed by using two different supports i.e., the Calgary Biofilm Device and the microplates. To the best of the Authors knowledge, this is the first study describing the application of specific anti-biofilm analyses to nanostructured coatings. In particular, anti-biofilm activities are tested against the following pathogenic strains: Escherichia (E.) coli NCTC12923, Staphylococcus (S.) aureus ATCC29213 and S. aureus 86. Among these, the strain 86 is not only pathogen but it also possesses several antibiotic resistance genes, allowing the evaluation of the utilization of nanostructured coatings as an alternative anti-microbial system to face the global threat of antibiotic resistance.

Results indicate that films deposited from silver targets are composed of nanosized aggregates of metallic silver, indicating a perfect transfer of composition from the deposition target to the coatings.

Results obtained here indicate that the films have significant antibacterial and antibiofilm activity. In addition, they prove that the system can be successfully applied for evaluation of coatings antibacterial efficacy for biomedical applications.

BIOACTIVE NANOSTRUCTURED THIN FILMS FOR 3D-PRINTED BIOMEDICAL DEVICES

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Calcium phosphates-based coatings have been widely studied to favour a firm bonding between orthopaedic implants and the host bone. To this aim, thin films (thickness below 1 μm) having high adhesion to the substrate and a nanostructured surface texture are desired, capable of boosting platelet, proteins and cells adhesion. In addition, a tunable composition is required to resemble as closely as possible the composition of mineralized tissues and/or to intentionally substitute ions having possible therapeutic functions. The authors demonstrated nanostructured films having high surface roughness and a composition perfectly resembling the deposition target one can be achieved by Ionized Jet Deposition (IJD). Highly adhesive nanostructured coatings were obtained by depositing bone-apatite like thin films by ablation of deproteinized bovine bone, capable of promoting host cells attachment, proliferation and differentiation. Here, biomimetic films are deposited by IJD, using biogenic and synthetic apatite targets. Since IJD deposition can be carried out without heating the substrate, application on heat sensitive polymeric substrate*, i.e.* 3D printed porous scaffolds, is investigated.

Biogenic apatite coatings are obtained by deposition of deproteinized bone (bovine, ovine, equine, porcine) and compared to ones of stoichiometry hydroxyapatite (HAp). Coatings composition (FT-IR-ATR, FT-IR microscopy, XRD, EDS) and morphology (SEM, AFM) are tested for deposition onto metallic and 3D-printed polymeric substrates (polyurethane (PU)). Different post-treatment annealing procedures for metallic substrates are compared (350-425°C), to optimize crystallinity. Then, uniformity of substrate coverage and possible damage caused to the polymeric substrate are studied by SEM, DSC and FT-IR microscopy.

Biogenic coatings are composed by carbonated HAp (XRD, FT-IR). Trace ions Na^+ and Mg^{2+} are transferred from deposition target to coating. All coatings are nanostructured, composed by nano-sized globular aggregates, of which morphology and dimensions depend on the target characteristics. As-deposited coatings are amorphous, but crystallinity can be tuned by post-treatment annealing. A bone-like crystallinity can be achieved for heating at ≥400°C, also depending on duration. When deposited on 3D-printed PU scaffolds, coatings, owing to sub-micrometric thickness, coat them entirely, without altering their fibre shape and porosity.

Obtained biomimetic bone apatite coatings can be deposited onto a variety of metallic and polymeric biomedical devices, thus finding several perspective applications in biomedical field.

VIRTUAL FRACTURE CLINIC: HAVE THE IMPLEMENTATION OF NEW GUIDELINES AND TEACHING IMPROVED THE SERVICE? A CLOSED LOOPED AUDIT.

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Virtual Fracture Clinic (VFC) is a consultant-led orthopaedic trauma outpatient triage and management service. The use of VFC has recently become commonplace in the United Kingdom. It allows multiple referral sources to the orthopaedic team, with clinical information and imaging reviewed by a consultant in VFC who formulates an appropriate management plan with the patient contacted; either to attend clinic for consultation or discharged with advice over the phone. The VFC is more efficient than a traditionally delivered outpatient fracture clinic service. We have utilized VFC for 1 year at our hospital, East Kent University Hospital Foundation Trust (EKHUFT), and undertook a closed loop audit to evaluate the service and highlight potential areas of improvement. The Objective of the study was to identify whether the implementation of new re-designed VFC referral guidelines together with teaching set across one of the hospitals in EKHUFT improved the effectiveness and standards of VFC referrals.

An initial audit was performed of all referrals made to VFC over a 2 weeks period in December 2018. Changes to the VFC referral pathway were implemented, and teaching sessions performed by the orthopaedic team to all referring units, including minor injury units (MIU) and the emergency department (ED). After implementation, re-audit of VFC referrals was performed in February 2019 over a similar 2 weeks period. Patient demographics, diagnosis and outcomes were collected from the online patient record with images reviewed using PACS software.

Following intervention, referral rates dropped by 27.7% (136 vs 188 patients) over the 2 weeks periods. Patient demographics, injury type and severity remained the same between the 2 groups. 51.5% (70/136) did not meet VFC pathway criteria after the intervention and were considered inappropriate, compared to 70% in the original group. 15.4% (21/136) referrals could have been managed in the emergency department using the new guidelines and leaflet discharge. 5.1% (7/136) of the referrals should have been referred to orthopaedic on-call acutely and 22% (30/136) of the referrals had a soft tissue injury or no injury identified. This did not change between the 2 groups despite intervention.

Referring MIU and ED units require continued support and teaching over a prolonged time period to hopefully see further improvements. Immediate hot reporting of radiographs may further benefit the service, but staffing and funding issues particularly out of hours, means this remains an aspiration.

DESIGN OF 3D PRINTED SCAFFOLDS MIMICKING THE NATURAL FEATURES OF HEALTHY BONE

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Bone tissue engineering is a promising strategy to treat the huge number of bone fractures caused by progressive population ageing and diseases i.e., osteoporosis. The bioactive and biomimetic materials design modulating cell behaviour can support healthy bone tissue regeneration. In this frame, type I collagen and hydroxyapatite (HA) have been often combined to produce biomimetic scaffolds. In addition, mesoporous bioactive glasses (MBGs) are known for their ability to promote the deposition of HA nanocrystals and their potential to incorporate and release therapeutic ions. Furthermore, the use of 3D printing technologies enables the effective design of scaffolds reproducing the natural bone architecture.

This study aims to design biomimetic and bioactive 3D printed scaffolds that mimic healthy bone tissue natural features in terms of chemical composition, topography and biochemical cues. Optimised collagenous hybrid systems will be processed by means of extrusion 3D printing technologies to obtain high resolution bone-like structures. Protocols of human co-cultures of osteoblasts and osteoclasts will be developed and used to test the 3D scaffolds.

Type I collagen has been combined with rod-like nano-HA and strontium containing MBGs (micro- and nano-sized particles) in order to obtain hybrid systems resembling the composition of native bone tissue. A comprehensive rheological study has been performed to investigate the potential use of the hybrid systems as biomaterial inks. Mesh-like structures have been obtained by means of extrusion-based technologies exploiting the freeform reversible embedding of suspended hydrogels (FRESH) approach. Different crosslinking methods have been tested to improve final constructs mechanical properties. Both crosslinked and non-crosslinked biomaterials were cultured with human osteoblasts and osteoclasts to assay the hybrid matrix biocompatibility as well as its influence on cell behaviour.

Homogeneous hybrid systems have been successfully developed and characterised, proving their suitability as biomaterial inks for 3D printing technologies. Mesh-like structures have been extruded in a thermo-reversible gelatine slurry, exploiting the sol-gel transition of the systems under physiological conditions. Covalent bonds between collagen molecules have been promoted by genipin treatment, leading to a significant increase in matrix strength and stability. The collagen methacrylation and the further UV-crosslinking are under investigation as alternative promising method to reinforce the 3D structure during the printing process. Biological tests showed the potential of the developed systems especially for genipin treated samples, with a significant adhesion of primary cells.

Collagenous hybrid systems proved their suitability for bioactive 3D printed structures design for bone tissue engineering. The multiple stimuli provided by the

scaffold composition and structure will be investigated on both direct and indirect human osteoblasts and osteoclasts co-culture, according to the developed protocols.

AN AUDIT ON CANCELLATION OF HIP FRACTURE PATIENTS AT A DISTRICT GENERAL HOSPITAL

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Hip fractures are a common injury in elderly patients. The UK has a National Hip Fracture Database to collect data on all patients presenting to hospital with a hip fracture. Literature evidence suggests that early surgery for hip fracture patients improves morbidity and mortality. UK national guidelines (BOA, NICE) recommend that surgery is performed within 36 hours of presentation and/or diagnosis for inpatients. Best Practice Tariffs ensure that hospitals are paid a set value if they meet this target of surgery within 36 hours. This study aims to look at reasons for delay to surgery for patients presenting to our busy level 2 trauma unit.

This is a retrospective review of prospectively collected data for patients referred to the orthopaedic team at our hospital with a diagnosis of a neck of femur fracture between 1st April and 31st December 2018. Patients under the age of 65 year of age were excluded from our study. Only patients who were operated on after 36 hours were included. The database for reasons of surgical delay was reviewed and electronic patient records were used to collect further data on length of stay and 30-day mortality.

A total of 249 patients were diagnosed with a hip fracture during the study period. 2 patients were too unwell for an operation and died within 24 hours of diagnosis/admission. 46 patients were included in the study. The primary reasons for surgical delay were patients not being fit for surgery (14/46) and the use of anti-coagulation (14/46). Other reasons included a lack of surgical capacity (7/46) and delayed diagnosis due to further imaging (CT). Mean delay to surgery was 51.8 hours (range 34.5 - 157.2 hours; median 42.9 hours), mean length of stay 20.4 days (range 5.3 - 55.7 days, median 15.6 days). 30-day mortality was 4/46 (8.6%) for patients who were delayed

Many of the issues we found in this study are unusual however these problems are commonly faced in many level 2 trauma units that serve an ever growing ageing population. Changing practice to provide improved out-of-hours medical care to facilitate medical optimisation and using current literature evidence that shows that the use of DOACs/NOACs does not adversely affect outcomes when patients are operated on within 24 hours of the last dose may help improve times to surgery.

THE ROLE OF INTRARTICULAR ADMINISTRATION OF FETUIN-A IN POST-TRAUMATIC KNEE OSTEOARTHRITIS. AN EXPERIMENTAL STUDY IN A RAT MODEL

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Osteoarthritis is a slowly progressive disease which includes the intervention of several cytokines and macrophage metalleinoproteinases reaction, leading to the degradation of the local cartilage but also having an impact on the serum acute phase proteins (APPs). Subsequently, biomarkers seem to be essential to estimate its progression and the need for any surgical intervention such as total arthroplasty, but also can be used as therapeutic agents. Recently, among APPs, fetuin-A drew attention regarding its possible anti-inflammatory role in animal models but also as a therapeutic agent in the inflammatory joint disease in clinical trials. The purpose of this study is to investigate the possible attenuating role of the intra-articular administration of Fetuin-A in post-traumatic induced secondary osteoarthritis in rats, and also its effect on the systematic levels of IL-2,4,7, BMPs 2,4,7, CRP and Fetuin-A.

30 male Sprague Dawley rats were separated in two groups where post-traumatic osteoarthritis was induced surgically by Anterior Cruciate Ligament Transection and the transection of the Medial Collateral Ligament of the right knee. In the Control Group, only surgical intervention took place. In Fetuin Group, along with the induction of osteoarthritis, a single dose of bovine fetuin was administrated intra-articularly intra-operatively in 5 and 8 weeks of the experimental protocol. Both groups were examined for 8 weeks. The levels of interleukins, bone morphogenetic proteins, Fetuin-A and C-Reactive Protein were evaluated by ELISA of peripheral blood in three time periods: preoperatively, 5 and 8 weeks post-operatively. Knee osteoarthritic lesions were classified according to Osteoarthritis Research Society International Grading System and Modified Mankin Score, by histologic examination.

IL-2 levels were significantly decreased in the Fetuin Group. No statistical difference was signed on the levels of IL-7, BMP-2,4,7 and Fetuin-A between the two groups. CRP levels were significantly increased in the Fetuin Group in 5 weeks of the experiment. Fetuin Group signed better scores according to the OARSI classification system and Modified Mankin Score, without any statistical significance.

Intra-articular administration of Fetuin-A restrictively affected the progression of post-traumatic arthritis in rats, as only the levels of IL-2 were decreased as well as limited osteoarthritic lesions were observed on the Fetuin Group.

SINGLE DOSE OF TRANEXAMIC ACID EFFECTIVELY REDUCED BLOOD LOSS AND TRANSFUSIONS IN ELDERLY PATIENTS UNDERGOING SURGERY FOR HIP FRACTURE

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This study aims to investigate that a single dose of tranexamic acid (TXA) will reduce blood loss and transfusion rates in elderly patients, undergoing intertrochanteric (IT) or femoral neck fractures surgery. Consecutive elderly patients receiving hip fracture surgery for stable or unstable IT fracture, treated with short intramedullary nail (IMN) insertion as well as cemented hemiarthroplasty for acute femoral neck (subcapital) hip fracture, were screened for inclusion in this single-centre randomized trial.

Patients were randomly allocated to a study group by sealed envelope. One TXA dose of 15 mg/kg i.v. diluted in 100 ml N/S or one placebo dose i.v. in 100 ml N/S were administered 5 mins before the skin cut. Haemoglobin (Hb) concentration was measured at admission time and prior to surgery. Post-operatively it was measured on a daily basis until day 4, giving a total of four Hb measurements (days 1 to 4). The transfusion trigger point was determined in accordance with the French guidelines for erythrocyte blood transfusion. The transfusion trigger was 10 g/dl for patients at risk, while in all other cases, it was 9 g/dl. Information regarding the transfusions number was assessed directly by the hospital blood bank database. Blood loss was calculated by the Hb dilution method. Nadler's formula was used to calculate patients' blood volume. For calculation of total blood loss (TBL) expressed to total Hb loss and total Volume loss, the number of transfusions (55 grams of Hb per transfusion), the Hb concentration on preoperatively (Hgbi) and the Hb concentration on the last measure (Hgbe) were used. (Hb balance method).

The primary efficacy outcome was the number of transfusions of allogeneic RBC from surgery up to day 4. The secondary ones were the total blood loss from surgery to day 4 as it was calculated by Hb-balance method. After randomization, 35 patients with femoral neck fracture and 30 patients with IT fracture received TXA prior to surgery. Respectively, 30 patients with femoral neck fracture and 55 with IT fracture didn't receive TXA. The groups did not differ significantly in their basic demographics (age, gender, BMI, injury mechanism, ASA score, co-morbidities). Results showed that patients undergoing hemiarthroplasty after receiving TXA, were transfused with less allogeneic RBC and had less total blood loss than patients that didn't receive TXA, but without statistical significance. While patients treated with IMN in the TXA group received a significantly lower number of RBC units than the control group (1.28 ± 1.049 vs 2.075 ± 1.685), (P = 0.0396), had a significantly lower loss of Hb (98.59 ± 55.24 vs 161.6 ± 141.7), (P = 0.0195) and a lower total blood volume loss (951.3 ± 598.9 ml vs 1513 ± 1247 ml), (P = 0.023).

This trial confirmed TXA administration efficacy in reducing blood loss and transfusion rate in elderly patients undergoing hip fracture surgery. A TXA single dose may be a safer option, taking into account these patients' physiological status and co-morbidities.

FID-134: A HYALURONAN-BISPHOSPHONATE MACROMOLECULAR DRUG DELIVERY SYSTEM FOR INTRA-ARTICULAR TREATMENT OF OSTEOARTHRITIS WITH POTENTIAL COMBINED CARTILAGE AND SUBCHONDRAL BONE TARGETING

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Osteoarthritis (OA) is a joint degenerative disease leading to chronic pain and disability, thus resulting in a major socioeconomic health burden. OA, which has long been believed to be a cartilage disease, is now considered a whole-joint disorder affecting various anatomical structures, including subchondral bone.

Hyaluronic Acid (HA) is commonly used as intra-articular viscosupplementation therapy for its mechanical features and biological effects. Bisphosphonates (BPs) are antiresorptive agents inhibiting recruitment and maturation of osteoclast precursors and activity of mature osteoclasts in the bone. Pre-clinical evidences in the literature, show that intra-articular BPs could impact on OA progression, slowing down or reversing it. The combination of HA biological and mechanical role and Alendronate (ALD) antiresorptive effect could be an interesting strategy for OA treatment. This study describes the synthesis and characterization of FID-134, a new chemical derivative of HA conjugated with ALD by means of a covalent bond, cleavable in physiological condition.

FID-134 was synthesized starting from 500 kDa HA: chemical structure and functionalization degree with ALD were investigated by NMR and ICP-OES. Kinetics of ALD release from FID-134 was determined in TRIS buffer at 37°C and compared to a simple mixture of HA+ALD. 20mg/mL formulations of FID-134 and HA+ALD were investigated for viscoelastic properties, in absence and presence of Ca^{2+} ions. The cytotoxicity of FID-134 and free ALD were tested on Saos-2 osteoblasts (ATCC HTB-85) and on primary bovine chondrocytes (PBC) at day 1, 3 and 7. The efficacy of FID-134 was assessed in an inflammatory arthritis *in vitro* model, where bovine cartilage biopsies were exposed to IL-1β/OSM (10ng/mL) for 3 weeks; at the same time, cartilage explants were treated with FID-134. Collagen release in the surnatants was quantified and compared to controls.

FID-134 structure was confirmed by NMR and the 20% mol/mol functionalization degree was determined by ICP-OES. Only about 50% of total bound ALD was released from FID-134 within 7 days, resulting slower compared to HA+ALD mixture. In presence of Ca^{2+} ions, viscoelastic properties of FID-134 dramatically improved, while HA+ALD formulation remained unaffected. The cytotoxicity of ALD was evident at 100 μM on Saos-2 and PBC after 3 days, while no cytotoxicity was observed at 7 days with FID-134. In the cartilage explant model, a strong collagen release was detected in inflammatory conditions after 3 weeks; this tendency was reversed, and collagen release halved when FID-134 was added to the biopsies.
The synthesized HA-ALD adduct, FID-134, opens the door for a new approach for OA treatment. The results suggest that FID-134 could be beneficial in cartilage degradation and in restoration of subchondral bone function. Finally, local

administration and controlled BP release would likely overcome the drawbacks of ALD oral administration, such as unspecific features and long-term toxic side effects.

DESIGN OF 3D PRINTED SCAFFOLDS MIMICKING THE NATURAL FEATURES OF HEALTHY BONE

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Bone tissue engineering is a promising strategy to treat the huge number of bone fractures caused by progressive population ageing and diseases i.e., osteoporosis. The bioactive and biomimetic materials design modulating cell behaviour can support healthy bone tissue regeneration. In this frame, type I collagen and hydroxyapatite (HA) have been often combined to produce biomimetic scaffolds. In addition, mesoporous bioactive glasses (MBGs) are known for their ability to promote the deposition of HA nanocrystals and their potential to incorporate and release therapeutic ions. Furthermore, the use of 3D printing technologies enables the effective design of scaffolds reproducing the natural bone architecture.

This study aims to design biomimetic and bioactive 3D printed scaffolds that mimic healthy bone tissue natural features in terms of chemical composition, topography and biochemical cues. Optimised collagenous hybrid systems will be processed by means of extrusion 3D printing technologies to obtain high resolution bone-like structures. Protocols of human co-cultures of osteoblasts and osteoclasts will be developed and used to test the 3D scaffolds.

Type I collagen has been combined with rod-like nano-HA and strontium containing MBGs (micro- and nano-sized particles) in order to obtain hybrid systems resembling the composition of native bone tissue. A comprehensive rheological study has been performed to investigate the potential use of the hybrid systems as biomaterial inks. Mesh-like structures have been obtained by means of extrusion-based technologies exploiting the freeform reversible embedding of suspended hydrogels (FRESH) approach. Different crosslinking methods have been tested to improve final constructs mechanical properties. Both crosslinked and non-crosslinked biomaterials were cultured with human osteoblasts and osteoclasts to assay the hybrid matrix biocompatibility as well as its influence on cell behaviour.

Homogeneous hybrid systems have been successfully developed and characterised, proving their suitability as biomaterial inks for 3D printing technologies. Mesh-like structures have been extruded in a thermo-reversible gelatine slurry, exploiting the sol-gel transition of the systems under physiological conditions. Covalent bonds between collagen molecules have been promoted by genipin treatment, leading to a significant increase in matrix strength and stability. The collagen methacrylation and the further UV-crosslinking are under investigation as alternative promising method to reinforce the 3D structure during the printing process. Biological tests showed the potential of the developed systems especially for genipin treated samples, with a significant adhesion of primary cells.

Collagenous hybrid systems proved their suitability for bioactive 3D printed structures design for bone tissue engineering. The multiple stimuli provided by the scaffold composition and structure will be investigated on both direct and indirect human osteoblasts and osteoclasts co-culture, according to the developed protocols.

CAN A MEDIALLY STABILIZED TKA DESIGN APPROACH A NATURAL KNEE KINEMATICS?

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Physiological kinematics is very difficult to restore after total knee arthroplasty (TKA). A new model of medial stabilized (MS) TKA prosthesis has a high spherical congruence of the internal compartment, which guarantees anteroposterior (AP) stability associated with a flat surface of the insert in the lateral compartment, that allows a greater AP translation of the external condyle during knee flexion. The aim of our study is to evaluate, by dynamic radiostereometric analysis (RSA), the knee *in vivo* kinematics after the implantation of a MS prosthesis during sit to stand and lunge movements. To describe the *in vivo* kinematics of the knee after MS Fixed Bearing TKA (GMK Sphere (TM) Medacta International AG, Castel San Pietro, Switzerland) using Model Based dynamic RSA.

A cohort of 18 patients (72.1 ± 7.4 years old) was evaluated by dynamic RSA 9 months after TKA. The kinematic evaluation was carried out using the dynamic RSA tool (BI-STAND DRX 2), developed at our Institute, during the execution of sit to stand and lunge movements. The kinematic data were processed using the Grood and Suntay decomposition and the Low Point method. The patients performed two motor tasks: a sit-to-stand and a lunge. Data were related to the flexion angle versus internal-external, varus-valgus rotations and antero-posterior translations of the femur with respect to the tibia.

During the sit to stand, the kinematic analysis showed the presence of a medial pivot, with a significantly greater ($p=0.0216$) anterior translation of the lateral condyle (3.9 ± 0.8 mm) than the medial one (1.6 ± 0.8 mm) associated with a femoral internal rotation (4.5 ± 0.9 deg). During the lunge, in the flexion phase, the lateral condyle showed a larger posterior translation than the medial one (6.2 ± 0.8 mm vs 5.3 ± 0.8 mm) associated with a femoral external rotation (3.1 ± 0.9 deg). In the extension phase, there is a larger anterior translation of the lateral condyle than the medial one (5.8 ± 0.8 mm vs 4.6 ± 0.8 mm) associated with femoral internal rotation (6.2 ± 0.9 deg). Analysing individual kinematics, we also found a negative correlation between clinical scores and VV laxity during sit to stand ($R= -0.61$) and that the higher femoral extra-rotation, the poorer clinical scores ($R= 0.65$).

The finding of outliers in the VV and IE rotations analysis highlights the importance of a correct soft tissue balancing in order to allow the prosthetic design to manifest its innovative features.

A HEALTH ECONOMIC ANALYSIS OF THE COST OF MANAGING OPEN FRACTURES IN THE ELDERLY.

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Open lower limb fractures are resource-intensive fractures, accounting for a significant proportion of the workload and cost of orthopaedic trauma units. A recent study has evaluated that the median cost of direct inpatient treatment of open lower-limb fractures in the National Health Service (NHS) is steep, at £19189 per patient. Healthcare providers are expected to be aware of the costs of treatments, although there is very limited dissemination of this information, neither on a national or local level. Older adults (>65 years old) are at an increased risk of the types of high-energy injuries that can result in open lower limb fractures. Generally, there remains a significant lack of literature surrounding the cost of open fracture management, especially in specific patient groups that are disproportionately affected by these fractures. This study has calculated the direct inpatient care costs of older adults with open lower limb fractures.

Open lower limb fractures in adult patients over 65 years old treated at Addenbrooke's Hospital of Cambridge University Hospitals NHS Trust were identified over the period of March 2014-March 2019. Isolated fractures of the femur, tibia and fibula over this time period were included. Direct inpatient care costs were calculated using information about the sustained fracture, operative time, implant(s) and theatre kit(s) used, the number of patient bed-days on the orthopaedic ward and critical care unit, and the number of hours of inpatient physiotherapy received. Direct inpatient care costs were compared with the income received by our centre for each of these cases, according to Healthcare Resource Group (HRG) cost codes. Our data was also compared with existing literature on Patient Level Costing (PLC) figures for open lower limb fractures.

We extracted data from 58 patients over the age of 65 years treated for open isolated lower limb fractures at Addenbrooke's Hospital, Cambridge University Hospitals NHS Trust, between March 2014 and March 2019. The median cost of inpatient care calculated in this study was £20,398 per patient, resulting in a financial loss to the hospital of £5113 per patient. When the results were disaggregated by sex, the median cost for an open lower limb fracture in a male patient was £20,886 compared to £19,304 in a female patient. Data were also disaggregated by the site of injury, which produced a median cost for an open femur fracture of £23,949, and £24,549 and £15,362 for open tibia and ankle fractures, respectively.

The absence of published primary literature and clinical audits on this topic continues to hinder the inclusion of cost-effectiveness as an important factor in clinical decision-making. This study provides valuable insight into the true cost of open lower limb fractures in a key patient population in a Major Trauma Centre in England and highlights the large losses incurred by hospitals in treating these cases. These results

support the revision of the remuneration structures in the NHS for the treatment of elderly patients with these injuries.

MESOPOROUS BIOACTIVE GLASSES AS SMART PLATFORM TO STIMULATE BONE REGENERATION

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Mesoporous bioactive glasses (MBGs) have been widely studied as bone regeneration systems, due to their bioactivity and ability to store and release therapeutic agents with specific biological functions. The incorporation of these nanomaterials into a thermosensitive hydrogel (TSH), in which a solution undergoes a sol-gel transition under physiological conditions, represents a promising approach to design multifunctional devices able to deliver selected molecules to pathological sites. In fact, this system can perfectly fit the defect cavity shape prior to the complete gelation, and acts as a carrier for therapeutic agents prolonged release *in situ*. This challenging concept is the underlying idea of the MOZART project, whose objective was to develop a library of MBGs containing different therapeutic ions and drugs, to be used as a new, smart platform technology for highly targeted therapies to enhance bone healing. The aim of this work is to investigate the bone regeneration potential of MBGs containing strontium ions (pro-osteogenic) and incorporated into thermosensitive poly(etherurethane)(PEU) based on Poloxamer407. In order to further increase the pro-osteogenic response, MBGs were also loaded with N-acetylcysteine (NAC).

MBGs containing 2%mol of Sr^{2+} were prepared by an aerosol-assisted spray-drying method and NAC was loaded post-synthesis via an incipient wetness method. The PEU hydrogel (SHP407) was synthesized via a two-step procedure in nitrogen atmosphere. Particles were characterized (FE-SEM, N_2 adsorption-desorption analysis, TGA, DSC, FT-IR and XRD) and then incorporated into the hydrogel. The hybrid systems rheological properties and stability in aqueous environment at 37°C, and its ability to co-release Sr^{2+} and NAC were analysed. After preliminary biological *in vitro* tests, a proof-of-concept rodent study was run to assess the ability of the resulting formulation as bone healing device. X-ray at 2 and 4-weeks post-surgery and µCT-analysis were used to evaluate the healing results in a rat osteotomy model of biologically impaired healing. Then, bones were processed for histological evaluation.

Preliminary *in vivo* results demonstrated that incorporation of MBGs into a TSH is a promising strategy to design a multifunctional injectable formulation for *in situ* and sustained delivery of pro-osteogenic species enhancing bone regeneration.

CAN AN ACTIVITY-BASED SCORE MEASURE HEALTH-GAIN IN HIGH PERFORMING PATIENTS AFTER HIP RESURFACING ARTHROPLASTY

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Patients undergoing hip resurfacing arthroplasty (HRA) is typically reserved for highly active patients. Patient Reported Outcome Measures (PROMs) such as the Oxford Hip Score (OHS) are reported to have ceiling effects, which may limit physicians' ability to measure health gain in these patients. The Metabolic Equivalent of Task (MET) index is a validated compendium assigning energy expenditure to a wide range of activities; for example, a slow walk expends 2.9 kcal/kg/hour, golf expends 4.0 kcal/kg/hour, while moderate lacrosse typically expends 8.1 kcal/kg/hour. We hypothesized that for patients with high OHS (47-48) after HRA, the MET index could better discriminate between high-performing individuals.

We evaluated 97 consecutive HRA patients performed by a single surgeon. They prospectively completed an online Oxford Hip Score. They also listed three activities which they had performed independently in the preceding 2 weeks with a Likert-scale slider denoting intensity of effort. Matched data-sets were obtained from 51 patients, from which 23 had OHS of 47-48 at 6-months. Their activity with the highest MET index was selected for analysis. The 23 patients' OHS improved from 29.3 ± 7.0 preoperatively to 47.6 ± 0.5 after 6-months, while their MET indices improved from 8.5 ± 3.7 to 12.9 ± 3.5 kcal/kg/hr. The activities performed by these high-performance individuals ranged from the lowest, pilates (8.05 kcal/kg/hour), to highest, running at 22km/hr (23 kcal/kg/hour). 45% of patients undergoing HRA in this cohort had OHS of 47 and 48 at 6-months after surgery.

Unlike the OHS, the MET index described variation in physical activity in these high-performance individuals, and did so on an objective measurable scale.

SURGICAL REPAIR OUTCOME FOR TENDINOPATHY IS AFFECTED IN OBESE PATIENTS MICROSCOPICALLY

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Obese patients show a higher incidence of tendon-related pathologies. These patients present a low inflammatory systemic environment and a higher mechanical demand which can affect the tendons. In addition, inflammation might have a role in the progression of the disease as well as in the healing process.

A systematic review was performed by searching PubMed, Embase and Cochrane Library databases. Inclusion criteria were studies of any level of evidence published in peer-reviewed journals reporting clinical or preclinical results. Evaluated data were extracted and critically analysed. PRISMA guidelines were applied, and risk of bias was assessed, as well as the methodological quality of the included studies. We excluded all the articles with high risk of bias and/or low quality after the assessment. Due to the high heterogeneity present among the studies, a metanalysis could not be done. Thus, a descriptive analysis was performed.

After applying the previously described criteria, thirty articles were included, assessed as medium or high quality. We analysed the data of 50865 subjects, 6096 of which were obese (BMI over 30 accordingly to the WHO criteria). The overall risk of re-tear after surgery is about the 10% more than normal BMI subjects. The rupture risk fluctuates in the studies without showing a significant trend.
Obese subjects have a higher risk to develop tendinopathy and a worse outcome after surgery as confirmed in several human studies. The obesity influence on tendon structure and mechanical properties may rely on the fat tissue endocrine proprieties and on hormonal imbalance.

Clinicians should consider obesity as a predisposing factor for the development of tendinopathies and for a higher risk of complications in patients who underwent surgical repair of tendons.

THE ROLE OF ADIPOKINES IN TENDINOPATHY: A SYSTEMATIC REVIEW

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Adipose tissue releases several bioactive peptides and hormones, like adipokines that promote a low inflammatory systemic state. Inflammation, affecting the tendon homeostasis, could play a role in tendon disease development as well as in the healing process. Obese patients show a dysregulated level of adipokines and considering the higher mechanical demand, this relates to higher incidence of tendinopathies among these subjects.

A systematic review was performed searching PubMed, Embase and Cochrane Library databases. Inclusion criteria were studies of any level of evidence published in peer-reviewed journals reporting clinical or preclinical results. Evaluated data were extracted and critically analysed. PRISMA guidelines were applied, and risk of bias was assessed, as was the methodological quality of the included studies. We excluded all the articles with high risk of bias and/or low quality after the assessment.

After applying the previously described criteria, we included 12 articles assessed as medium or high quality. Leptin, others adipokines and in general changes in the hormones delicate equilibrium affect the tendon either qualitatively and/or quantitatively. The evidence still lacks consensus on their role which is probably involved in both anabolic and catabolic pathways.

The role of adipokines in the structure and healing of tendons is still debated. Further studies are needed to clarify the relation between deregulated levels of adipokines and the development of tendinopathy. A better understanding of the molecular interactions could allow us to individuate future therapeutic targets.

THE ROLE OF TYPE I DIABETES IN INTERVERTEBRAL DISC DEGENERATION

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Intervertebral disc degeneration (IDD) is a major cause of low back pain, which affects 80% of the adult population at least once in their life. The pathophysiological conditions underlying IDD are still poorly understood. Genetic makeup, aging, smoking, physical inactivity and mechanical overloading, especially due to obesity, are among the strongest risk factors involved. Moreover, IDD is often associated with chronic inflammation within disc tissues, which increases matrix breakdown, glycosaminoglycan (GAG) loss and cell death. This micro-inflammatory environment is typical of several metabolic disorders, including diabetes mellitus (DM). As the etiopathogenesis of IDD in diabetic subjects remains scarcely understood, we hypothesised that this may be driven by a DM-induced inflammation leading to a combination of reduced GAG levels, decreased proteoglycan synthesis and increased matrix breakdown within the disc. The objective of the study was to investigate the pathogenesis of IDD in a murine model of type 1 DM (T1DM), namely non-obese diabetic (NOD) mouse.

Total disc glycosaminoglycan (GAG) content, proteoglycan synthesis, aggrecan fragmentation mediated by matrix metalloproteinases (MMPs) and a Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS), glucose transporter (mGLUT1) gene expression and apoptosis (TUNEL assay) were assessed in NOD mice and wild-type euglycemic control mice. Spinal structural and molecular changes were analysed by micro-computed tomography (mCT), histological staining (Safranin-O and fast green) and quantitative immunofluorescence (anti-ADAMTS-4 and 5 antibodies). Statistical analysis was conducted considering the average of 35 samples ± standard error for each measurement, with 95% confidence intervals calculated to determine statistical significance (p-value < 0.05).

IVDs of NOD mice showed increased disc apoptosis ($p < 0.05$) and higher aggrecan fragmentation mediated by ADAMTS ($p < 0.05$). However, ADAMTS-4 and -5 did not appear to be involved in this process. The total GAG content normalized to DNA and PG synthesis showed no statistically significant alterations, as well as Safranin O staining. Although not significantly, NOD mice showed reduced glucose uptake. In addition, the vertebral structure of NOD mice at mCT seemed not to be altered.

These data demonstrate that DM may contribute to IDD by increasing aggrecan degradation and promoting cell apoptosis, which may represent early indicators of the involvement of DM in the pathogenesis of IDD.

DOES A SINGLE HAEMCUE TEST REDUCE THE NEED FOR EXTENSIVE PRE-OPERATIVE BLOOD TESTS AND THEIR ASSOCIATED COSTS IN ELECTIVE ARTHROPLASTY?

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Pre-operative anaemia can present in up to 30% of elective arthroplasty patients. The presence of anaemia increases the risk of requiring blood transfusion post-operatively as well as acts as an independent risk factor for poor outcome such as prosthetic joint infection. Recent international consensus on this topic has recommended a specific care pathway for screening patients with pre-operative anaemia using a simple bedside Heaemacue finger-prick test to detect in a simple and cost-effective manner, and then allow treatment of preoperative anaemia. This pathway was therefore incorporated in our trust.

This was a retrospective study done at a single tertiary-referral arthroplasty centre. Our data collection included the Heamacue test results and formal haemoglobin levels if they were performed as well as compliance and costs of each of the tests for patients listed for an elective shoulder, hip and knee arthroplasty between September and December 2018. Medical records and demographics were also collected for these patients for subgroup analysis. Our exclusion criteria comprised patients listed for revision arthroplasty surgery.

87 patients were included in this study. Our compliance rate was 15%. The mean difference between a Haemacue test and a formal FBC result was only 17.6g/L suggesting that it has a reasonably high accuracy. With regards to costs, we found that a Haemacue test costs £2, compared to £7.50 for a full blood count and Haematinics combined. This gave an overall cost saving of £5.50 per patient. Extrapolation of this date locally for 2017 at our hospital, where 1575 primary joint arthroplasties were done, a cost saving of £8,662.5 could have been achieved. Within the UK using data extrapolated from the National Joint Registry a total of £1,102,205.5 (1,221,894 Euros) could have been saved.

The use of a single, Haemacue test to screen for pre-operative anaemia in elective arthroplasty patients is more cost effective compared to a formal full count and haematinics tests. However, we found that compliance with the care pathway is variable due to system limitations. This may be addressed through implementing changes to our electronic system in which patients are booked for surgery. We also noted a significant cost reduction if this pathway were to be used Nation-wide. Thus, we encourage other centres to consider the use of the Haemacue test pre-operatively in elective arthroplasty instead of formal full blood counts at the time of decision to treat with arthroplasty; this allows sufficient time for correction of pre-operative anaemia thus improving patient outcomes from arthroplasty.

POST-OPERATIVE URINARY RETENTION FOLLOWING LOWER LIMB ARTHROPLASTY: INCIDENCE AND ANALYSIS OF THE ASSOCIATED FACTORS

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Acute post-operative urinary retention (POUR) is a recognized complication following lower limb arthroplasty. Its occurrence may have patient and ultimately medico-legal implications. Identifying high-risk patients and the associated risk factors pre-operatively, is vital to tackle this issue and reduce its occurrence, which ultimately, may enhance the overall success of our operations. Our aim was to assess the incidence of POUR following elective lower limb arthroplasty and analyze the related factors that could potentially predict the likelihood of developing POUR in our patient cohort. A prospective audit of 158 patients was conducted in our department. POUR was defined as inability to pass urine voluntarily within the first 24 hours following elective lower limb arthroplasty leading to the insertion of a urinary catheter. Surgical-related factors including intra-operative fluid use, type of spinal anesthetic, duration of surgery, time from surgery till insertion of a urinary catheter as well as patient-related factors including medication, urological history and Body Mass index (BMI) was collected and analyzed. 21 (13.3%) patients developed post-operative urinary retention, 11 (52%) and 10 (48%) following knee and hip replacements respectively. Of which, 19 (90.5%) were male and 2 (9.5%) were female with an average age of 66 yrs. 13 (62%) had a previous urological history and 10 (48%) were on retention associated medication. Bupivacaine as a spinal anesthetic was associated with an increased risk of developing post-operative urinary retention. The average time till catheter insertion was 14 hrs. Only 2 (10%) had an unsuccessful TWOC on discharge. Bupivacaine as a spinal anesthetic and a previous urological history can be considered as risk factors for the development of POUR. Pre-operative urinary catheterization should be considered in this high-risk group of patients.

PREDICTORS OF TRANSFUSION FOR ADULTS UNDERGOING ELECTIVE POSTERIOR THORACO-LUMBAR SPINAL FUSION

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Posterior spinal surgery is associated with a significant amount of blood loss. The factors predisposing the patient to excessive bleeding-and therefore transfusion- are not well established nor is the effect of transfusion on the outcomes following spinal surgery. We had two goals in this study. First, we were to investigate any suspected risk factors of transfusion in posterior thoraco-lumbar fusion patients. Second, we wanted to observe the negative impact-if one existed- of transfusion on the outcomes of surgery

All adults undergoing posterior thoraco-lumbar spine fusion in our institution from May 2015 to May 2018 were included. Data collected included demographic data as well as BMI, preoperative hemoglobin, American Society of Anesthesiologists classification (ASA), delta Hemoglobin, estimated blood loss, incidence of transfusion, number of units transfused, number of levels fused, length of stay and re-admission within 30 days. The data was analyzed to correlate these variables with the frequency of transfusion and then to assess the association of adverse outcomes with transfusion.

125 patients were included in the study. Only 6 patients (4.8%) required re-admission within the first 30 days after discharge. Length of stay averaged 8.4 days (3-74). 18 patients (14.4%) required transfusion peri-operatively. When multiple variables were analyzed for any correlation, the number of levels fused, age and BMI had statistically significant correlation with the need for transfusion ($P < 0.005$)

Patients undergoing posterior thoraco-lumbar fusion are more likely to require blood transfusion if they were older, over-weight & obese or had a multi-level fusion. Receiving blood transfusion is associated with increased complication rates.

EXAMINATION OF THE SITE OF BONE BRUISES IN ANTERIOR CRUCIATE LIGAMENT INJURY

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We report bone bruises on Anterior Cruciate Ligament (hereinafter referred to as ACL) injury. We also investigated the relationship among the presence or absence of bone bruises, localization, and the presence or absence of meniscal injury according to the period of MRI scan from injury.

We underwent the study used a total of 76 knees who underwent ACL reconstruction at our hospital and related hospitals from January 2014 to December 2017. We investigated on MRI images taken after injury. Meniscal injuries were evaluated by intraoperative findings.

The average age at injury was 25.8 years old (13-48 years old) in 44 males and 32 females. Bone bruises were found in 54 of 76 knees (71%). Among them, the ratio of non-contact type was much higher in the group with bone bruises than in the contact group (83% in the group with bone bruises, 64% in the group without bone bruises), resulting in a shorter period from injury to MRI (bone bruises group: 12.4 days, non-bone bruises group: 23 days). Looking at the appearance frequency of bone bruises according to the period from injury to MRI imaging, the appearance frequency of bone bruises decreased as the time to imaging became longer (within 2 weeks of injury: 76%, injury from 2 weeks to 1 month: 65%, injury 1-3 months: 53%). With regard to the localization of bone bruises, in the coronal section, both femurs and tibiae frequently had bone bruises on the outside. In the sagittal section, it occurred in front of the femur, in particular. On the tibial side, many cases of bone bruises occurred in the rear. In addition, the association between bone bruises and meniscal injuries were significantly complicated with lateral meniscal injury in the group without femoroconstriction in the group with lateral femoral bone bruises and in the group with posterior tibia bone bruises. There was no significant association between bone bruises and meniscal injury among the other groups.

Bone bruises were found in 54 of 76 knees (71%). Regarding the occurrence of many lateral developments, it is thought that the tibia is sub-dislocated anteriorly due to mild flexion, valgus force, and external rotation injury, and injury is caused by axial pressure applied to the outside of the femur and posterior of the tibia It was done. As a result, it was considered that the external meniscal injury was injured. The medial unilateral development of bone contusion was observed in 3 knees on the medial femur and 1 knee on the medial tibia. All internal single-cased cases are contact-type injuries, the result of which may be different in the mechanism of bone contusion development.

DIFFERENCES BETWEEN HORN AND ROOT CELLS OF HUMAN MEDIAL MENISCUS IN CELLULAR CHARACTERISTICS AND RESPONSES TO MECHANICAL STRESS

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The meniscus is a fibrocartilaginous tissue that plays an important role in controlling the complex biomechanics of the knee. Many histological and mechanical studies about meniscal attachment have been carried out, and medial meniscus (MM) root repair is recommended to prevent subsequent cartilage degeneration following MM posterior root tear. However, there are only few studies about the differences between meniscus root and horn cells. The goal of this study was to clarify the differences between these two cells.

Tissue samples were obtained from the medial knee compartments of 10 patients with osteoarthritis who underwent total knee arthroplasty. Morphology, distribution, and proliferation of MM root and horn cells, as well as gene and protein expression levels of Sry-type HMG box (SOX) 9 and type II collagen (COL2A1) were determined after cyclic tensile strain (CTS) treatment.

Horn cells had a triangular morphology, whereas root cells were fibroblast-like. The number of horn cells positive for SOX9 and COL2A1 was considerably higher than that of root cells. Although root and horn cells showed similar levels of proliferation after 48, 72, or 96 h of culture, more horn cells than root cells were lost following 2-h CTS (5% and 10% strain). SOX9 and COL2A1 mRNA expression levels were significantly enhanced in horn cells compared with those in root cells after 2- and 4-h CTS (5%) treatment.

This study demonstrates that MM root and horn cells have distinct characteristics and show different cellular phenotypes. Our results suggest that physiological tensile strain is important for activating extracellular matrix production in horn cells. Restoring physiological mechanical stress may be useful for promoting healing of the MM posterior horn.

GUIDED MOTION TOTAL KNEE ARTHROPLASTY (TKA) IN PATIENTS WITH BMI OF 40 KG/M2 OR MORE: RESULTS FROM THE INTERNATIONAL MULTICENTER STUDY OF 2,059 PRIMARY TKAS WITH UP TO 6 YEARS FOLLOW-UP

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Outcomes for guided motion primary total knee arthroplasty (TKA) in obese patients are unknown. 1,684 consecutive patients underwent 2,059 primary TKAs with a second-generation guided motion implant between 2011-2017 at three European and seven US sites. Of 2,003 (97.3%) TKAs in 1,644 patients with BMI data: average age 64.5 years; 58.4% females; average BMI 32.5 kg/m2; 13.4% had BMI $\geq$ 40 kg/m2. Subjects with BMI $\geq$ 40 kg/m2 had longest length of hospital stay (LOS) at European sites; LOS similar at US sites. Subjects with BMI $\geq$ 40 kg/m2 (P=0.0349) had longest surgery duration. BMI $\geq$ 40 kg/m2 had more re-hospitalizations or post-TKA reoperations than BMI < 40 kg/m2 (12.7% and 9.2% at five-year post-TKA, P<0.0495). Surgery duration and long-term complication rates are higher in patients with BMI $\geq$ 40 kg/m2, but device revision risk is not elevated.

GUIDED MOTION TOTAL KNEE ARTHROPLASTY (TKA) SYSTEM IN YOUNGER PATIENTS HAS A LOWER REVISION RATE THAN REGISTRY CONTROLS: RESULTS FROM THE INTERNATIONAL MULTICENTER STUDY WITH UP TO 6 YEARS FOLLOW-UP

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Patients $\leq$ 55 years have a high primary TKA revision rate compared to patients >55 years. Guided motion knee devices are commonly used in younger patients yet outcomes remain unknown. In this sub-group analysis of a large multicenter study, 254 TKAs with a second-generation guided motion knee implant were performed between 2011-2017 in 202 patients $\leq$ 55 years at seven US and three European sites. Revision rates were compared with Australian Joint Registry (AOANJRR) 2017 data. Average age 49.7 (range 18-54); 56.4% females; average BMI 34 kg/m2; 67.1% obese; patellae resurfaced in 98.4%. Average follow-up 4.2 years; longest follow-up six years; 27.5% followed-up for $\geq$ five years. Of eight revisions: total revision (one), tibial plate replacements (three), tibial insert exchanges (four). One tibial plate revision re-revised to total revision. Revision indications were mechanical loosening (n=2), infection (n=3), peri-prosthetic fracture (n=1), and instability (n=2). The Kaplan-Meier revision estimate was 3.4% (95% C.I. 1.7% to 6.7%) at five years compared to AOANJRR rate of 6.9%. There was no differential risk by sex. The revision rate of the second-generation guided motion knee system is lower in younger patients compared to registry controls.

A CLOUD-BASED PLATFORM TO DRIVE TECHNOLOGY TRANSFER OF MODELING AND SIMULATIONS TOOLS ACROSS ORTHOPEDICS RESEARCH AND DEVELOPMENT

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For decades, universities and research centers have been applying modeling and simulation (M&S) to problems involving health and medicine, coining the expression *in silico clinical trials*. However, its use is still limited to a restricted pool of specialists.

It is here proposed an easy-to-use cloud-based platform that aims to create a collaborative marketplace for M&S in orthopedics, where developers and model creators are able to capitalize on their work while protecting their intellectual property (IP), and researcher, surgeons and medical device companies can use M&S to accelerate time and to reduce costs of their research and development (R&D) processes.

Digital libraries on *InSilicoTrials.com* are built on collaborations among first-rate research center, model developers, software, and cloud providers (*partners*). Their access is provided to life science and healthcare companies, clinical centers, and research institutes (*users*), offering them with several solutions for the different steps of the orthopedics and medical devices R&D process. The platform is built using the Microsoft Azure cloud services, conforming to global standards of security and privacy for healthcare, ensuring that clinical data is properly managed, protected, and kept private. The environment protects the IP of partners against the downloading, copying, and changing of their M&S solutions; while providing a safe environment for users to seamlessly upload their own data, set up and run simulations, analyze results, and produce reports in conformity with regulatory requirements.

The proposed platform allows exploitation of M&S through a *Software-as-a-Service* delivery model. The pay-per-use pricing: 1. provide partners with a strong incentive to commercialize their high-quality M&S solutions; 2. enable users with limited budget, such as small companies, research centers and hospitals, to use advanced M&S solutions. Pricing of the M&S tools is based on specific aspects, such as particular features and computational power required, in agreement with the developing partner, and is distinct for different types of customers (i.e., academia or industry).

The first medical devices application hosted on InSilicoTrials.com is *NuMRis* (Numerical Magnetic Resonance Implant Safety), implemented in collaboration with the U.S. F.D.A. Center for Devices and Radiological Health, and ANSYS, Inc. The automatic tool allows the investigation of radiofrequency (RF)-induced heating of passive medical implants, such as orthopedic devices (e.g., rods and screws), pain management devices (e.g., leads), and cardiovascular devices (e.g., stents), following the *ASTM F2182-19e2* Standard Test Method. *NuMRis* promotes the broader adoption of digital evidence in preclinical trials for RF safety analysis, supporting the device submission process and pre-market regulatory evaluation.

InSilicoTrials.com aims at defining a new collaborative framework in healthcare, engaging research centers to safely commercialize their IP, i.e., model

templates, simulation tools and virtual patients, by helping clinicians and healthcare companies to significantly expedite the pre-clinical and clinical development phases, and to move across the regulatory approval and HTA processes.

THE EFFECT OF DURATION OF VITAMIN D3 APPLICATION ON THE OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS

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Critical size bone defects pose a serious clinical problem, as the intrinsic healing capacity of bone fails due to the size of the defect. Bone healing might be aided by addition of 1,25(OH)2 vitamin D3 (vitD3) to bone tissue engineering scaffolds. VitD3can promote osteogenic differentiation of human stem cells such as adipose stem cells (hASCs), which is a clinical-relevant source of mesenchymal stem cells. However, it is unknown which release kinetics of vitD3, i.e. short or sustained release from scaffolds, leads to the most optimal osteogenic differentiation of hASCs. We hypothesized that sustained release of vitD3 leads to more osteogenic differentiation of hASCs than shorter applications.

hASCs (1x105, passage 3-4) were seeded on 20 Â± 1 mg of calcium phosphate particles (day 0), cultured for 20 days, and treated with a total amount of 124 ng vitD3. This treatment was provided either during 30 min before seeding (pre-incubation, short stimulation: [200 nM]), after seeding, over the first 2 days (burst- release high: [100 nM]), or over the total culture period of 20 days (sustained-release: [10 nM]). In the extra condition: burst-release low the hASCs were treated for 2 days after seeding with 6.2 ng vitD3 ([10 nM]) per day.

Live/dead staining followed by fluorescent microscopy showed that hASCs attached to the calcium phosphate particles and were mostly viable (Â±75 %) at day 2. VitD3 applied for any duration did not affect the proliferation of hASCs at day 7 and day 20, measured with an alamar blue assay. At day 7, sustained-release increased the release of active alkaline phosphatase on average by 3.5-fold, compared to all the other conditions. At day 20, this was increased 4.3-fold. At both day 7 and day 20 total protein levels were similar in all conditions.

Our results suggest that sustained release of VitD3 from bone tissue engineered scaffolds may be beneficial for the osteogenic differentiation of human stem cells for the treatment of critical bone size defects.

UNDERSTANDING WHY PEOPLE WITH CHRONIC POST-SURGICAL PAIN FOLLOWING KNEE REPLACEMENT DON'T CONSULT HEALTHCARE PROFESSIONALS

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In the UK and USA in 2016 more than 263,000 primary knee replacements were performed. Around 20% of patients report chronic post-surgical pain (CPSP) at three or more months after total knee replacement (TKR). A large proportion of adults with all types of chronic musculoskeletal pain do not use services for a number of reasons, despite being in constant or daily pain. Given the high prevalence of CPSP, there is potentially a large hidden population with an unexpressed need for care, experiencing ongoing pain and disability; understanding why they do not use health services may herald further insight into why many remain dissatisfied with knee replacement surgery. The aim of this study is to understand why some people with CPSP after TKR do not access services or make little use of healthcare. We conducted face-to-face in-depth interviews with 34 patients from 2 high-volume orthopaedic hospitals in England, to investigate their experience of long-term pain after knee replacement; their knowledge and understanding of CPSP; and their decisions about consulting for CPSP. The sample size was based on achievement of saturation and participants provided written informed consent. Interviews were transcribed and analysed using an inductive thematic approach with double coding for rigor. Ethical approval for the study was granted by the West Midlands Solihull Research Ethics Committee (15/WM/0469). A core theme within the analysis suggests that participants do not seek healthcare because they believe that nothing further can be done, either by themselves or by healthcare professionals. Surgeons' satisfaction with the knee surgery and reassurances that pain would improve, left patients feeling uncertain about whether to re-consult, and some assumed that further consultation could lead to further surgery or medication, which they wish to avoid. Some participants' comorbidities took precedence over their knee pain when seeking healthcare. Others felt they had received their "share" of healthcare resources and that others were more deserving of treatment. People's descriptions of pain varied, from dull, or aching to shooting pains. Many described their pain as "discomfort" rather than pain. The majority described pain that was better than their pre-surgical pain, though others described pain that was worse, which they believed to be nerve damage. Many expressed disappointment in the outcome of their TKR. Expectations of pain varied, where most had expected some post-surgical pain, others underestimated it, and some had expected to be completely pain free following their TKR. Our analysis suggests that the reasons that some people with CPSP after TKR do not consult are varied and complex, spanning psychosocial, structural, moral, and organisational domains. There was an overriding sense that further consultation would be futile or may lead to unwanted treatment. Results suggest that improved information for patients about CPSP and appropriate post-surgical healthcare services may help patients and clinicians to manage this condition more effectively.

PATIENT EXPERIENCES OF RECOVERY FROM ONE- AND TWO-STAGE REVISION SURGERY FOR PROSTHETIC HIP INFECTION

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Prosthetic joint infection (PJI) is an uncommon but serious complication of hip replacement. Over 1,000 operations are performed annually in the United Kingdom for PJI following hip replacement, using either one- or two-stage revision arthroplasty. It is unclear which is preferred by patients and which has the best long-term outcome. This qualitative study aims to describe patient experiences of treatment and recovery following one- and two-stage revision arthroplasty for PJI within the context of a pragmatic randomised controlled trial comparing these two approaches. Semi-structured interviews were conducted with 32 patients undergoing one- or two-stage revision treatment for PJI as part of a UK multi-centre randomised controlled trial. Patients were recruited from 12 participating National Health Service (NHS) Orthopaedic Departments and were interviewed 2-4 months after their first revision surgery and again approximately 18 months later. Final sample size was justified on the basis of thematic saturation. All patients consented to the interview being audio-recorded, transcribed, anonymised and analysed using an inductive thematic approach. Ethical approval was provided by NRES Committee South-West Frenchay, 14/SW/116. Patients in both the one- and two-stage treatment groups described prolonged hospital stays, with burdensome antibiotics and brief physiotherapy treatment. However, following discharge home and during recovery, participants undergoing two-stage revision with an ‘empty hip’ or with a spacer reported being physically restricted in almost every aspect of their daily life, resulting in inactivity and confinement to home. Mobility aids were not sufficiently available through the health service for these patients. A key difference is that those with a spacer reported more pain than those without. Approximately one year following their second-stage revision, participants described being more independent and active, but two directly attributed muscle weakness to the lengthy period without a hip and described resulting falls or dislocations that had complicated their recovery. In contrast, those undergoing one-stage revision and CUMARS appeared to be more alike, reporting better mobility, functionality and independence, although still limited. Participants in these groups also reported minimal or no pain following their revision. A key difference between CUMARS and one-stage revision was the uncertainty of whether a second operation was necessary, which participants described as “hanging over them”, while those in the two-stage empty hip or spacer group described a more positive anticipation of a second definitive operation as it marked an end to what was described as a detachment from life. Our findings highlight the differences between patient experiences of recovery following revision arthroplasty, and how this is influenced by the surgical approach and presence or lack of spacers. An understanding of lived experiences following one- and two-stage surgical interventions will complement knowledge about the clinical effectiveness of these different types of revision surgery.

PLACEMENT OF POLLER SCREWS DURING INTRAMEDULLARY NAILING

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Various technical tips have been described on the placement of poller screws during intramedullary nailing however studies reporting outcomes are limited. Overall, there is no consistent conclusion about whether intramedullary nailing alone, or intramedullary nails augmented with poller screws is more advantageous. In a systematic review, we asked: (1) What is the proportion of non-unions with poller screw usage? (2) What is the proportion of malalignment, infection and secondary surgical procedures with poller screws usage?

We conducted a systematic review of multiple databases including Pubmed, EMBASE, and the Cochrane Library. Seventy-four records were identified, twelve met our inclusion criteria.

Twelve studies with a total of 348 participants and 353 fractures were included. Mean follow up time was 21.4 months and mean age of included patients was 40.1 year. Seven studies had heterogenous population of non-unions and/ or malunions in addition to acute fractures. Three studies included only acute fractures and two studies examined non unions only. Four of the twelve studies reported non unions with an overall outcome proportion of 4%. Six studies reported coronal malalignment with an overall outcome proportion of 6%. The secondary surgical procedures rate ranged from 2 - 40% with an overall outcome proportion of 8% and included grafting, revisions and any reported cases of removal of metal work.

When compared with existing literature our review suggests intramedullary nailing with poller screws has lower rates of non-unions and coronal malalignment than those reported in the literature for intramedullary nailing alone. Prospective randomized control trial is necessary to fully determine outcome benefits.

HISTONE DEMETHYLASE UTX PROTECTS AGAINST GLUCOCORTICOID-INDUCED OSTEOGENESIS LOSS AND OSTEOPOROSIS

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Chronic glucocorticoid use causes osteogenesis loss, accelerating the progression of osteoporosis. Histone methylation is shown to epigenetically increase repressive transcription, altering lineage programming of mesenchymal stem cells (MSC). This study is undertaken to characterize the action of histone demethylase UTX to osteogenic lineage specification of bone-marrow MSC and bone integrity upon glucocorticoid treatment.

Bone-marrow MSC were incubated in osteogenic medium containing supraphysiological dexamethasone. Osteogenic gene expression and mineralized nodule formation were probed using RT-PCR and von Kossa staining. The enrichment of trimethylated lysine 27 at histone 3 (H3K27me3) in Dkk1 promoter was quantified using chromatin immunoprecipitation-PCR. Bone mass and trabecular morphometry in methylprednisolone-treated skeletons were quantified using microCT analysis.
Supraphysiological dexamethasone decreased osteogenic genes Runx2 and osteocalcin expression and mineralized matrix production along with reduced UTX expression in MSC. Forced UTX expression attenuated the glucocorticoid-mediated loss of osteogenic differentiation, whereas UTX knockdown provoked osteogenesis loss and cytoplasmic oil overproduction. UTX demethylated H3K27 and reduced the glucocorticoid-mediated the H3K27 enrichment in Dkk1 promoter, reversing beta-catenin signal, but downregulating Dkk1 production by MSC. In vivo, treatment with UTX inhibitor GSK-J4 significantly suppressed bone mineral density, trabecular volume, and thickness along with porous trabecular, fatty marrow and disturbed beta-catenin/Dkk1 histopathology comparable with glucocorticoid-induced osteoporosis condition.

This study offers a productive insight into how UTX protects MSC from methylated histone-mediated osteogenesis repression in the development of glucocorticoid-induced osteoporosis.

INHIBITION OF BROMODOMAIN-CONTAINING PROTEIN 4 COUNTERACTS GLUCOCORTICOID-INDUCED BONE LOSS AND FATTY MARROW

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Glucocorticoid excess is shown to deteriorate bone tissue integrity, increasing the risk of osteoporosis. Marrow adipogenesis at cost of osteogenesis is a prominent feature of this osteoporosis condition. Epigenetic pathway histone deacetylase (HDAC)-mediated histone acetylation regulates osteogenic activity and bone mass. This study is aimed to figure out what role of acetylated histone reader bromodomain-containing protein 4 (BRD4) did play in glucocorticoid-induced osteoporosis.

Bone-marrow mesenchymal stem cells were incubated in osteogenic medium with or without 1 μM dexamethasone. Mineralized matrix and adipocyte formation were probed using von Kossa and Nile Red O staining, respectively. Osteogenic and adipogenic marker expression were quantified using RT-PCR. The binding of acetylated histone to promoter of transcription factors were detected using chromatin immunoprecipitation-PCR. Bone mineral density and microstructure in osteoporotic bone were quantified with microCT system.

Glucocorticoid repressed osteogenic transcription factor Runx2 expression and mineralized matrix formation along with a low level of acetylated lysine 9 at histone 3 (H3K9ac), whereas BRD4 signaling and adipocytic formation were increased in cell cultures. BRD4 knockdown reversed the H3K9ac enrichment in Runx2 promoter and osteogenesis, but downregulated adipogenic differentiation. Silencing BRD4 attenuated H3K9ac occupancy in forkhead box P1 (Foxp1) relevant to lipid metabolism upon glucocorticoid stress. Foxp1 interference downregulated adipogenic activities of glucocorticoid-treated cells. In vivo, treatment with BRD4 inhibitor JQ-1 compromised the glucocorticoid-induced bone mineral density loss, spare trabecular structure, and fatty marrow, as well as improved biomechanical properties of bone tissue.

Taken together, BRD4-mediated Foxp1 pathways drive mesenchymal stem cells shifting toward adipocytic cells rather than osteogenic cells to aggravates excessive marrow adipogenesis in the process of glucocorticoid-induced osteoporosis. Pharmacological inhibition of BRD4 signaling protects bone tissue from bone loss and fatty marrow in glucocorticoid-treated mice. This study conveys a new molecular insight into epigenetic regulation of osteogenesis and adipogenesis in osteoporotic skeleton and highlight the remedial effect of BRD4 inhibitor on glucocorticoid-induced bone loss.

TOP 100 CITED ARTICLES ON LUMBAR SPONDYLOLISTHESIS: BIBLIOGRAPHIC ANALYSIS

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Spondylolisthesis is common recognized spine pathology. A lot of studies targeted spondylolisthesis in the recent years, few of which have made a major influential impact on the clinical practice. To the extent our knowledge this is the first study to highlight and analyze the top 100 cited articles on spondylolisthesis through a systematic search strategy used previously in published studies in different medical specialty. The aim of this study is to identify the most cited studies on spondylolisthesis and report their impact in spine field.

Thomson Reuters Web of Science-Science Citation Index Expanded was searched using title-specific search “spondylolisthesis”. All studies published in English language between 1900 and 2019 were included with no restrictions. The top 100 cited articles were identified using “Times cited” arranging articles from high to low according to citation count. Further analysis was made to obtain the following items: Article title, author’s name and specialty, country of origin, institution, journal of publication, year of publication, citations number, study design.

The citation count of the top 100 articles ranged from 69 to 584. All published between 1950 - 2016. Among 20 journals, Spine had the highest number of articles 47, with citation number of 5964 out of 13644. Second ranked was Journal of Bone and Joint Surgery with 16 articles and a total citation of 3187. In respect to the primary author’s specialty, Orthopedic surgeons contributed to the majority of top 100 list with 82 articles, Neurosurgery was the second specialty with 10 articles. United states had produced more than half of the list by 59 articles. England was the second country with 7 articles. Surgical management of lumbar spondylolisthesis was the most common discussed topic.

This article identifies the top 100 influential papers on spondylolisthesis and recognizes an important aspect of knowledge evolution served by leading researchers as they guide today’s clinical decision making in spondylolisthesis.

GENERATION OF A 3D GRADIENT INDUCING A PRECISE CONTROL OVER PHENOTYPE AND PRE-VASCULATURE FOR OSTEOCHONDRAL TISSUE MODELLING

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Gradients of three-dimensional (3D) hierarchical tissues are common in nature and present specific architectures, as this is the case of the anisotropic subchondral bone interfaced with articular cartilage. While diverse fabrication techniques based on 3D printing, microfabrication, and microfluidics have been used to recreate tailored biomimetic tissues and their respective microenvironment, an alternative solution is still needed for improved biomimetic gradient tissues under dynamic conditions with control over pre-vasculature formation. Here, we engineered a gradient osteochondral human-based tissue with precise control over both cell/tissue phenotype and pre-vasculature formation, which opens-up possibilities for the study of complex tissues interfaces, with broader applications in drug testing and regenerative medicine.

The fabrication of 3D gradients of microparticles was performed combining methacrylated gelatin (GelMA) and gellan gum (GG) (3:1, w:w ratio) with hydroxyapatite microparticles (HAp, 30% w/w). The mixing of the interface was controlled by the temperature of two polymeric layers, being the second added at 10 ºC higher than the first one. This subsequent addition of polymeric solutions at different temperatures promoted convection, which drove the microparticles through the interface from the first to the second layered gel forming the HAp gradient. After ionic and photo-crosslinking, the freezing step was programmed using an external cover of styrofoam forcing the ice crystals to grow linearly, generating an anisotropic architecture in a gradient scaffold. A dual-chamber microreactor device was designed (figure 1A) to culture fat pad adipose-derived stem cells and microvascular endothelial cells under two biochemical microenvironments.
Using control over temperature and crosslinking, hydrogel-like structures were built in 3D anisotropic HAp gradients. Then, an in vitro osteochondral tissue model was obtained using a dual-chamber platform. Results showed a significant difference of SOX9 ($p < 0.05$), Osteocalcin and RUNX2 ($p < 0.05$) from the top to the bottom regions of the 3D gradient structures under dynamic conditions. Finally, a pre-vasculature was controlled over 7 days, stimulating the endothelization of the subchondral bone-like region 35% more ($p < 0.05$) when compared to the cartilage-like region.

In this work, microparticle and biochemical gradients were fabricated into anisotropic architectures. The obtained outcomes enable the precise control of 3D

gradients in programmable architectures, such as anisotropic structures, with broad applications in interfaced tissue engineering, regenerative medicine and drug testing.

IMPROVING FRACTURE CLINIC SERVICES AND BECOMING COMPLIANT WITH BOAST 7 GUIDELINES WITH THE USE OF VIRTUAL FRACTURE CLINIC

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The British Orthopedic Association recommends that patients referred to fracture clinic are reviewed within 72 hours. With the increase in referrals and limited clinic capacity it is becoming increasingly difficult to see every referral with in a 72 hour time frame. Some patients are waiting 2 weeks or more before they can be seen in a fracture clinic. With the aim of improving care by seeking to meet BOAST 7 target, waiting times for fracture clinic appointments at the Homerton University Hospital were audited prospectively against this national guideline, before virtual fracture clinic was implemented and 6 weeks after the implementation of virtual fracture clinic at our hospital. Virtual fracture clinic is where an Orthopedic consultant reviews a patients x-rays and A&E documentation and decides if that patients needs to be seen in a face to face fracture clinic to discuss operative vs. non-operative management of their injury or if a treatment plan can be delivered without the patient having to come back to hospital.

The study was conducted as a prospective closed-loop audit in which the second cycle took place after the implementation of the new virtual fracture clinic service.

The first cycle showed a non-compliant waiting time with only 18% of patients being seen within 72 hours. Following the implementation of virtual fracture clinic, 84% of all patients were reviewed within 72 hours.

Virtual fracture clinic delivered a significant reduction in waiting times. Virtual fracture clinic has only just been implemented at the Homerton University Hospital and hopefully at the next audit we will be 100% compliant with the BOA BOAST 7 Guideline. We would recommend that virtual fracture clinics being rolled out in Orthopedic departments in all hospitals which have Orthopedic services.

CALCIUM HOMEOSTASIS IN CHONDROCYTES: MECHANISMS AND DYSFUNCTION IN OSTEOARTHRITIS

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Calcium is an important element for a wide range of physiological functions including muscle contraction, neuronal activity, exocytosis, blood coagulation and cell communication. In the musculoskeletal system calcium is crucial for the structural integrity of bones, teeth, intervertebral disc and articular cartilage. At the cellular level calcium acts as a second messenger. Calcium signalling uses intracellular calcium ions to drive intracellular communication and signal transduction processes. When calcium enters the cell it exerts allosteric regulatory effects on many enzymes and proteins. Examining the role of calcium in chondrocyte biology is important for understanding the role for this divalent ion in the metabolic modulation of chondrocyte function in health and disease. This includes the study of calcium transport systems such as channels, transporters and pumps involved in calcium homeostasis in chondrocytes and how existing pharmacological drugs act on these transport systems. L-type calcium channel blockers are drugs used as cardiac antiarrhythmics or antihypertensives, depending on whether the drugs have higher affinity for the heart (the phenylalkylamines, like verapamil), or for the blood vessels (the dihydropyridines, like nifedipine). L-type calcium channels are present in many musculoskeletal tissues including skeletal muscle, smooth muscle, bone and cartilage. L-type calcium channel inhibitors like nifedipine used for the treatment of some forms of hypertension modulate calcium-mediated events in chondrocytes under dynamic loading, thus affecting metabolism, osmotic responses and extracellular matrix turnover in cartilage. The aim of our work is to understand the impact of L-type calcium channel inhibitors used for the treatment of hypertension on chondrocytes and on the chondrogenic differentiation of bone marrow derived mesenchymal stem cells (MSCs). This knowledge will enhance our understanding of the development of osteoarthritis (OA) and may lead to new opportunities for chondroprotection and regenerative medicine for OA. We have used electrophysiology to demonstrate L-type calcium currents in chondrocytes immediately after pharmacological activation with the calcium channel opener Bay-K8644. We have also used immunohistochemistry to demonstrate expression of the a1C subunit $Ca_v1.2$ (CACNA1C) in human chondrocytes and MSCs. Inhibitors of L-type calcium channels such as nifedipine downregulate mitochondrial respiration and ATP production in MSCs but not in chondrocytes. Nifedipine inhibits proliferation of chondrocytes and enhances glycolytic capacity in

chondrocytes, promoting glycolytic reserve in both MSCs and chondrocytes. Nifedipine can also stimulate chondrogenic differentiation in MSCs (with or without growth factors). Metabolic responses to nifedipine differs in mesenchymal stem cells and chondrocytes highlighting important metabolic differences between these cells. In summary, antihypertensive drugs such as nifedipine can affect the biological function of chondrocytes and MSCs and may modulate the course of OA progression and impact on cartilage repair.

SELF-ASSEMBLED COLLAGEN-RICH CONSTRUCTS FOR TENDON ENTHESIS REPAIR

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The fibrocartilaginous enthesis displays a complex interface between two mechanically dissimilar tissues, namely tendon and bone. This graded transition zone consists of parallel collagen type I fibres arising from the tendon and inserting into bone across zones of fibrocartilage with aligned collagen type I and collagen type II fibres and mineralised fibrocartilage. Due the high stress concentrations arising at the interface, entheses are prone to traumatic and chronic overuse injuries such as rotator cuff and anterior cruciate ligament (ACL) tears. Treatment strategies range from surgical reattachment for complete tears and conservative treatments (physiotherapy, anti-inflammatory drugs) in chronic inflammatory conditions. Generally, the native tissue architecture is not re-established and mechanically inferior scar tissue is formed. Current interfacial tissue engineering approaches pose scaffold-associated drawbacks and limitations, such as foreign body response. Using a thermo-responsive electrospun scaffold that provides architectural signals similar to native tissues and can be removed prior to implantation, we aim to develop an ECM-rich, cell-based implant for tendon-enthesis regeneration. Alcian blue staining revealed highest sGAG deposition in cell (human adipose derived stem cells) sheets grown on random electrospun fibres and lowest sGAG deposition in collagen type I sponges. Cells did not show an equal distribution throughout the collagen type II scaffolds but tended to form localised aggregates. Thermo-responsive electrospun fibres with random and aligned fibre orientation provided an adequate three-dimensional environment for chondrogenic differentiation of multilayer hADSC-sheets shown by high ECM-production, especially high sGAG deposition. Chondrogenic cell sheets showed increased expression of SOX9, COL2A1, COL1A1, COMP and ACAN after 7 days of chondrogenic induction when compared to pellet culture. Anisotropic fibres enabled the generation of aligned chondrogenic cell sheets, shown by cell and collagen fibre alignment. Thermoresponsive electrospun fibres showed high chondro-inductivity due to their three-dimensionality and therefore pose a promising tool for the generation of scaffold-free multilayer constructs for tendon-enthesis repair within short culture periods. Aligned chondrogenic cell sheets mimic the zonal orientation of the native enthesis as the fibrocartilaginous zone exhibits high collagen alignment.

DEVELOPMENT OF ANISOTROPIC COLLAGEN SUBSTRATES WITH CONTROLLED RIGIDITY FOR TENDON TISSUE ENGINEERING

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Adherent cells are known to respond to physical characteristics of their surrounding microenvironment, adapting their cytoskeleton and initiating signaling cascades specific to the type of cue encountered. Scaffolds mimicking native biophysical cues have proven to differentiate stem cells towards tissue-specific lineages and to maintain the phenotype of somatic cells for longer periods of time in culture. Biomaterial-based tendon implants are designed to withstand high physiological loads but often lack the appropriate biochemical, biophysical and biological structure to drive tendon regeneration by populating cells. The objective of this study is to use tendon main component, collagen type I, to create scaffolds that reproduce tendon natural anisotropy and rigidity, in an effort to engineer functional tendon tissue with native organization and strength, able to maintain tenocyte phenotype and to differentiate stem cells towards the tenogenic lineage. Porcine collagen type I in solution was treated with one of the following cross-linkers: glutaraldehyde, genipin or 4-arm polyethylene glycol (4SP). The resulting mixture was poured on micro-grooved (2x2x2 um) or planar PDMS moulds and air-dried to obtain 5 mg/ml collagen films. Surface topography and elastic modulus were analyzed using SEM/AFM and rheometry, respectively. Human tendon cells were cultured on the micro-grooved/planar scaffolds for up to 10 days. Cell morphology, collagen III and tenascin C expression were analyzed by immunocytochemistry. Among the different cross-linkers used, only the treatment with 4SP resulted in scaffolds with a recognizable micro-grooved surface topography. Precise control over the micro-grooved topography and the rigidity of the scaffolds was achieved by cross-linking the collagen with varying concentrations of 4SP (0, 0.5, 1 and 1.5mM) at low pH and temperature. The elastic modulus of the scaffolds cross-linked with 4SP (0.5mM) matched the values previously reported to induce tenogenic differentiation in stem cells (50-90 kPa). Approximately eighty percent of the human tendon cells cultured on the micro-grooved collagen films aligned in the direction of the anisotropy for 10 days in culture, mimicking the alignment of tenocytes in the native tissue. Cell nuclei morphology, known to play a central role in the process of mechanotransduction, was significantly more elongated for the tenocytes cultured on the micro-grooved scaffolds after 4 days in culture for all the 4SP concentrations. Synthesis, deposition and alignment of collagen III and tenascin C, two important tenogenic markers, were up regulated selectively on the micro-grooved and rigid scaffolds after 10 days in culture, respectively. These results highlight the synergistic effect of matrix rigidity and cell alignment on tenogenic cell lineage commitment. Collectively, this study provides new insights into how collagen can be modulated to create scaffolds with precise imprinted topographies and controlled rigidities.

DESIGN AND CHARACTERISATION OF A THREE-LAYER COLLAGEN-BASED SCAFFOLD TO MODULATE BMSC BEHAVIOUR FOR ENTHESIS 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, namely tendon, fibrocartilage, mineralized fibrocartilage and bone. Following injuries and surgical repair, the enthesis is often not reestablished and so far, traditionally used tissue substitutes have lacked to reproduce the complexity of the native tissue. In this work, we hypothesised that a collagen-based three-layer scaffold that mimic the composition of the enthesis, in combination with bioactive molecules, will enhance the functional regeneration of the enthesis. A three-layer sponge composed of a tendon-like layer (collagen I), a cartilage-like layer (collagen II) and a bone-like layer (collagen I and hydroxyapatite) was fabricated by an iterative layering freeze-drying technique. Scaffold porosity and structural continuity at the interfaces were assessed through SEM analysis. Bone-marrow derived stem cells (BMSCs) were seeded by syringe vacuum assisted technique on the scaffold. Scaffolds were cultured in basal media for 3 days before switching to differentiation media (chondrogenic, tenogenic and osteogenic). BMSCs metabolic activity, proliferation and viability were assessed by alamarBlue, PicoGreen and Live/Dead assays. At D21 the scaffolds were fixed, cryosectioned and Alizarin Red and Alcian Blue stainings were performed in order to evaluate BMSC differentiation towards osteogenic and chondrogenic lineage. The presence of collagen I and tenascin in the scaffolds was evaluated by immunofluorescence staining at D21 in order to assess tenogenic differentiation of BMSCs. Subsequently, the cartilage-like layer was functionalized with IGF-1, seeded with BMSCs and cultured in basal media up to D21. Structural continuity at the interfaces of the scaffolds was confirmed by SEM and scaffold porosity was assessed as >98%. The scaffolds supported cell proliferation and infiltration homogeneously throughout all the layers up to D21. Osteogenic differentiation of BMSC selectively in the bone-like layer was confirmed by Alizarin red staining in scaffolds cultured in basal and osteogenic media. Alcian blue staining revealed the presence of proteoglycans selectively in the cartilage-like layer in scaffolds cultured in chondrogenic media but not in basal media. Increased expression of the tenogenic markers collagen I and tenascin were observed in the tendon-like layer of scaffolds cultured in tenogenic but not in basal media for 21 days. The presence of IGF-1 increased osteogenic and chondrogenic differentiation of BMSCs, whereas no difference was observed for tenogenic differentiation. In conclusion, a 3-layer collagen sponge was successfully fabricated with distinct but integrated layers; the different collagen composition of the non-functionalized 3-layer sponge was able to regulate BMSC differentiation in a localized manner within the scaffold. The scaffold functionalization with IGF-1 accelerated chondrogenic and osteogenic BMSC differentiation. Overall,

functionalization of the 3-layer scaffolds holds promising potential in enthesis regeneration.

THE EFFECT OF DURATION OF VITAMIN D3 APPLICATION ON THE OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS

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Critical size bone defects pose a serious clinical problem, as the intrinsic healing capacity of bone fails due to the size of the defect. Bone healing might be aided by addition of 1,25(OH)2 vitamin D3 (vitD3) to bone tissue engineering scaffolds. VitD3can promote osteogenic differentiation of human stem cells such as adipose stem cells (hASCs), which is a clinical-relevant source of mesenchymal stem cells. However, it is unknown which release kinetics of vitD3, i.e. short or sustained release from scaffolds, leads to the most optimal osteogenic differentiation of hASCs. We hypothesized that sustained release of vitD3 leads to more osteogenic differentiation of hASCs than shorter applications.
hASCs (1x105, passage 3-4) were seeded on 20 Â± 1 mg of calcium phosphate particles (day 0), cultured for 20 days, and treated with a total amount of 124 ng vitD3. This treatment was provided either during 30 min before seeding (pre-incubation, short stimulation: [200 nM]), after seeding, over the first 2 days (burst- release high: [100 nM]), or over the total culture period of 20 days (sustained-release: [10 nM]). In the extra condition: burst-release low the hASCs were treated for 2 days after seeding with 6.2 ng vitD3 ([10 nM]) per day.
Live/dead staining followed by fluorescent microscopy showed that hASCs attached to the calcium phosphate particles and were mostly viable (Â±75 %) at day 2. VitD3 applied for any duration did not affect the proliferation of hASCs at day 7 and day 20, measured with an alamar blue assay. At day 7, sustained-release increased the release of active alkaline phosphatase on average by 3.5-fold, compared to all the other conditions. At day 20, this was increased 4.3-fold. At both day 7 and day 20 total protein levels were similar in all conditions.
Our results suggest that sustained release of VitD3 from bone tissue engineered scaffolds may be beneficial for the osteogenic differentiation of human stem cells for the treatment of critical bone size defects.

LIGAMENTOCYTE CELL SHEETS FOR ANTERIOR CRUCIATE LIGAMENT (ACL) TISSUE ENGINEERING

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Cell sheets are manufactured from a high-density cell layer stabilized by its own freshly produced extracellular matrix (ECM). They could serve as versatile scaffolds for tissue repair. Unfortunately, their production often remains time-consuming requiring weeks of culturing. Ligament cell sheets are so far barely available. Regarding musculoskeletal tissues exposed to high repetitive biomechanical forces, the stability of cell sheets is insufficient. It could help to combine them with a biomechanical competent scaffold e.g. produced by an embroidering technique. Hence, we wanted to (1) develop a very rapid strategy to produce ACL ligamentocyte sheets within 24 h by using a thermoresponsive polymer surface, (2) use the sheets for scaffold seeding and (3) reflect the fibrocartilaginous transition zone of an ACL enthesis by combining sheets of ligamentocytes with chondrocytes or chondrogenic precursor cells as a strategy for directed seeding of two cell types on topologically different scaffold areas. Different cell numbers of lapine ACL ligamentocytes (L-ACLs), lapine articular chondrocytes (L-ACs) and human mesenchymal stromal cells (H-MSCs) were used for sheet formation. Experiments were performed with novel, self-assembled poly(glycidyl ether) (PGE) brushes based on random glycidyl methyl ether and ethyl glycidyl ether copolymers on polystyrene 12-well cell culture plates, which allow rapid sheet formation within 24 h. Uncoated plates served as controls. Temperature-triggered detachment was performed by 10 min incubation with PBS at ambient temperature before treatment with fresh warm PBS for 5 min at 37 degrees Celsius. Harvested cell sheets were transferred on polyglycolic acid (PGA) or embroidered poly-lactic acid / poly-co-caprolactone (PLA/P[LA-CL]) scaffolds, functionalized with collagen foam and fluorine gas treatment (prepared at the IPF in Dresden and the FILK in Freiberg). Cell distribution, growth, vitality and synthesis of ECM components were monitored up to 7 days. Cell numbers required for sheet preparation (3.9 cm2) depended strongly on the cell type (L-ACLs: 0.395 mio/cm2, L-AC: 0.342 mio/cm2, H-MSCs: 0.131 mio/cm2) and was highest for L-ACLs. The majority of cells survived sheet assembly, detachment, transfer onto the scaffolds and culturing. Cells migrated from the sheets into the scaffolds and spread through the scaffolds. L-ACLs and L-ACs produced ECM and maintained their phenotypes (type II collagen and sulfated glycosaminoglycans in L-AC sheets, decorin and tenascin C in L-ACL sheets). The presence and distribution of two cell types in scaffold cocultures (L-ACLs and H-MSCs) was proven by anti-human vimentin labeling. Hence, the PGE brush surface allows rapid formation (24 h) of cell sheets.

CYCLIC STRETCHING OF ACL-DERIVED FIBROBLASTS IN TWO-DIMENSIONAL AND THREE-DIMENSIONAL BIOMATERIAL-FREE AND BIOMATERIAL CULTURES LEADS TO SYNCHRONIZED CELL ORIENTATION AND ACTIVATION

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Ligament fibroblasts must be mechanosensitive and possess sufficient adaptability to a novel mechanomilieu ensuring the permanent load capacity of the tissue. Once mechanoreceptors are activated, the fibroblasts react with a specific signal transmission (mechanotransduction), which ultimately leads to an adaption of their cytoskeletal organization and protein synthesis. However, the cellular response of anterior cruciate ligament (ACL) fibroblasts to cyclic mechanical stretching is still unclear. Hence, this study should allow a deeper understanding of the reaction profile of mechanically stretched ACL cells in two- (2D) and three-dimensional (3D) biomaterial-free and biomaterial cultures with respect to cell survival, size, orientation, migration and distribution. For the 2D approach consisting of monolayers with 6000 lapine (L) ACL cells per cm2 and for the 3D cultures using preformed LACL cell spheroids (2.5-4/cm2) with 25.000 cells per spheroid, silicone chambers were coated with geltrex and statically colonized with the LACL cells for 24 h before cyclically stretched for 48 h (14 percent uniaxial stretch). A second approach using 3D scaffold cultures was performed which were seeded dynamically for 24 h with LACL cells before cyclically stretched in a novel custom-made mechanostimulator. The scaffolds [polylactic acid (PLA) and polycaprolactone (PCL)] were functionalized with 10 percent gas fluorination and a collagen foam. Scaffolds (120 mm2) were precolonized dynamically with an LACL cell suspension (1 mio cells/mL) for 24 h before stretched for 72 h (4 percent uniaxial stretch). Cell vitality and numbers were monitored. The cytoskeleton orientation was shown by cytochemistry (F-actin) and evaluated (ImageJ). Cell proliferation, based on the DNA content was measured. Cell viability in stretched samples (2D, 3D and scaffold) remained above 90 percent. Stretching on the silicone chambers led to increased cell counts, length and significantly higher colonized areas than in unstretched controls. Higher numbers of LACL cells migrated out of the 3D spheroids under stretching conditions. In response to intermittent stretching, cells oriented in a 70 degrees` angle against the stretch direction in silicone chambers, whereas cell arrangement was more compact on the threads of the scaffolds than in unstretched cultures. In summary, stretching induced a rapid (48 h) cell and cytoskeletal alignment in 2D as well as in 3D cultures. The natural ACL is characterized by a strongly uniaxial cell and extracellular matrix organization which might be achieved in tissue engineered constructs by a suitable cyclic stretching protocol in future.

INFLUENCE OF LOW-FREQUENCY ALTERNATING ELECTRICAL FIELDS ON THE BONE REMODELLING CAPACITY OF HUMAN OSTEOBLAST

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Several electrical fields are known to be present in bone tissue as originally described by Fukada and Yasuda in the year 1957. Intrinsic voltages can derive from bone deformation and reversely lead to mechanical modifications, called the piezoelectric effect. This effect is used in the clinic for the treatment of bone defects by applying electric and magnetic stimulation directly to the bone supplied with an implant such as the electroinductive screw system. Through this system a sinusoidal alternating voltage with a maximum of 700 mV can be applied which leads to an electric field of 5-70 V/m in the surrounding bone. This approach is established for bone healing therapies. Despite the established clinical application of electrical stimulation in bone, the fundamental processes acting during this stimulation are still poorly understood. A better understanding of the influence of electric fields on cells involved in bone formation is important to improve therapy and clinical success.
To study the impact of electrical fields on bone cells in vitro, Ti6Al4V electrodes were designed according to the pattern of the ASNIS III s screw for a 6-well system. Osteoblasts were seeded on collagen coated coverslip and placed centred on the bottom of each well. During four weeks the cells were stimulated 3x45 min/d and metabolic and alkaline phosphatase (ALP) activity as well as gene expression of cells were analysed. Furthermore, supernatants were collected and proteins typical for bone remodelling were examined.
The electrical stimulation did not exert a significant influence on the metabolic activity and the ALP production in cells over time using these settings. Gene expression of BSP and ALP was upregulated after the first 3 days whereas OPG was increased in the second half after 14 days of electrical stimulation. Moreover, the concentration of the released proteins OPG, IL-6, DKK-1 and OPN increased when cells were cultivated under electrical stimulation. However, no changes could be seen for essential markers, like RANKL, Leptin, BMP-2, IL-1beta and TNF-alpha.
Therefore, further studies will be done with osteoblasts and osteoclasts to study bone remodelling processes under the influence of electrical fields more in detail. This study was supported by the German Research Foundation (DFG) JO 1483/1-1.

CELL RESPONSE TO NOVEL NANOSTRUCTURED TITANIUM SCAFFOLDS A PILOT STUDY

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Numerous implanted hip and knee joint arthroplasties have to be replaced due to early or late loosening of the implant, a failure of osteointegration with fibrous tissue at the bone-implant-interface. This could be counteracted by ensuring that cells which attach to the implant surface differentiate towards bone cells afterwards. For this reason, human mesenchymal stem cells (hMSCs) will be included in this study. These cells are naturally available at the bone-implant-interface, multipotent and therefore ideal to study the osteoinductivity of a material. The goal of this pilot study was to test the cell response towards three different titanium grades with a novel surface structuring, as a first step towards achieving an improved implant surface for enhanced osteointegration. Disk-shaped titanium scaffolds with a diameter of 12 mm and a height of 1.2 mm were used. The surface topography (500 µm x 500 µm x 300 µm pores) was generated via laser treatment of the surface. By using nanosecond pulsed laser technique, a rough surface with micro- and nanostructural (titanium droplets) features was automatically formed. Three different batches made of commercially pure titanium grades 1 and 2 (Ti1/Ti2) or Ti6Al4V alloy grade 5 (Ti5) were produced. Four cell types were analysed on these batches: primary hMSCs from one donor (m, 25 y), periosteum derived cells (PDCs), human osteoblasts (hOBs) and periodontal ligament cells (PDLs). Cells were seeded on Ti1, Ti2 and Ti5 scaffolds in triplicates. Resazurin assay to examine cell viability was conducted with all cell types. Measurements were executed on several days after seeding, from day one up to day 14. Actin staining as well as live/dead staining was performed with hMSCs cultured on titanium for 1, 3, 5 or 7 days. The cell viability assay revealed early turning points of growth for osteogenic hOBs (day 3) and PDCs (day 7). HMSCs grew steadily on the material and non-osteogenic PDLs stayed in plateau throughout the cultivation period. With respect to the material, cells demonstrated better proliferation on Ti1 and Ti2 than on Ti5. Live/dead staining showed a high survival rate of hMSCs at each time point and on all three titanium grades, with a neglectable number of dead cells. Actin staining confirmed an enhanced spreading and stretching of hMSCs on Ti1 and Ti2 compared to hMSCs on Ti5. Our pilot data indicates that cells react to different titanium compositions, revealed by increased proliferation on commercially pure titanium (Ti1/2). Furthermore, our results demonstrate that osteogenic cells prefer the novel surface structuring in comparison to non-osteogenic PDL cells, which stayed in plateau. The turning points of growth (hOBs/PDCs) suggest an osteosupportiveness of the surface. Although hMSCs did not show a turning point in growth, their growth was steady and resulted in the highest number of cells along with a well stretched morphology. Due to their good proliferation and response to the material, hMSCs are currently being used for evaluating the osteogenic potential of the novel scaffolds.

PHYSIOXIA PRECONDITIONED MESENCHYMAL STEM CELLS IMPROVE CARTILAGE REGENERATION FOR THE TREATMENT OF EARLY OSTEOARTHRITIS DEFECTS

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Osteoarthritis (OA) is a progressive and degenerative joint disease resulting in changes to articular cartilage. In focal early OA defects, autologous chondrocyte implantation (ACI) has a 2-fold failure rate due to poor graft integration and presence of inflammatory factors (e.g. Interleukin-1β). Bone marrow derived mesenchymal stem cells (MSCs) are an alternative cell source for cell-based treatments due to their chondrogenic capacity, though in vivo implantation leads to bone formation. In vivo, chondrocytes reside under an oxygen tension between 2-7% oxygen or physioxia. Physioxia enhances MSC chondrogenesis with reduced hypertrophic marker (collagen X and MMP13) expression compared to hyperoxic conditions (20% oxygen). This study sought to understand whether implantation of physioxic preconditioned MSCs improves cartilage regeneration in an early OA defect model compared to hyperoxic MSCs. Bone marrow extracted from New Zealand white rabbits (male: 5-6 months old; n = 6) was split equally for expansion under 2% (physioxia) or 20% (hyperoxia) oxygen. Chondrogenic pellets (2 x 105 cells/pellet) formed at passage 1 were cultured in the presence of TGF-Î²1 under their expansion conditions and measured for their wet weight and GAG content after 21 days. During bone marrow extraction, a dental drill (2.5mm diameter) was applied to medial femoral condyle on both the right and left knee and left untreated for 6 weeks. Following this period, physioxia and hyperoxia preconditioned MSCs were seeded into a hyaluronic acid (TETEC) hydrogel. Fibrous tissue was scraped and then MSC-hydrogel was injected into the right (hyperoxic MSCs) and left (physioxia MSCs) knee. Additional control rabbits with drilled defects had fibrous tissue scrapped and then left untreated without MSC-hydrogel treatment for the duration of the experiment. Rabbits were sacrificed at 6 (n = 3) and 12 (n = 3) weeks post-treatment, condyles harvested, decalcified in 10% EDTA and sectioned using a cryostat. Region of interest was identified; sections stained with Safranin-O/Fast green and evaluated for cartilage regeneration using the Sellers scoring system by three blinded observers. Physioxic culture of rabbit MSCs showed significantly shorter doubling time and greater cell numbers compared to hyperoxic culture (*p < 0.05). Furthermore, physioxia enhanced MSC chondrogenesis via significant increases in pellet wet weight and GAG content (*p < 0.05). Implantation of physioxic preconditioned MSCs showed significantly improved cartilage regeneration (Mean Sellers score = 7 $\pm$ 3; *p < 0.05) compared to hyperoxic MSCs (Sellers score = 12 $\pm$ 2) and empty defects (Sellers score = 17 $\pm$ 3). Physioxia enhances in vitro rabbit MSC chondrogenesis. Subsequent in vivo implantation of physioxia preconditioned MSCs improved cartilage regeneration in an early OA defect model compared to hyperoxic MSCs. Future studies will investigate the mechanisms for enhanced in vivo regeneration using physioxia preconditioned MSCs.

WATER JET DRILLING CAN SAFELY BE APPLIED TO MICROFRACTURE TALAR CHONDRAL DEFECTS IN THE GOAT

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Surgical microfracture is considered a first line treatment for talar osteochondral defects. Pain reduction, functional improvement and patient satisfaction are described to be 61-86% in both primary and secondary osteochondral defects. However, limited research is available whether improvement of the surgical technique is possible. We do know that the current rigid awls and drills limit the access to all locations in human joints and increase the risk of heat necrosis of bone. Application of a flexible water jet instrument to drill the microfracture holes can improve the reachability of the defect without inducing thermal damage. The aim of this study is to determine whether water jet drilling is a safe alternative compared to conventional microfracture awls by studying potential side effects and perioperative complications, as well as the quality of cartilage repair tissue in a caprine model. 6 mm diameter talar chondral defects were created bilaterally in 6 goats (12 samples). One defect in each goat was treated with microfracture holes created with conventional awls. The contralateral defect was treated with holes created with 5 second water jet bursts at a pressure of 50 MPa. The pressure was generated with a custom-made setup using an air compressor connected to a 300 litre accumulator that powered an air driven high-pressure pump (P160 Resato, Roden, The Netherlands, www.resato.com). Postoperative complications were recorded. After 24 weeks, analyses were performed using the ICRS macroscopic score and the modified O'Driscoll histological score. Wilcoxon ranked sum tests were used to assess significant differences between the two instrument groups using each goat as its own control ($p \leq 0.05$). One postoperative complication was signs of a prolonged wound healing with swelling and reluctance to weight bearing starting two days after surgery on the water jet side. Antibiotics were administered which resolved the symptoms. The median total ICRS score for the tali treated with water jets was 9,5 (range: 6-12) and 9 (range 2-11) for Observer 1 and 2 respectively; and for the tali treated conventionally this was 9,5 (range 5-11) and 9 range (2-10). The median total Modified O'Driscoll score for the tali treated with water jets was 15 (range: 7-17) and 13 (range: 3-20) for Observer 1 and 2 respectively; and for the tali treated conventionally was 13 (range: 11-21) and 15 (range: 9-20). No differences were found in complication rate or repair tissue quality between the two techniques. The results suggest that water jet drilling can be a safe alternative for conventional microfracture treatment. Future research and development will include the design of an arthroscopic prototype of the water jet drill. The focus will be on stability in nozzle positioning and minimized sterile saline consumption to further the decrease the risk of soft tissue damage.

ELASTIC AND VISCOELASTIC CHARACTERIZATION OF ILIOTIBIAL BAND AND GRACILIS TENDON GRAFTS FOR ANTEROLATERAL LIGAMENT RECONSTRUCTION

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The anterolateral ligament (ALL) has been recently recognized as a distinct stabilizer for internal rotation in the ACL-deficient knee and it has been hypothesized that ALL reconstruction may play an important role in improving anterolateral instability following ACL reconstruction. Both the gracilis tendon (GT) and a portion of the iliotibial band (ITB) have been suggested as graft materials for ALL reconstruction, however, there is an ongoing debate concerning whether GT or ITB are appropriate grafting materials. Furthermore, there is limited knowledge in how the mechanical properties of these potential grafts compare to the native ALL. Consequently, the aim of this study was to characterize the elastic (Young's modulus and failure load) and viscoelastic (dynamic and static creep) mechanical properties of the ALL and compare these results with the characteristics of the grafting materials (GT and ITB), in order to provide guidance to clinicians with respect to graft material choice.

Fourteen fresh-frozen cadaveric knees (85.2±12.2 yr) were obtained. The ALL, ITB, and the distal (GTD) and proximal gracilis tendons (GTP) (bisected at mid portion) were harvested from each donor and tested with a dynamic material testing frame. Prior to testing, the cross-sectional area of each tissue was measured using a casting method and the force required to achieve a min-max stress (1.2-12 MPa) for the testing protocol was calculated (preconditioning (20 cycles, 3-6 MPa), sinusoidal cycle (200 cycles, 1.2-12 MPa), dwell at constant load (100 s, 12 MPa), and load to failure (3%/s)). Kruskall-Wallis tests were used to compare all tissue groups ($p<0.05$).

The Young's modulus of both ALL (181.3±63.9 MPa) and ITB (357.6±94.4 MPa) are significantly lower than GTD (835.4±146.5 MPa) and GTP (725.6±227.1 MPa). In contrast, the failure load of ALL (124.5±40.9 N) was comparable with GTD (452.7±119.3 N) and GTP (433±133.7 N), however, significantly lower than ITB (909.6±194.7 N). Dynamic creep of the ALL (0.5±0.3 mm) and ITB (0.7±0.2 mm) were similar ($p>0.05$) whereas the GTD (0.26±0.06 mm) and GTP (0.28±0.1 mm) were significantly lower. Static creep progression of the ALL (1.09±0.4 %) was highest across all tissues, while GTD (0.24±0.05 %) and GTP (0.25±0.0.04 %) were lowest and comparable with ITB (0.3±0.07 %) creep progression.

Since grafts from the ITB, GTD and GTP were comparable to the ALL only for certain mechanical properties, there was no clear preference for using one over another for ALL reconstruction. Therefore, further studies should be performed in order to evaluate which parameters play a vital role to determine the optimum grafting choice.

SCAPULAR RECONSTRUCTIONS USING STATISTICAL SHAPE MODELING: DESIGN AND VALIDATION

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During shoulder arthroplasty the native functionality of the diseased shoulder joint is restored, this functionality is strongly dependent upon the native anatomy of the pre-diseased shoulder joint. Therefore, surgeons often use the healthy contralateral scapula to plan the surgery, however in bilateral diseases such as osteoarthritis this is not always feasible. Virtual reconstructions are then used to reconstruct the pre-diseased anatomy and plan surgery or subject-specific implants. In this project, we develop and validate a statistical shape modeling method to reconstruct the pre-diseased anatomy of eroded scapulae with the aim to investigate the existence of predisposing anatomy for certain shoulder conditions.

The training dataset for the statistical shape model consisted of 110 CT images from patients without observable scapulae pathologies as judged by an experienced shoulder surgeon. 3D scapulae models were constructed from the segmented images. An open-source non-rigid B-spline-based registration algorithm was used to obtain point-to-point correspondences between the models. The statistical shape model was then constructed from the dataset using principle component analysis. The cross-validation was performed similarly to the procedure described by Plessers et al. Virtual defects were created on each of the training set models, which closely resemble the morphology of glenoid defects according to the Wallace classification method. The statistical shape model was reconstructed using the leave-one-out method, so the corresponding training set model is no longer incorporated in the shape model. Scapula reconstruction was performed using a Monte Carlo Markov chain algorithm, random walk proposals included both shape and pose parameters, the closest fitting proposal was selected for the virtual reconstruction. Automatic 3D measurements were performed on both the training and reconstructed 3D models, including glenoid version, critical shoulder angle, glenoid offset and glenoid center position.

The root-mean-square error between the measurements of the training data and reconstructed models was calculated for the different severities of glenoid defects. For the least severe defect, the mean error on the inclination, version and critical shoulder angle (°) was 2.22 (± 1.60 SD), 2.59 (± 1.86 SD) and 1.92 (± 1.44 SD) respectively. The reconstructed models predicted the native glenoid offset and centre position (mm) an accuracy of 0.87 (± 0.96 SD) and 0.88 (± 0.57 SD) respectively. The overall reconstruction error was 0.71 mm for the reconstructed part. For larger defects each error measurement increased significantly.

A virtual reconstruction methodology was developed which can predict glenoid parameters with high accuracy. This tool will be used in the planning of shoulder surgeries and investigation of predisposing scapular morphologies.

TREATMENT OF OSTEOCHONDRAL DEFECTS OF THE KNEE USING BILAYERED SCAFFOLD-FREE CONSTRUCTS IN RATS

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Joint surface restoration of deep osteochondral defects represents a significant unmet clinical need. Moreover, untreated lesions lead to a high rate of osteoarthritis. The current strategies to repair deep osteochondral defects such as osteochondral grafting or sandwich strategies combining bone autografts with ACI/MACI fail to generate long-lasting osteochondral interfaces. Herein, we investigated the capacity of juvenile Osteochondral Grafts (OCGs) to repair osteochondral defects in skeletally mature animals. With this regenerative model in view, we set up a new biological, bilayered, and scaffold-free Tissue Engineered (TE) construct for the repair of the osteochondral unit of the knee.

Skeletally immature (5 weeks old) and mature (11 weeks old) Lewis rats were used. Cylindrical OCGs were excised from the intercondylar groove of the knee of skeletally immature rats and transplanted into osteochondral defects created in skeletally mature rats. To create bilayered TE constructs, micromasses of human periosteum-derived progenitor cells (hPDCs) and human articular chondrocytes (hACs) were produced in vitro using chemically defined medium formulations. These constructs were subsequently implanted orthotopically *in vivo* in nude rats. At 4 and 16 weeks after surgery, the knees were collected and processed for subsequent 3D imaging analysis and histological evaluation. Micro-computed tomography (µCT), H&E and Safranin O staining were used to evaluate the degree of tissue repair.

Our results showed that the osteochondral unit of the knee in 5 weeks old rats exhibit an immature phenotype, displaying active subchondral bone formation through endochondral ossification, the absence of a tidemark, and articular chondrocytes oriented parallel to the articular surface. When transplanted into skeletally mature animals, the immature OCGs resumed their maturation process, i.e., formed new subchondral bone, partially established the tidemark, and maintained their Safranin O-positive hyaline cartilage at 16 weeks after transplantation. The bilayered TE constructs (hPDCs + hACs) could partially recapitulate the cascade of events as seen with the immature OCGs, i.e., the regeneration of the subchondral bone and the formation of the typical joint surface architecture, ranging from non-mineralized hyaline cartilage in the superficial layers to a progressively mineralized matrix at the interface with a new subchondral bone plate.

Cell-based TE constructs displaying a hierarchically organized structure comprising of different tissue forming units seem an attractive new strategy to treat osteochondral defects of the knee.

PROTEOMICS CHARACTERIZATION OF HMSC CHONDROGENIC AND OSTEOGENIC DIFFERENTIATION IN 3D PRINTED SCAFFOLDS

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The extracellular matrix (ECM) is the non-cellular structural support that provides cells with a network of biochemical and biomechanical factors for cellular processes. The ECM regulates cell function, differentiation and homeostasis. Here, we present a proteomics characterization of three commonly used additive manufactured polymers: polylactic acid (PLA), polyactive (PEOT/PBT) and polycaprolactone (PCL).
We cultured human mesenchymal stromal cells (hMSCs) and make them undergo chondrogenic and osteogenic differentiation on 3D printed PCL, PEOT/PBT and PLA scaffolds. hMSCs were cultured in basal, chondrogenic and osteogenic media (200000 cells/scaffold) and analyzed after 35 days of culture. Differentiation was proved through biochemical assays, immunofluorescence and histology. The protein content was explored using label free liquid chromatography mass spectrometry (LC-MS), which revealed upregulated proteins and their related pathways.

A higher difference was found among different media compared to the scaffold type through principal component analysis (PCA). Interestingly, in all three materials, chondrogenesis was characterized by a lower but more diverse amount of proteins. PCL induced ECM production in both differentiation media, but it led to more apoptosis and GAG degradation in the chondrogenic medium compared to the osteogenic one. During chondrogenesis in PEOT/PBT and PLA, cell differentiation resulted in the activation of stress response cascades, collagen formation and ECM remodelling. On the other hand, in osteogenesis, PCL enhanced insulin-like growth factor pathway and fibrin clot related pathways.

EVALUATION OF INTER- AND INTRA-OPERATOR RELIABILITY OF MANUAL SEGMENTATION OF FEMORAL METASTATIC LESIONS

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Patients with advanced cancer can develop bone metastases in the femur which are often painful and increase the risk of pathological fracture. Accurate segmentation of bone metastases is, amongst others, important to improve patient-specific computer models which calculate fracture risk, and for radiotherapy planning to determine exact radiation fields. Deep learning algorithms have shown to be promising to improve segmentation accuracy for metastatic lesions, but require reliable segmentations as training input. The aim of this study was to investigate the inter- and intra-operator reliability of manual segmentation of femoral metastatic lesions and to define a set of lesions which can serve as a training dataset for deep learning algorithms. F

CT-scans of 60 advanced cancer patients with a femur affected with bone metastases (20 osteolytic, 20 osteoblastic and 20 mixed) were used in this study. Two operators were trained by an experienced radiologist and then segmented the metastatic lesions in all femurs twice with a four-week time interval. 3D and 2D Dice coefficients (DCs) were calculated to quantify the inter- and intra-operator reliability of the segmentations. We defined a DC>0.7 as good reliability, in line with a statistical image segmentation study.

Mean first and second inter-operator 3D-DCs were 0.54 (±0.28) and 0.50 (±0.32), respectively. Mean intra-operator I and II 3D-DCs were 0.56 (±0.28) and 0.71 (±0.23), respectively. Larger lesions (>60 cm^3) scored higher DCs in comparison with smaller lesions.

This study reveals that manual segmentation of metastatic lesions is challenging and that the current manual segmentation approach resulted in dissatisfying outcomes, particularly for lesions with small volumes. However, segmentation of larger lesions resulted in a good inter- and intra-operator reliability. In addition, we were able to select 521 slices with good segmentation reliability that can be used to create a training dataset for deep learning algorithms. By using deep learning algorithms, we aim for more accurate automated lesion segmentations which might be used in computer modelling and radiotherapy planning.

RULING OUT UNDERLYING INFECTION IN 200 UNSUSPECTED REVISION HIP AND KNEE ARTHROPLASTIES USING A MULTIPLEX PCR SYSTEM

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Ruling out an infection in one-stage knee and hip revisions for presumed aseptic failure by conventional tissue cultures takes up to 14 days. Multiplex polymerase chain reaction (PCR) is a quick test (4-5 hours) for detecting infections. The purpose of this study was to evaluate the negative predictive value of an automated multiplex PCR for the detection of microorganisms in synovial fluid obtained intraoperatively in unsuspected knee and hip revisions.

The NPV of the multiplex PCR U-ITI system of synovial fluid compared to tissue cultures of knee and hip revisions was 95.7% and 92.5%, respectively. Cultures required several days for growth whereas the automated mPCR U-ITI system provided results within five hours.

The multiplex PCR U-ITI system is a quick and reliable test in ruling out infection in presumed aseptic knee and hip revisions. With this test the number of unsuspected infected revisions can be lowered and antibiotic overtreatment as well as undertreatment after one-stage revision arthroplasty can be avoided. This directly results in a reduction in length of hospital stay, hospital costs and possible antibiotic resistance development.

PULLOUT TESTING OF SPINAL INSTRUMENTATION IN AN IN VIVO MIMICKING EXPERIMENTAL SETUP

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Complications after spinal fusion surgery are common, with implant loosening occurring in up to 50% of osteoporotic patients. Pedicle screw fixation strength reduces as a result of decreased trabecular bone density, whereas sublaminar wiring is less affected by these changes. Therefore, pedicle screw augmentation with radiopaque sublaminar wires (made with Dyneema Purity® Radiapque fibers, DSM Biomedical, Geleen, the Netherlands) may improve fixation strength. Furthermore, sublaminar tape could result in a gradual motion transition to distribute stress over multiple levels and thereby reduce implant loosening. The objective of this study is to test this hypothesis in a novel experimental setup in which a cantilever bending moment is applied to individual human vertebrae.

Thirty-eight human cadaver vertebrae were stratified into four different groups: ultra-high molecular weight polyethylene sublaminar tape (ST), pedicle screw (PS), metal sublaminar wire (SW) and pedicle screw reinforced with sublaminar tape (PS+ST). The vertebrae were individually embedded in resin, and a cantilever bending moment was applied bilaterally through the spinal rods using a universal material testing machine. This cantilever bending setup closely resembles the loading of fixators at transitional levels of spinal instrumentation.

The pull-out strength of the ST (3563 ± 476N) was not significantly different compared to PS, SW or PS+ST. The PS+ST group had a significantly higher pull-out strength (4522 ± 826N) compared to PS (2678 ± 292N) as well as SW (2931 ± 250N).

The higher failure strength of PS + ST compared to PS indicates that PS augmentation with ST may be an effective measure to reduce the incidence of screw pullout, even in osteoporotic vertebrae. Moreover, the lower stiffness of sublaminar fixation techniques and the absence of damage to the cortices in the ST group suggest that ST as a stand-alone fixation technique in adult spinal deformity surgery may also be clinically feasible and offer clinical benefits.

AN OSTEOCHONDRAL CULTURE PLATFORM TO SCREEN THE BONE FORMING POTENTIAL STIMULATED BY BIOMATERIALS

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Novel biomaterials are being developed and studied, intended to be applied as bone graft substitute materials. Typically, these materials are being tested in in vitro setups, where among others their cytotoxicity and alkaline phosphatase activity (as a marker for osteoblastic differentiation) are being evaluated. However, it has been reported that in vitro tests correlate poorly with in vivo results and therefore many promising biomaterials may not reach the clinic as a bone graft substitute product. One of the reasons for the poor correlation, may be the minimal complexity of the in vitro tests, as compared to the in vivo environment. Ex vivo models, mimicking the natural tissue environment whilst maintaining control of culture parameters, may be a promising alternative to assess biomaterials for bone formation. Assess the possibility of an ex vivo culture platform to test biomaterials on their potential to stimulate new bone formation. Osteochondral plugs (cylinders n=10, Ø 10 mm, height 15 mm) were drilled from fresh porcine knees, from the slaughterhouse. A bone defect (Ø 6 mm) was created and which was filled with a biomaterial graft (S53P4 bioactive glass (n=3); collagen sponges loaded with BMP-2 (n=3, as positive control)) or kept empty (n=4). The explants were cultured in custom-made two-chamber bioreactors for six weeks (LifeTec Group BV). Cartilage and bone were physically separated, similar to the in vivo situation, by a sealing ring. The two tissues were cultured in separate compartments, allowing for specific culture medium for each tissue. Medium was changed every 2-3 days and weekly micro computed tomography (µCT) images were obtained to longitudinally monitor the formation of new bone. An MTT assay was performed on half of the samples after six weeks of culture. The other samples were fixed for histology, to determine which cells were present after six weeks. The MTT metabolic assay showed that a number of cells in the bone were viable after six weeks. The further away from the border, the fewer living cells were observed. The cells in the cartilage also survived. No significant bone formation was observed with µCT in either of groups, even though abundant bone formation was expected in the BMP-2 group. Explanations of the negative results of the positive group might be that too few viable cells remain after six weeks, or that the cells that are still present are not able to form bone. No significant bone formation was observed in the bone defects in osteochondral explants that were cultured with, or without, biomaterials for six weeks. However, the platform showed that it is capable to successfully culture osteochondral explants for six weeks.

Histology needs to be performed to evaluate which cells were present at the end of the culture and this will be compared to the cells present directly after drilling the explants.

CALCULATION OF FORCES IN UHMWPE CABLES USED FOR CORRECTION OF ADULT SPINAL DEFORMATIES

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Currently, between 17% of patients undergoing surgery for adult spinal deformity experience severe instrumentation related problems such as screw pullout or proximal junctional failure necessitating revision surgery. Cables may be used to reinforce pedicle screw fixation as an additive measure or may provide less rigid fixation at the construct end levels in order to prevent junctional level problems. The purpose of this study is to provide insight into the maximum expected load during flexion in UHMWPE cable in constructs intended for correction of adult spine deformity (degenerative scoliosis) in the PoSTuRe first-in-man clinical trial.

Following the concept of toppinoff, a new construct is proposed with screw/cable fixation of rods at the lower levels and standalone UHMWPE cables at the upper level (T11). A parametric FE model of the instrumented thoracolumbar spine, which has been previously validated, was used to represent the construct. Pedicle screws are modeled by assigning a rigid tie constraint between the rod and the lamina of the corresponding spinal level. Cables are modeled using linear elastic line elements, fixing the rod to the lamina medially at the cranial laminar end and laterally at the caudal laminar end. A Youngs modulus was assigned such that the stiffness of the line element was the same as that of the cable. An 8 Nm flexion moment was applied to the cranial endplate.

The maximum value of the force in the wire (80 N) is found at the T11 (upper) level. At the other levels, forces in the cable are very small because most of the force is carried by the screw (T12) or because the wires are force shielded by the contralateral and adjacent level pedicle screws (L2, L3).

The model provides first estimates of the forces that can be expected in the UHMWPE cables in constructs for kyphosis correction during movement. It is expected that this approach can help in defining the number of wires for optimal treatment.

ENVIRONMENTAL STIMULI TO GUIDE MESENCHYMAL STROMAL CELLS IN BONE TISSUE ENGINEERING

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It is well known that environmental cues such as mechanical loading and/or cell culture medium composition affect tissue-engineered constructs resembling natural bone. These studies are mostly based on an initial setting of the influential parameter that will not be further changed throughout the study. Through the growth of the cells and the deposition of the extracellular matrix (ECM) the initial environmental conditions of the cells will change, and with that also the loads on the cells will change. This study investigates how changes of mechanical load or media composition during culture influences the differentiation and ECM production of mesenchymal stromal cells seeded on porous 3D silk fibroin scaffolds. ECM formation, ECM mineralization and cell differentiation in 3D tissue-engineered bone were analyzed using microscopic tools. Our results suggest that mechanical stimuli are necessary to differentiate human mesenchymal stromal cells of both bone marrow and adipose tissue origin into ECM producing osteoblasts which ultimately become ECM-embedded osteocytes. However, the influence of this stimulus seems to fade quickly after the onset of the culture. Constructs which were initially cultured under mechanical loading continued to deposit minerals at a similar growth rate once the mechanical stimulation was stopped. On the other hand, cell culture medium supplementation with FBS was identified as an extremely potent biochemical cue that influences the mechanosensitivity of the cells with regards to cell differentiation, ECM secretion and mineral deposition.

Only through a thorough understanding on these influences over time will we be able to predictably control tissue development in vitro.

BIOLOGY VERSUS CLINICAL OUTCOME: A HUMAN ACHILLES TENDON STUDY

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Tendon healing is a complex process that often results in compromised healing of the tendon tissue. It has recently been shown that temporal changes in the expression profile and the histological tissue quality of the tendons occur during the early healing process after acute Achilles tendon rupture. Whether these changes are accompanied by an altered healing process, is not yet known and was the aim of the present study.

Tendon biopsies were obtained from 24 patients with acute Achilles tendon rupture at the time of surgery (2-9 days after rupture) and examined histologically as well as on RNA level. Histologically, the tendon architecture, the amount of aligned collagen, glycosaminoglycan and fat as well as the cellularity, vascularity and immune cell infiltration were determined. On RNA level the expression of markers for the modeling/remodeling (MMPs and TIMPs), collagens (1, 3, 5), tendon markers (scleraxis, tenomodulin), pro- and anti-inflammatory markers (IL-1beta, IL6, IL10, IL33, TNFa, TGF-beta1, COX2) and immune cell markers (CD3, CD68, CD80, CD206) were analyzed by Real-Time PCR. To determine the clinical outcome, the patients were followed up 12 months after the operation and the following scores were recorded: Subjective score, Tegner score, Visual Analog Scale (VAS) pain, VAS function, Matles Test, Achilles tendon total rupture score (ATRS), Therman 100-points score, Heel rise test. Statistics: Spearman correlation analysis.

Correlation analysis shows that early post-rupture surgery is associated with better clinical outcome (ATRS Score: $p=0.022$). Histologically, a good functional healing outcome shows a positive correlation to the amount of aligned collagen (Heel Rise Test: $p = 0.009$) and glycosaminoglycans in the tendon (Heel Rise Test: $p = 0.026$, Matles difference: $p = 0.029$), as well as a negative correlation to the fat content (Thermann score: $p = 0.018$, subjective score: $p = 0.027$, VAS function: $p = 0.031$). On RNA level, a good healing outcome correlates with increased expression of MMP13, collagen 1, 3, 5 (Heel Rise Test: $p = 0.019$, $p = 0.048$, $p = 0.030$), and TIMP2 (Tegner Score: $p = 0.040$), TGF-beta1 (Thermann Score: $p = 0.032$) and CD80 (ATRS: $p = 0.025$, Thermann score:, $p = 0.032$). Whereas a limited healing outcome is associated with an increased expression of MMP2 (Heel Rise Test: $p = 0.033$), MMP3 (Matles Test: $p=0.001$, Heal Rise test $p = 0.017$), and IL33 (Tegner Score: $p = 0.047$).

The results of the study show a clear relationship between the tendon biology at the time of the surgery and the clinical and functional healing outcome 12 months after the operation. Especially matrix formation and remodeling play a crucial role, while the examined immunological factors seem to influence the tendon healing to a lesser extent. The modulation of matrix formation could potentially lead to improved treatment options in the future.

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SOLUBLE BIOCHEMICAL MARKERS OF SARCOPENIA: WHERE ARE WE NOW AND WHERE DO WE NEED TO GO?

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Sarcopenia is a progressive and generalized skeletal muscle disorder that involves loss of muscle mass and function. It is associated with increased adverse outcomes including falls, functional decline, frailty and mortality and affects 65% of people over the age of 65 more than half of people aged 80 and above. The factors that cause and worsen sarcopenia are categorised into two groups. The primary aetiological factor is ageing and the secondary factors include disease, physical inactivity, and poor nutrition. Sarcopenia is considered to be 'primary' (or age-related) when no other specific cause is evident. However, a number of 'secondary' factors may be present in addition to ageing. Sarcopenia can occur secondary to a systemic or inflammatory disease, including malignancy and organ failure. Physical inactivity is one of the major contributors to the development of sarcopenia, whether due to a sedentary lifestyle or to disease related immobility or disability. Furthermore, sarcopenia can develop as a result of inadequate protein consumption. Biomarkers are objective and quantifiable characteristics of physiological and pathophysiological processes. Biomarkers can be used to predict the development of sarcopenia in older susceptible adults and enable early interventions that can reduce the risk of physical disability, the co-morbidities associated with the loss of muscle mass and the poor health outcomes that result from sarcopenia. Non-invasive imaging technologies can be used as biomarkers to detect loss of skeletal muscle mass in sarcopenia include bone densitometry, computed tomography, ultrasound and magnetic resonance imaging. However, imaging requires sophisticated and expensive equipment that is not available in a resource poor setting. Therefore, markers of skeletal muscle strength and fitness and soluble biochemical markers in blood may be used as alternative biomarkers. Studies on sarcopenia have identified numerous soluble biochemical biomarkers. These biomarkers can be divided into two groups: "muscle-specific" and "non-muscle-specific" biomarkers. Since sarcopenia is associated with rapid skeletal muscle wasting, the skeletal muscle-specific isoform of troponin T may be considerate a useful biomarker of sarcopenia, since high troponin levels in blood are an expression of muscle wasting. Peptides derived from collagen type VI turnover may be potential biomarkers of sarcopenia. We have recently conducted a systematic review to summarize the data from recent mass-spectrometry based proteomic studies of the secretome of skeletal muscle cells

in response to disease, exercise or metabolic stress in order to identify the proteins involved in muscle breakdown. Developing robust *in vitro* models for the study of sarcopenia using primary muscle cells is a high priority as is exploiting the *in vitro* models to understand catabolic and inflammatory processes and molecular mechanisms involved in sarcopenia. Co-cultures with adipose-derived and other cells may be used to screen for small molecules and biologicals capable of inhibiting the catabolic and inflammatory pathways involved in sarcopenia. This presentation reviews recent progress in this area and outlines opportunities for future research on sarcopenia.

PRELIMINARY STUDY ON THE USE OF 3D PRINTED MODELS IN THE PRE-OPERATIVE PLANNING OF REVISION ACL RECONSTRUCTION

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Revision anterior cruciate ligament (ACL) reconstruction is a technically demanding procedure, reporting poorer outcomes compared to the primary procedure. Identification of the cause of primary failure and a thorough pre-operative evaluation is required to plan the most appropriate surgical approach. 3D printing technology has become increasingly commonplace in the surgical setting. In particular, patient-specific anatomical models can be used to aid pre-operative planning of complicated procedures. We have conducted a qualitative study to gauge the interest amongst orthopaedic knee surgeons in using a 3D-printed model to plan revision ACL reconstructions.

A tibia and femur model was printed from one patient who is a candidate for the procedure. The binder jetting printing technique was performed, using Visijet PXL Core powder. 12 orthopaedic knee surgeons assessed the usefulness of the 3D-printed model compared to conventional CT images on a likert scale. 6 key steps of preoperative planning were assessed, including the size and location of the tunnel defects, the need for notchplasty, and whether a staged revision was required.

We found that surgeons preferred the 3D-printed model to conventional CT images only, and 83% of them would use such a model for both pre-operative simulation, and as an intra-operative reference. However, there were some variation in the perceived usefulness of the model in several areas assessed. This may reflect differences in individual approach towards planning of the procedure.

Our findings suggest that 3D-printed models could be a versatile pre-operative and intra-operative tool for complicated arthroscopic knee surgery. While 3D printing technology is becoming increasingly accessible and affordable, in-depth cost-effectiveness studies need to be conducted before it can be integrated into clinical. Further study would be needed to determine the clinical utility and economic cost-effectiveness of the 3D-printed model in revision ACL reconstruction.

AN AUDIT OF DISTAL RADIUS FRACTURE MANAGEMENT AT A LEVEL 2 TRAUMA CENTRE

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Distal radius fractures (DRF) are very common injuries. National recommendations (British Orthopaedic Association, National Institute for Health and Care Excellence (NICE)) exist in the UK to guide the management of these injuries. These guidelines provide recommendations about several aspects of care including which type of injuries to treat non-operatively and surgically, timing of surgery and routine follow-up. In particular, current recommendations include considering immobilizing patients for 4 weeks in plaster for those managed conservatively, and operating on fractures within 72 hours for intra-articular injuries and 7 days for extra-articular fractures. With increased demands for services and an ageing population, prompt surgery for those presenting with distal radius fractures is not always possible. A key factor is the need for prompt surgery for hip fracture patients.

This study is an audit of the current standard of care at a busy level 2 trauma unit against national guidelines for the management of DRFs. This retrospective audit includes all patients presenting to our emergency department from June to September 2018. Patients over 18 years of age with a diagnosis of a closed distal radius fracture and follow-up in our department were included in the study. Those with open fractures were excluded. Data was retrieved from clinical coding, electronic patient records, and IMPAX Client (Picture archiving and communication system). The following data was collected on patients treated conservatively and those managed surgically:- (1)Time to surgery for surgical management; (2)Period of immobilization for both conservative and operative groups.

45 patients (13 male, 32 female) with 49 distal radius fractures (2 patients had bilateral injuries) were included. Patients had mean age 63 years (range 19 to 92 years) 30 wrists were treated non-operatively and 19 wrists treated surgically (8 K-wires, 10 ORIF, 1 MUA). Mean time to surgery in the operative group was 8 days (range 1 - 21 days, median 7 days). Mean time to surgery for intra-articular fractures was 7 days (range 1 - 21) and 12 days for extra-articular fractures (range 4 - 20). Mean immobilization period in those treated in plaster is 6 weeks (range 4 - 13 weeks, median 5.6 weeks).

At busy level 2 trauma units with limited theatre capacity and a high volume of hip fracture admissions, time to surgery for less urgent injuries such as wrist fractures is often delayed. National guidelines are useful in helping to guide management however their standards are often difficult to achieve in the context of increasing populations in urban areas and an ageing population.

WNT SIGNALING AND FIBRONECTIN FRAGMENTS IMPAIR CHONDROCYTE RESPONSE TO MECHANICAL COMPRESSION

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In a healthy joint, mechanical loading increases matrix synthesis and maintains cell phenotype, while reducing catabolic activities. It activates several pathways, most of them yet largely unknown, with integrins, TGF-β, canonical (Erk 1/2) and stress-activated (JNK) MAPK playing a key role. Degenerative joint diseases are characterized by Wnt upregulation and by the presence of proteolytic fibronectin fragments (FB-fs). Despite they are known to impair some of the aforementioned pathways, little is known on their modulatory effect on cartilage mechanoresponsiveness. This study aims at investigating the effect of mechanical loading in healthy and in vitro diseased cartilage models using pro-hypertrophic Wnt agonist CHIR99021 and the pro-catabolic FB-fs 30 kDa.
Human primary chondrocytes from OA patients have been grown in alginate hydrogels for one week, prior to be incubated for 4 days with 3μM CHIR99021 or 1 μM FB-fs. Human cartilage explants isolated from OA patients have incubated 4 days with 3 Î¼M CHIR99021 or 1 μM FB-fs. Both groups have then been mechanically stimulated (unconfined compression, 10% displacement, 1.5 hours, 1 Hz), using a BioDynamic bioreactor 5270 from TA Instruments. Expression of collagen type I, II and X, aggrecan, ALK-1, ALK-5, αV, α5 and β1 integrins, TGF-β1 have been assessed by Real Time-PCR and normalized with the expression of S29. Percentage of phosphorylated Smad2, Smad1 and JNK were determined through western blot. TGF-β1 content was quantified by sandwich ELISA; MMP-13 and GAG by western blot and DMMB assay, respectively. At least three biological replicates were used. ANOVA test was used for parametric analysis; Kruskal-Wallis and Mann-Whitney post hoc test for non-parametric.
Preliminary data show that compression increased collagen II expression in control, but not in CHIR99021 and FB-fs pre-treated group (Fig. 1A-B). This was associated with downregulation of β1-integrin expression, which is the main collagen receptor and further regulates collagen II expression, suggesting inhibition of Erk1/2 pathway. A trend of increase expression of collagen type X after mechanical loading was observed in CHIR and FB-fs group. ALK-1 and ALK-5 showed a trend toward stronger upregulation in CHIR99021 group after compression, suggesting the activation of both Smad1/5/8 and Smad 2/3 pathways. To further investigate pathways leading to these different mechano-responses, the phosphorylation levels of Smad1 and Smad2, Erk1/2 and JNK proteins are currently being studied. Preliminary results show that Smad2, Smad1 and JNK protein levels increased in all groups after mechanical loading, independently of an increase in TGF-β1 expression or content. Compression further increased phosphorylation of Smad2, but not of Smad1, in all groups.

MATERIAL PROPERTIES AND MULTIAXIAL MECHANICAL LOADING TO OPTIMIZE CHONDROGENESIS BUT LIMIT PROTEOGLYCAN LOSS IN CELL-ENRICHED HYDROGEL CONSTRUCTS

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To unravel the relation between mechanical loading and biological response, cell-seeded hydrogel constructs can be used in bioreactors under multi-axial loading conditions that combines compressive with torsional loading. Typically, considerable biological variation is observed. This study explores the potential confounding role of mechanical factors in multi-directional loading experiments. Indeed, depending on the material properties of the constructs and characteristics of the mechanical loading, the mechanical environment within the constructs may vary. Consequently, the local biological response may vary from chondrogenesis in some parts to proteoglycan loss in others.
This study uses the finite element method to investigate the effects of material properties of cell-seeded constructs and multiaxial loading characteristics on local mechanical environment (stresses and strains) and relate these to chondrogenesis (based on maximum compressive principal strain (MCPS) - Zahedmanesh et al., 2014) and proteoglycan loss (based on fluid velocity (FV) - Orozco et al., 2018).
The construct was modelled as a homogenized poro-hyperelastic (using a Neohookean model and Darcys law) cylinder of 8mm diameter and equal height using Abaqus. The bottom surface was fully constrained and dynamic unconfined compression and torsion loading were applied to the top surface. Free fluid flow was allowed through the lateral surface. We studied the sensitivity of the maximum values of the target parameters at 9 key locations to the material parameters and loading characteristics. Six input parameters were varied in preselected ranges: elastic modulus (E=[20,80]kPa), Poissons ratio (nu=[0.1,0.4]), permeability (k=[1,4]e-12m4/Ns), compressive strain (Comp=[5,20]%), rotation (Rot=[5,20]°) and loading frequency (Freq=[1,4]Hz). A full-factorial design of experiment method was used and a first-order polynomial surface including the interactions fitted the responses.
MCPS varies between 7.34% and 33.52% and is independent of the material properties (E, nu and k) and Freq but has a high dependency on Comp and a limited dependency on Rot. The maximum value occurs centrally in the construct, except for high values of Rot and low Comp where it occurs at the edges. FV vary between 0.0013mm/sec and 0.1807mm/sec and dominantly depends on E, k and Comp, while its dependency on Rot and Freq is limited. The maximum value usually occurs at the edges, although at high Freq it may move towards the center of the superficial and deep zones. This study can be used as a guideline for the optimized selection of mechanical parameters of hydrogel for cell-seeded constructs and loading conditions in multi-axial bioreactor studies. In future work, we will study the effect in intact and injured cartilage explants.

LOADING OF CHONDROCYTES IN A MULTI-AXIAL BIOREACTOR PLATFORM SHOWS DIFFERENTIAL RESPONSES BETWEEN CHONDROGENIC PRECURSOR CELLS AND OSTEOARTHRITIC CHONDROCYTES

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Mechanical loading regulates the metabolism of chondrocytes in cartilage1. Nowadays, studies exploring the in vitro response of cartilage towards loading often rely on bioreactor experiments applying only compressive loading. This is likely not sufficiently representative for the complex multi-directional loading profile in vivo (i.e. where typical compressive and shear loading are both present). The impact of multi-axial loading is specifically relevant in the context of the onset of osteoarthritis (OA) due to joint destabilization. Here, alterations in the 3D loading profile, and in particular increased shear forces, are suggested to initiate catabolic molecular responses leading to cartilage degeneration3. However, in vitro/ex vivo data confirming this hypothesis are currently lacking. Therefore, we aim to investigate how increased shear loading affects the metabolism and ECM deposition of a healthy chondrogenic cell line and if this response is different in osteoarthritic primary chondrocytes.
A murine chondrogenic precursor cell line (ATDC5) and primary human osteoarthritic articular chondrocytes (hOACs) were encapsulated in 2.2% alginate disks and cultured in DMEM medium for three days. Hydrogels seeded with the different cell groups were loaded in the TA ElectroForce BioDynamic Bioreactor and subjected to following loading conditions: (a) 10% compression at 1Hz for 1h, (b) 10% compression and 10° shear loading at 1Hz for 1h. Unloaded constructs were used as control. After loading, hydrogel constructs were stabilized in culture medium for 2 hours, to facilitate adequate gene expression responses, before being dissolved and snap frozen. RNA was isolated and gene expression levels specific for anabolic pathways, characterized by extracellular matrix (ECM) genes (Col2a1, Aggrecan and Perlecan), catabolic processes (MMP-3 and MMP-13) and chondrogenic transcription factor (Sox9) were evaluated using RT-qPCR. The TA ElectroForce BioDynamic Bioreactor was successfully set-up to mimic cartilage loading.
In ATDC5 cells, compression elicits an increase in all measured ECM genes (Col2a1, Aggrecan and Perlecan) compared to unloaded controls, suggesting an anabolic response. This upregulation is decreased when adding additional shear strain. In contrast to ATDC5 cells, the anabolic response of proteoglycans Aggrecan and Perlecan to compressive loading was lower in osteoarthritic chondrocytes, and Col2a1 expression appeared decreased. Adding shear strain reversed this effect on Col2a1 expression. Multi-directional loading increased transcription factor Sox9 expression compared to compression in both ATDC5 and OA chondrocytes. In OA chondrocytes, both loading regimens increased MMP-3 and MMP-13 expression. Shear loading reduces the anabolic effect of compressive loading in both cell types. OA cells presented more catabolic response to mechanical loading compared to precursors, given the increase in catabolic enzymes MMP-3 and MMP-13.

IMPROVING FRACTURE CLINIC SERVICES AND BECOMING COMPLIANT WITH BOAST 7 GUIDELINES WITH THE USE OF VIRTUAL FRACTURE CLINIC

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The British Orthopedic Association recommends that patients referred to fracture clinic are reviewed within 72 hours. With the increase in referrals and limited clinic capacity it is becoming increasingly difficult to see every referral with in a 72 hour time frame. Some patients are waiting 2 weeks or more before they can be seen in a fracture clinic. With the aim of improving care by seeking to meet BOAST 7 target, waiting times for fracture clinic appointments at the Homerton University Hospital were audited prospectively against this national guideline, before virtual fracture clinic was implemented and 6 weeks after the implementation of virtual fracture clinic at our hospital. Virtual fracture clinic is where an Orthopedic consultant reviews a patients x-rays and A&E documentation and decides if that patients needs to be seen in a face to face fracture clinic to discuss operative vs. non-operative management of their injury or if a treatment plan can be delivered without the patient having to come back to hospital.

The study was conducted as a prospective closed-loop audit in which the second cycle took place after the implementation of the new virtual fracture clinic service.

The first cycle showed a non-compliant waiting time with only 18% of patients being seen within 72 hours. Following the implementation of virtual fracture clinic, 84% of all patients were reviewed within 72 hours.

Virtual fracture clinic delivered a significant reduction in waiting times. Virtual fracture clinic has only just been implemented at the Homerton University Hospital and hopefully at the next audit we will be 100% compliant with the BOA BOAST 7 Guideline. We would recommend that virtual fracture clinics being rolled out in Orthopedic departments in all hospitals which have Orthopedic services.

MICROCOMPUTED TOMOGRAPHIC, BIOMECHANICAL AND HISTOLOGICAL ANALYSES OF LUMBAR INTERBODY FUSION IN PIG MODEL: COMPARISON OF ILIAC CREST BONE GRAFT AND NEWLY DEVELOPED HYBRID BIODEGRADABLE NANOCOMPOSIT POROUS IMPLANT.

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The use of lumbar fusion procedures in the USA and Europe has rapidly increased over the last decade and a large number of these procedures involve the use of bone grafts. Despite of technical progress of spinal surgery and operative materials the risk of vertebral fusion failure occurs in 5 – 35 % of cases. Autografting has been considered the gold standard for bone graft procedures. However, the harvesting from the iliac crest can be associated with short and long-term morbidity in up to 22 % of cases. Main goal of this experimental study was to compare newly developed hybrid biodegradable nanocomposit porous implant (HBNPI) against bone craft from iliac crest as a new and better alternative for lumbar interbody fusion.

24 male pigs 4 months old weighting around 40 Kg were included in our study. These pigs were divided into two study groups depending on fusion method. Group A – 12 pigs underwent lateral lumbal interbody fusion (L2/3) with implantation of iliac crest bonegraft. Group B - 12 pigs underwent lateral lumbal interbody fusion (L2/3) with newly developed HBNPI. Each group were divided into two subgroups from these 6 spines were harvested 8 weeks (subgroup A1, B1) and 6 spines 16 weeks (group A2, B2) after surgery. After sacrifice, the lumbar spines were taking out and micro-CT, biomechanical testing and histomorphological analysis in all groups were performed to evaluate a quality of intervertebral fusion. As controls (group N), 6 cadaveric intact lumbar spines underwent biomechanical, micro-CT and histological testing.

All 24 animals recovered from general anesthesia without unusual events. The operations lasted between 50-90 minutes (mean 70) in Group A and between 35-72 minutes (mean 43) in Group B. All of the pigs from group A could stand up and were mobile within 20 hours (range 7-20). When bone graft harvesting was not necessary (group B) this time was shortened, ranging from 1 – 1,5 hour. All pigs from Group A were limping on the first postoperative day. No limping animal was observed in group B. Total body weight of the pigs increased from 37 kg (range 36-40) at the start to 85 (range 80-89) at sacrifice. Biomechanics evaluation shows that extension flexural stiffness values are statistically significantly different between A2 (16 weeks post-implant) and A1 (8 weeks post-implant). Group A2 achieves higher values than Group A1, which is attributed to the adhesion of the implant to the surrounding vertebrae.

Similarly, this also applies to groups B2 and B1. The flexural stiffness at group B2 extension is statistically significantly higher than the A2 group and also than the native N group. Biomechanical evaluation supports findings on micro-CT and histological specimens, where both adjacent vertebrae are completely fused in groups B2, unlike in group A2, where there is no or incomplete fusion.

Newly developed HBNPI represents new possibility how to do intervertebral fusion, and simultaneous become chance how to improve and accelerate bone healing process against standard procedures.

AN AUDIT ON THE USE OF A CALIBRATION MARKER FOR NECK OF FEMUR FRACTURE X-RAYS: A USEFUL BUT FORGOTTEN TOOL.

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Neck of femur fractures are a common presentation and certain patients can be managed with a total hip replacement. To receive a total hip replacement the pelvic X-rays should be templated as per AO guidelines and a common way this is performed is by including a calibration marker on the X-ray. The aim of this study is to assess and improve upon the use of the calibration marker.

Details of patients admitted with a neck of femur fracture from January 1st 2018 until December 31st 2018 were gathered and used to review each initial X-ray and determine if a calibration marker was included. 376 patients were admitted with a neck of femur fracture over the one year period. 36% of patients did not have a calibration marker on their initial pelvic X-ray and 11% did not have a chest X ray. 215 patients had an intracapsular fracture and 39 went on to have a total hip replacement. 12 patients were lacking a calibration marker on their original X ray and required a repeat X ray. After a poster was placed in the radiographer booth acting as a visual aid, the use of a calibration marker improved from 62% to 70%.

Calibration markers are useful tools which can aid the pre-operative planning for hip replacement surgeries shortening operative time, increase precision and reduce prosthetic loosening, lowers the risk of peri-prosthetic fractures, reduce leg length discrepancy and ensure the required implants are available. If a marker is not included on the initial X-rays, and a patient has a neck of femur fracture which requires a joint replacement, they may have to have additional X-rays performed as was the case for 12 patients in this study. This process leads to possible delays in surgery, additional radiation and increased healthcare costs.

COMPUTATIONAL OPTIMIZATION AND BIODEGRADATION OF 3D-PRINTED PATIENT-SPECIFIC ACETABULAR IMPLANTS

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3D-printed orthopedic implants have been gaining popularity in recent years due to the control this manufacturing technique gives the designer over the different design aspects of the implant. This technique allows us to manufacture implants with material properties similar to bone, giving the implant designer the opportunity to address one of the main complications experienced after total hip arthroplasty (THA), i.e. aseptic loosening of the implant. To restore proper function after implant loosening, the implant needs to be replaced. During these revision surgeries, some extra bone is removed along with the implant, further increasing the already present defects, and making it harder to achieve proper mechanical stability with the revision implant. A possible way to limit the increasing loss of bone is the use of biodegradable orthopedic implants that optimize long-term implant stability. These implants need to both optimize the implant such that stress shielding is minimized, and tune the implant degradation rate such that newly formed bone is able to replace the degrading metal in order to maintain a proper bone-implant contact. The hope is that such (partly) degradable implants will lead to a reduction in the size of the bone defects over time, making possible future revisions less likely and less complex.

We focused on improving the long-term implant stability of patient-specific acetabular implants for large bone defects and the modeling of their biodegradable behavior. To improve long-term implant stability we implemented a topology optimization approach. A patient-specific finite element model of the hip joint with and without implant was derived from CT-scans to evaluate the performance of the designs during the optimization routine. To evaluate the biodegradation behavior, a quantitative mathematical model was developed to assess the degradation rates of the biodegradable part of the implant. Currently, the biodegradation model has been implemented for magnesium (Mg) implants as a first proof of concept.

For a first test case, an optimized implant was found with stress shielding levels below 20% in most regions. The highest stress shielding levels were found at the bone implant interface. The biodegradation model has been validated using experimental data, which includes immersion tests of simple scaffolds created from Commercial Pure Mg. The mass loss of the scaffold is about 0.8 mg/cm^2 for the first day of immersion in simulated body fluid (SBF) solution. After the formation of a protective film on the surface of the simple scaffold, the degradation rate starts to slow down.

Initial results presented serve as a proof of concept of the developed computational framework for the implant optimization and the implant biodegradation behavior. Currently, timing calibration, benchmarking and validation are taking place.

Reducing implant-induced stress shielding, obtaining a better implant integration and reduction of bone defects, by allowing for bone to partially replace

the implant over time, are crucial design factors for large bone defect implants. In this research, we have developed in-silico models to investigate these factors. Once validated and coupled, the models will serve as an important tool to find the appropriate biodegradable implant designs and biodegradable metal properties for THA applications, that improve current implant lifetime while ensuring proper mechanical functioning.

LIPID PROFILES IN HOFFA'S FAT PAD OF OSTEOARTHRITIC VS OSTEOCHONDRAL DEFECT PATIENTS

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Early detection of knee osteoarthritis (OA) is critical for possible preventive treatment, such as weight loss, physical activity and sports advice and restoring biomechanics, to postpone total knee arthroplasty (TKA). Specific biomarkers for prognosis and early diagnosis of OA are lacking. Therefore, in this study, we analyzed the lipid profiles of different tissue types within Hoffa's fat pad (HFP) of OA and cartilage defect (CD) patients, using matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI). The HFP has already been shown to play an important role in the inflammatory process in OA by prostaglandin release. Additionally, MALDI-MSI allows us to investigate on tissue lipid distribution at molecular level, which makes it a promising tool for the detection of disease specific biomarkers for OA development.

Samples of HFP were obtained of patients undergoing surgical treatment for OA (n=3) (TKA) or CD (n=3) (cartilage repair). In all cases, tissue was obtained without patient harm. HFP samples were washed in phosphate buffered saline (PBS) and snap-frozen directly after surgical dissection to remove redundant blood contamination and to prevent as much tissue degradation as possible. Tissue sections were cut at 15 µm thickness in a cryostat (Leica Microsystems, Wetzlar) and deposited on indium tin oxide glass slides. Norharmane (Sigma-Aldrich) matrix was sublimed onto the tissue using the HTX Sublimator (HTX Technologies, Chapel Hill). µMALDI-MSI was performed using Synapt G2Si (Waters) at 50 µm resolution in positive ion mode. MS/MS fragmentation was performed for lipid identification. Data were processed with in-house Tricks for MATLAB and analyzed using principle component analysis (PCA) and verlan

OA and CD HFP specific lipid profiles were revealed by MALDI-MSI followed by PCA and DA. With these analyses we were able to distinguish different tissue types within HFP of different patient groups. Further discriminant analysis showed HFP intra-tissue heterogeneity with characteristic lipid profiles specific for connective and adipose tissues, but also for synovial tissue and blood vessels, revealing the high molecular complexity of this tissue. As expected, lipid signals were lower at the site of the connective tissue, compared to the adipose tissue. In particular, tri-acyl glycerol, di-acyl glycerol, sphingomyelin and phosphocholine species were differently abundant in the adipose tissue of HFP of OA compared to CD.

To our knowledge, this is the first study comparing lipid profiles in HFP of OA patients with CD patients using MALDI-MSI. Our results show different lipid profiles between OA and CD patients, as well as intra-tissue heterogeneity within HFP, rendering MALDI-MSI as a useful technology for OA biomarker discovery. Future research will focus on expanding the number of subjects and the improvement of lipid detection signals.

ORTHOPAEDICS FORWARD TO PRECISION MEDICINE: ELECTRONIC TOOLS USING LIFESTYLE PARAMETERS MAY LOWER THE PERSONAL RISK OF EARLY REOPERATIONS IN TOTAL KNEE ARTHROPLASTY

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Precision medicine tailoring the patient pathway based on the risk, prognosis, and treatment response may bring benefits to the patients. To identify risk factors contributing to the early failure of treatment (development of events of interest) and when possible to change the prognosis via modifying these factors may improve the outcome and/or lower the risk of complications. There is an emerging goal to identify such parameters in total knee arthroplasty (TKA) thus lower the risk of revision surgery. The goal of this study was to identify factors explaining the risk for early revision of TKA using an artificial intelligence method appropriate for this task.

We applied a patient similarity network (PSN) for the identification of risk factors associated with early reoperations (n=109, 5.8%) in patients with TKA (n=1885). Next, an algorithm based on formal concept analysis was developed to support the patient decision on how to change modifying personal characteristics with respect to the estimated probability of reoperations.

The early reoperations were less frequent in women (4.4%, median time to reoperation 4.5 mo) than in men (8.2%, 10 mo), reaching the highest incidence in younger men (10.9%).

COLLAGEN FIBRIL DIAMETER DISTRIBUTION OF BOVINE ANTERIOR CRUCIATE LIGAMENT CHANGES FROM BIMODAL TO UNIMODAL UPON INJURY WITH SUBSEQUENT DECREASE IN MEAN DIAMETER

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More than 250,000 people are suffering from Anterior Cruciate Ligament (ACL) related injuries each year in the US, with a cost of $17-25K/patient. There is an unmet clinical demand for improving grafts/scaffolds to provide biological integration in addition to mechanical support. Currently, no data is available for the utilization of fibrous scaffolds with bimodal distribution for ACL regeneration. The novelty in this study is that it proposes for the first time to investigate the collagen fibril diameter distribution in healthy and injured bovine ACL tissue, and utilization of such structure for scaffold design. Objectives are 1) developing a bovine ACL tear model and measuring the collagen fibril diameter distribution of both healthy and injured ACL tissues, and 2) fabricating scaffolds to mimic the structural properties of healthy and injured ACL tissue.

Bovine ACL tissues (1-3 years old) were harvested and characterized for their fibril diameter distribution using Transmission Electron Microscopy (TEM) and biomechanical properties under tension. The electrospun polycaprolactone (PCL) scaffolds were characterized using SEM and mechanical testing.

Healthy and injured ACL fibril diameter, and that of PCL scaffolds representing healthy and injured ACL are compared using unpaired student t-test.

The proposed fibrous scaffold design represents a significant departure from the conventional unimodal approach, and is expected to have significant contribution to ACL regeneration. These discoveries will serve as the foundation for the development of biomimetic tissue engineering substrates aimed at promoting biological graft fixation.

RETROGRADE FEMORAL NAILING THROUGH AN OPEN PHYSIS DOES NOT IMPAIR GROWTH IN PIGS

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The use of retrograde femoral intramedullary nails in children for deformity correction is controversial. It is unknown if the injury to the central part of the growth plate results in premature bony union, leading to limb deformities or discrepancies. The aim of this study was to assess physeal healing and bone growth after insertion of a retrograde femoral nail thorough the centre of the physis in a skeletally immature experimental porcine model. Eleven immature pigs were included in the study. One leg was randomised for operation with a retrograde femoral nail (diameter 10.7 mm), whilst the non-operated contralateral remained as control. All nails were inserted centrally in coronal and sagittal plane under fluoroscopic guidance, and the nails spanned the physis. The nails were removed at 8 weeks. Both femora in all animals underwent MRI at baseline (pre-operatively), 8 weeks (after nail removal) and 16 weeks (before euthanasia). Femoral bone length was measured at 5 sites (anterior, posterior, central, lateral and medial) using 3d T1-weighted MRI. Growth was calculated after 8 weeks (growth with nail) and 16 weeks (growth without nail). Physeal cross-sectional area and percentage violated by the nail was determined on MRI. Operated side was compared to non-operated. Corresponding 95% confidence intervals were calculated. No differences in axial growth were observed between operated and non-operated sides. Mean growth difference was 0,61 mm [-0,78;2,01] whilst the nail was inserted into the bone and 0,72 mm [-1,04;1,65] after nail removal. No signs of angular bone deformities were found when comparing operated side to non-operated side. No premature bony healing at the physis occurred. Histology confirmed fibrous healing. Mean physeal violation was 5.72% [5.51; 5.93] by the femoral nail. The insertion of a retrograde femoral nail through the centre of an open physis might be a safe procedure with no subsequent growth arrest. However, experiments assessing the long term physeal healing and growth are needed.

HYPERMOBILITY AMONG PATIENTS WITH GREATER TROCHANTERIC PAIN SYNDROME

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Greater trochanteric pain syndrome (GTPS) is a common and disabling hip condition. Hypermobility has been suggested as a possible cause of GTPS. The purpose of this study was to report the prevalence of hypermobility and to investigate its impact on hip-related function and awareness in patients with GTPS. This cross-sectional study was based on a cohort of patients diagnosed with GTPS in the 2013-2015 period. Hypermobility was investigated with the Beighton Score and defined by a cut-off score ≥5. Data on patients' current hip function and awareness were collected with the questionnaires the Copenhagen Hip and Groin Outcome Score and the Forgotten Joint Score. A total of 612 patients with GTPS were identified based on the diagnosis system; out of those, 390 patients were assessed for eligibility, and 145 (37%) were included. The prevalence of hypermobility within this cohort was estimated to be 11% (95% confidence interval (CI): 3-26%) for males and 25% (95% CI: 17-34%) for females. No significant association was found between hypermobility and self- reported hip function and awareness. We recommend that future studies of GTPS will include hypermobility and investigate the consequences of hypermobility among patients with GTPS.

PIXEL VALUE SCORE PRIOR TO FRAME REMOVAL IN TIBIAL LENGTHENING

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The regenerative potential of bone is enormous, and it is possible to lengthen limbs by bone distraction. However, there remains a major risk of fracture after lengthening the bone. Previous studies have described how the pixel value ratio may be used for determining the time for frame removal.

The aim of this study was to investigate the intrarater and the interrater reliability of pixel value scores from radiographs in tibial lengthening prior to frame removal. Moreover, the study aimed to determine the overall number of X- rays obtained during circular frame treatment.

Retrospective study. Patients treated with tibial lengthening by a circular frame at Aalborg University Hospital from January 1st 2000 to December 31st 2017 and a minimum of 12 months after frame removal were included. The bone was divided in proximal-, regenerate- and distal bone zone. These 3 zones were in AP x-ray divided in an anterior and posterior zone and in sagittal X-ray medial and lateral zone producing 6 zones in which the pixel value was measured. Pixel value ratio was calculated as: (Proximal pixel value+Distal pixel value)/2/Regenerate pixel value. Interrater correlations were calculated from measurements obtained by an orthopaedic registrar and an orthopaedic specialist. Intrarater correlation was calculated from repeated measurements obtained by an orthopaedic specialist.

Mean duration of circular frame treatment was: 6 (+-3) months. Median number of x-ray controls during frame treatment were: 9 (+-4). Out of 90 tibial lengthening it was only possible to measure pixel value in all six areas of interest on 20 lengthening prior to frame removal. Major reasons for inability to obtain measurements were metal hardware crossing the areas of bone interest on x-rays. The mean (95 % confidence intervals) pixel ratios values were: 1) lateral: 0.96 (0.93-1.00); 2) medial: 0.95 (0.92-0.99); 3) anterior: 0.94 (0.90-0.97); 4) posterior: 0.96 (0.93-0.99). The mean (95 % confidence intervals) inter ratter ICC estimates were: 1) lateral: 0.8 (0.5-0.9); 2) medial: 0.8 (0.4-0.9); 3) anterior: 0.4 (-0.5-0.8); 4) posterior: 0.6 (0.1-0.9). The mean (95 % confidence intervals) intra ratter ICC estimates were: 1) lateral: 1.0 (0.9-1.0); 2) medial: 1.0 (1.0-1.0); 3) anterior: 0.9 (0.9-1.0); 4) posterior: 1.0 (1.0-1.0). Out of the 20 lengthening examined one fracture occurred in the bone regenerate after frame removal.

Prospective studies are warranted to determine whether the pixel value ratio can be used as an indicator for frame removal.

FROM FAILURE TO SUCCESS: THE IN VIVO TRANSLATION OF A NOVEL BONE ADHESIVE TEST MODEL

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An *ex vivo* biomechanical test model for evaluating a novel bone adhesive has been developed. However, at day 1 in the in vivo pilot, high blood flow forced the study to halt until the solution presented here was developed.

The profuse bleeding after bone core removal affected the bond strength and was reflected in the lower mean peak value 1.53N. After considering several options, we were successful in sealing the source of blood flow by pressing adhesive into place after bone core removal. After the initial adhesive had cured additional adhesive was used to secure the bone core in place. The animals were sacrificed after 24 h and a tensile test was undertaken on the bone core to failure.

The *ex vivo* study produced mean peak tensile loads of 7.63N SD 2.39N (n=8, 4 rats 8 femurs). Whilst the mean peak tensile loads in the day 1 in vivo pilot were significantly lower 1.53N SD1.57 (n=8, 6 rats 8 femurs - 4 used for other tests). The subsequent layered adhesive bone cores showed a mean peak tensile force of 6.79N SD =3.13 (n=8, 4 rats 8 femurs). 7/8 failed at the bone to glue interface. This is the first successful demonstration of bonding bone *in vivo* for this class of adhesives.

The development of a double adhesive method of fixing a bone core in the distal femur enabled mean peak tensile forces to be achieved *in vivo* at 24 hours that were comparable with the *ex vivo* results previously demonstrated. This method supports application in further animal series and over longer time scales. Biomaterials researchers that intend to use gel or paste like preparations in distal femur defects in the rat should be aware of the risks of biomaterial displacement by local blood flow.

HYDROXYAPATITE: A PLATFORM FOR RECRUITING SYSTEMICALLY ADMINISTERED DRUGS

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Targeted delivery of drugs is a major challenge in diseases such as infections and tumors. The aim of this study was to demonstrate that hydroxyapatite (HA) particles can act as a recruiting moiety for various bioactive molecules and as a proof-of-concept demonstrate that the affinity of drugs to hydroxyapatite can exert a biological effect. A bisphosphonate, zoledronic acid (ZA), was used as a model drug. Experiment 1 (ZA seeks HA): Calcium sulphate (CaS)/hydroxyapatite (HA) biomaterial pellets (diameter¸=5 mm, height=2 mm) were implanted in the abdominal muscle pouch of rats. After 2-weeks of implantation, a sub-cutaneous injection of 14C-ZA (0.1 mg/kg) was given. 24 h later, the animals were sacrificed and the uptake of ZA determined in the pellets using scintillation counting. Experiment 2 (Systemically administered ZA seeks HA and exerts a biological effect): A fenestrated implant was filled with the CaS/HA biomaterial and inserted in the proximal tibia of rats. 2-weeks post-op, a subcutaneous injection of ZA (0.1 mg/kg) was given. Animals were sacrificed at 6-weeks post-op. Empty implant was used as a control. Peri-implant bone formation was evaluated using different techniques such as micro-CT, mechanical testing and histology. Welch's t-test was used for mechanical testing and Mann-Whitney U test for micro-CT data analysis. Experiment 1: Uptake of radioactive ZA in the CaS/HA biomaterial was confirmed. Almost no ZA was present in the surrounding muscle. These results show high specific binding between systemically administered ZA and synthetic particulate HA. Experiment 2: Significantly higher peri-implant bone was measured using micro-CT in the group wherein the implant contained the CaS/HA biomaterial and ZA was administered systemically (This study presents a method for biomodulating HA in situ by different bioactive molecules. The approach of implanting a biomaterial capable of recruiting systemically given drugs and thereby activate the material is novel and may present a possibility to treat bone infections or tumors.

PRE OPERATIVE PLANNING STRATEGY FOR THE FIXATION OF 'COMPLEX' ANKLE FRACTURES AT A MAJOR TRAUMA CENTRE: A 5 YEAR STUDY

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Management of complex posterior malleolar fractures requires a detailed appreciation of ligamentous and bony anatomy for optimal fracture fixation and restoration of articular congruency. Pre operative planning is vitally important to determine the surgical strategy for complex ankle fractures. We evaluated pre operative planning strategy pre and post implementation of BOAST 12 guidelines (2016) focussing on pre operative CT scans prior to definitive fixation at a major trauma centre.

A multi-surgeon retrospective review of prospectively collected data from 2013 to 2018 was performed at a major trauma centre. Patients who had sustained a posterior malleolar fracture and definitive fixation were identified. Information was collated from PICS, PACS, the trauma database and operative notes.

134 patients were identified over a 5 year period who had sustained a posterior malleolar fracture and had definitive fixation. (Pre BOAST guidelines = 61, Post BOAST guidelines = 73). Prior to the implementation of BOAST guidelines ¼ with posterior malleolar fractures did not have a pre operative CT scan (15/61). Post implementation of BOAST 12 90% (66/73)patients with fixation of posterior malleolus fractures had a pre operative CT scan. Posterior malleolus surgery most commonly took place In patients between 18-30 years.

Following implantation of BOAST 12 guidelines there was a 15% increase in pre operative CT scanning for 'complex ankle fractures'. Changes in national guidelines have heavily influenced pre operative planning strategy for ankle fractures at University Hospitals Birmingham. A detailed appreciation of fracture pattern pre operatively helps guide surgical strategy.

SINGLE INJECTION OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2-LOADED ARTIFICIAL COLLAGEN-LIKE PEPTIDE ACCELERATES CONSOLIDATION AND BONE UNION AT THE DOCKING SITE IN A MOUSE SEGMENTAL BONE TRANSPORT MODEL

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Segmental bone transport (SBT) using an external fixator is currently a standard treatment for large-diameter bone defects at the donor site with low morbidity. However, long-term application of the device is needed for bone healing. In addition, patients who received SBT treatment sometimes fail to show bone repair and union at the docking site, and require secondary surgery. The objective of this study was to investigate whether a single injection of recombinant human bone morphogenetic protein 2 (rhBMP-2)-loaded artificial collagen-like peptide gel (rhBMP-2/ACG) accelerates consolidation and bone union at the docking site in a mouse SBT model.
Six-month-old C57BL/6J mice were reconstructed by SBT with external fixator that has transport unit, and a 2.0-mm bone defect was created in the right femur. Mice were divided randomly into four treatment groups with eight mice in each group, Group CONT (immobile control), Group 0.2mm/d, Group 1.0mm/d, and Group BMP-2. Mice in Group 0.2mm/d and 1.0mm/d, bone segment was moved 0.2 mm per day for 10 days and 1.0 mm per day for 2 days, respectively. Mice in Group BMP-2 received an injection of 2.0 μg of rhBMP-2 dissolved in ACG into the bone defect site immediately after the defect-creating surgery and the bone segment was moved 1.0 mm/day for 2 days.

All animals were sacrificed at eight weeks after surgery. Consolidation at bone defect site and bone union at docking site were evaluated radiologically and histologically.
At the bone defect site, seven of eight mice in Group 0.2mm/d and two of eight mice in Group 1.0mm/d showed bone union. In contrast, all mice in Group CONT showed non-union at the bone defect site. At the docking site, four of eight mice in Group 0.2 mm/d and three of eight mice in Group 1.0 mm/d showed non-union. Meanwhile, all mice in Group BMP-2 showed bone union at the bone defect and docking sites. Bone volume and bone mineral content were significantly higher in Group 0.2mm/d and Group BMP-2 than in Group CONT. HE staining of tissue from Group 0.2mm/d and Group BMP-2 showed large amounts of longitudinal trabecular bone and regenerative new bone at eight weeks after surgery at the bone defect site. Meanwhile, in Group CONT and Group 1.0mm/d, maturation of regenerative bone at the bone defect site was poor. Differences between groups were analyzed using one-way ANOVA and a subsequent Bonferroni's post-hoc comparisons test. $P < 0.05$ was considered significant.

rhBMP-2/ACG combined with SBT may be effective for enhancing bone healing in large bone defects without the need for secondary procedures.

CLINICAL AND RADIOLOGICAL OUTCOMES AFTER OPEN REDUCTION AND INTERNAL FIXATION OF LISFRANC INJURIES: A SINGLE CENTRE EXPERIENCE

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Lisfranc injuries are uncommon and can be challenging to manage. There is considerable variation in opinion regarding the mode of operative treatment of these injuries, with some studies preferring primary arthrodesis over traditional open reduction and internal fixation (ORIF). We aim to assess the clinical and radiological outcomes of the patients treated with ORIF in our unit.

This is a retrospective study, in which all 27 consecutive patients treated with ORIF between June 2013 and October 2018 by one surgeon were included with an average follow-up of 2.4 years. All patients underwent ORIF with joint-sparing surgery by a dorsal bridging plate (DBP) for the second and third tarsometatarsal (TMT) joint, and the first TMT joint was fixed with trans-articular screws. Patients had clinical examination and radiological assessment, and completed American Orthopaedic Foot and Ankle Society (AOFAS) midfoot score and Foot Function Index (FFI) questionnaires.

Our early results of 22 patients (5 lost to follow-up) showed that 16 (72%) patients were pain free, walking normally without aids, and wearing normal shoes and 68% were able to run or play sports. The mean AOFAS midfoot score was 78.1 (63–100) and the average FFI was 19.5 (0.6–34). Radiological assessment confirmed that only three patients had progression to posttraumatic arthritis at the TMT joints though only one of these was clinically symptomatic.

Good clinical and radiological outcomes can be achieved by ORIF in Lisfranc injuries with joint-sparing surgery using DBP.

SYMMETRY OF THE NORMAL ANKLE SYNDESMOSIS ANALYZED BY A 3D WEIGHTBEARING AND NON-WEIGHTBEARING CT

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Syndesmotic ankle injuries are present in one fourth of all ankle trauma and may lead to chronic syndesmotic instability as well as posttraumatic ankle osteoarthritis. The main challenge remains distinguishing them from other types of ankle trauma. Currently, the patient's injured and non-injured ankles are compared using plain radiographs to determine pathology. However, these try to quantify 3D displacement using 2D measurements techniques and it is unknown to what extent the 3D configuration of the normal ankle syndesmosis is symmetrical.

We aimed to assess the 3D symmetry of the normal ankle syndesmosis between the right and left side in a non- and weightbearing CT.

In this retrospective comparative cohort study, patients with a bilateral non-weightbearing CT (NWBCT; N=28; Mean age=44, SD=17.4) and weight-bearing CT (WBCT; N=33; Mean age=48 years; SD=16.3) were analyzed. Consecutive patients were included between January 2016 and December 2018 when having a bilateral non-weightbearing or weightbearing CT of the foot and ankle. Exclusion criteria were the presence of hindfoot pathology and age less than 18 years or greather than 75 years. CT images were segmented to obtain 3D models. Computer Aided Design (CAD) operations were used to fit the left ankle on top of the right ankle. The outermost point of the apex of the lateral malleolus (AML), anterior tubercle (ATF) and posterior tubercle (PTF) were computed. The difference in the coordinates attached to these anatomical landmarks of the left distal fibula in the ankle syndesmosis with respect to right were used to quantify symmetry. A Cartesian coordinate system was defined based on the tibia to obtain the direction of differences in all six degrees of freedom. Statistical analysis was performed using the Mann-Whitney U test to allow comparison between measurements from a NWBCT and WBCT. Reference values were determined for each 3D measurement in a NWBCT and WBCT based on their 2SD. The highest difference in translation could be detected in the anterior-posterior direction (Mean AP_{NWBCT}= -0.01mm; 2SD=3.43/Mean AP_{WBCT}=-0.1mm; 2SD=2.3) and amongst rotations in the external direction (Mean AP_{NWBCT}=-0.3°; 2SD=6.7/Mean AP_{WBCT}=-0,2°; 2SD=5.2). None of these differences were statistically significant in the normal ankle syndesmosis when obtained from a NWBCT compared to a WBCT (P>0.05).

This study provides references values concerning the 3D symmetry of the normal ankle syndesmosis in weightbearing and non-weightbearing CT-scans. These novel data contribute relevantly to previous 2D radiographic quantifications. In clinical practice they will aid in distinguishing if a patient with a syndesmotic ankle lesion differs from normal variance in syndesmotic ankle symmetry.

STUDY OF OSTEOARTHRITIS (OA) DEVELOPMENT AND PREDICTION OF POTENTIAL INTERVENTION TARGETS BY AN *IN SILICO* APPROACH

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During OA the homeostasis of healthy articular chondrocytes is dysregulated, which leads to a phenotypical transition of the cells, further influenced by external stimuli. Chondrocytes sense those stimuli, integrate them at the intracellular level and respond by modifying their secretory and molecular state. This process is controlled by a complex interplay of intracellular factors. Each factor is influenced by a myriad of feedback mechanisms, making the prediction of what will happen in case of external perturbation challenging. Hampering the hypertrophic phenotype has emerged as a potential therapeutic strategy to help OA patients (Ripmeester et al. 2018). Therefore, we developed a computational model of the chondrocyte's underlying regulatory network (RN) to identify key regulators as potential drug targets

A mechanistic mathematical model of articular chondrocyte differentiation was implemented with a semi-quantitative formalism. It is composed of a protein RN and a gene RN(GRN) and developed by combining two strategies. First, we established a mechanistic network based on accumulation of decades of biological knowledge. Second, we combined that mechanistic network with data-driven modelling by inferring an OA-GRN using an ensemble of machine learning methods. This required a large gene expression dataset, provided by distinct public microarrays merged through an in-house pipeline for cross-platform integration.

We successfully merged various micro-array experiments into one single dataset where the biological variance was predominant over the batch effect from the different technical platforms. The gain of information provided by this merge enabled us to reconstruct an OA-GRN which subsequently served to complete our mechanistic model. With this model, we studied the system's multi-stability, equating the model's stable states to chondrocyte phenotypes. The network structure explained the occurrence of two biologically relevant phenotypes: a hypertrophic-like and a healthy-like phenotype, recognized based on known cell state markers. Second, we tested several hypotheses that could trigger the onset of OA to validate the model with relevant biological phenomena. For instance, forced inflammation pushed the chondrocyte towards hypertrophy but this was partly rescued by higher levels of TGF-β. However, we could annihilate this rescue by concomitantly mimicking an increase in the ALK1/ALK5 balance. Finally, we performed a screening of *in-silico* (combinatorial) perturbations (inhibitions and/or over-activations) to identify key molecular factors involved in the stability of the chondrocyte state. More precisely, we looked for the most potent conditions for decreasing hypertrophy. Preliminary validation experiments have confirmed that PKA activation could decrease the hypertrophic phenotype in primary chondrocytes. Importantly the in-silico results

highlighted that targeting two factors at the same time would greatly help reducing hypertrophic changes.

A priori testing of conditions with in-silico models may cut time and cost of experiments via target prioritization and opens new routes for OA combinatorial therapies.

TRABECULAR BONE SCORE OF POSTMENOPAUSAL WOMEN

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Osteoporosis is a common disorder characterized by low bone mass and reduced bone quality that affects the bone strength negatively and leads to increased risk of fracture. Bone mineral density (BMD) has been the standard instrument for the diagnosis of osteoporosis and the determination of fracture risk. Despite the approximation of the bone mass, BMD does not provide information about the bone structure. Trabecular bone score (TBS), which provides an indirect evaluation of skeletal microarchitecture, is calculated from dual X-ray absorptiometry and a simple and noninvasive method that may contribute to the prediction of osteoporotic fractures in addition to the measure of bone density. The goal of this study was to determine the mean TBS values in healthy postmenopausal women and the overall association between TBS and demographic features, bone mineral density of the lumbar spine and femoral neck and bone mineral density to body mass index ratio (BMD/BMI) of the lumbar spine. Fifty-three postmenopausal healthy women participated. The bone mineral density of the lumbar spine and femoral neck were measured dual X-ray absorptiometry. Anteroposterior lumbar spine acquisitions were used to calculate TBS for L1-L4. Age, height, weight, BMI and the ratio of BMD to BMI, which was considered to be a simple tool for assessing fracture risk in especially obese individuals, were calculated. The relationship between TBS and other variables was examined using Spearman's rank correlation coefficients. Mean BMD of the lumbar spine and the femoral neck were 0.945 Â± 0.133 and 0.785 Â± 0.112 g/cm2, respectively (Table 1). Mean TBS was 1.354 Â± 0.107. There was a significant positive moderate correlation between TBS and total lumbar BMD/BMI ratio (r=0.595, pTBS values of postmenopausal women were negatively correlated with age and BMI and positively with bone mineral density and BMD/BMI ratio. The ratio between lumbar BMD and BMI presented a stronger correlation with TBS than that of BMD with TBS. Because of the better correlation, the BMD/BMI ratio may be used as a simple tool for the assessment of the risk of fractures. Further investigation may be needed to evaluate the factors influencing exercise intervention on TBS on this population of patients.

ISOLATION AND COMPARATIVE CHARACTERIZATION OF EXOSOMES FROM HUMAN MESENCHYMAL STEM CELLS BY ULTRACENTRIFUGE AND MACS METHODS TO ENHANCE BONE HEALING

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Mesenchymal stem cell (MSC) exosomes are intracellular vesicles, which can regulate transcription and control gene expression through the molecules they carry, easily enter into the target cell, contain no regenerative effect, and do not produce an immune response. There are different methods in the literature to obtain these vesicles. However, studies on the isolation of MSC-derived exosomes and their comparative characterization using magnetically active cell sorting (MACS) and ultracentrifugation methods are lacking. The most appropriate isolation method for MSC-derived exosomes can be determined by comparing the isolation and characterization parameters of mesenchymal stem cells using magnetically active cell sorting and ultracentrifugation methods. The aim of this study was to define the advantages and disadvantages of the methods used for determining the purpose-oriented method. Human bone marrow-derived mesenchymal stem cells were cultured in standard MSC culture conditions (37ºC and 5% CO 2). Exosomal contamination was prevented by removal of exosomes from the serum that used in the standard growth medium. For exosome isolation of the cells reaching sufficient density, the media were replaced with new ones every two days, the old media were collected in liquid refrigerated with liquid nitrogen and stored at -80ºC. Part of the accumulated exosomes were isolated by using the MACS method, while the other was isolated by using the ultracentrifugation method, which included serial centrifugation steps. The amount of protein contained in the phosphate buffer solution in which the exosomes were reconstituted was determined by microplate reader using the BCA kit. Based on the protein concentration obtained, exosomes were read by means of a dye flow cytometer with fluorescent antibodies attached to surface markers specific to CD9, CD63, and CD81 specific for exosomes by latex beads. Finally, the exosomes were stained with uranyl acetate and phosphotungstic acid and then placed on 200 mesh and formvar-carbon film coated grids. Exosomes were isolated using both ultracentrifugation and MACS methods. While ultra-large amounts of exosomes can be isolated by ultracentrifugation method, MACS method provides a lower amount of isolation. Exosomes with magnetically active cell sorting are selected with specific surface markers, therefore, exosomal purity is thought to be higher. Exosomes which were isolated by both ultracentrifugation and MACS methods were monitored by using transmission electron microscopy and they were not found to be morphologically different. In conclusion, MACS and ultracentrifugation are effective methods for the isolation of human bone marrow-derived MSC exosomes. Both methods have advantages and disadvantages. Exosomes can be isolated together with magnetic beads using the MACS method. In the ultracentrifuge method, cleaner

exosomes can be isolated. While the exosomes are isolated by MACS, they can also be characterized by beads.

PROSPECTIVE STUDY OF PATELLAR TENDON CHANGES WITH ULTRASONOGRAPHIC INVESTIGATION AND THE EFFECT ON CLINICAL OUTCOMES AFTER KNEE PROSTHESIS

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Shortening of patellar tendon after total knee arthroplasty (TKA) was previously reported by several studies. Its etiology still remains controversial. Patellar tendon shortening, a direct cause of patella baja, has a dramatic negative impact in terms of clinical outcomes after TKA. Main objective of this study is to assess the feasibility of utilizing a different technique with Ultrasound that is easy to use, cost-effective and able to eliminate the problem of differential magnification occurring in other techniques which count on standard x-rays and to establish the correlation between clinical outcomes and changes in patellar tendon length and thickness after TKA.

The study was designed as prospective cohort and, after a minimum of 4-year-follow up period, 47 knees of 24 patients who had undergone primary TKA without patellar resurfacing were included in the study. All patients were scored with Kujala and HSS scores and all patellar tendons were evaluated with USG regarding their length and thickness. We used conventional grey-scale ultrasound imaging (US) to determine any changes in patellar tendon morphology. All cases were evaluated by the same radiologist. The patellar tendon was examined with the knee in 30° flexion. The flexion angle helped to stretch the extensor mechanism and avoid anisotropy (concavity) of the patellar tendon. The transducer was placed along the long axis of the tendon. The patellar tendon was initially examined in the longitudinal plane in order to measure the total length. Then, total length was divided into three parts and sagittal thickness was calculated at the proximal, median, and distal thirds of the patellar tendon. Both the length and thickness of the tendon were measured before surgery and at the 4th year of follow-up.

Of the 47 knees that were included in our study, the mean pre-operative and postoperative length of the patellar tendon was 40.78±6.15 mm and 35.93±4.52 mm. Our results suggested significant shortening of the patellar tendon after primary TKA surgery ($p<0.05$). Intergroup analysis suggested that reduced sagittal thickness in the proximal third of the tendon was more strongly correlated with an increase in functional outcomes ($p<0.05$). Our results suggested no significant difference in clinical outcome scores between patients with increased or decreased length of the patellar tendon after TKA ($p>0.05$).

We suggest that determining morphologic changes in sagittal thickness as well as length is important in explaining some of the ambiguous causes of anterior knee pain and impaired clinical outcomes after TKA. More accurate documentation of morphologic changes in the patellar tendon after TKA will certainly help to develop new techniques by surgeons or avoid some existing routines that may harm the tendon. USG is a feasible method for evaluating patellar tendon morphology after TKA but more future studies are needed.

ENZYMATICALLY CROSSLINKED NATURAL POLYMERS FOR CARTILAGE REPAIR

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Osteoarthritis is the most common chronic condition of the joints. It is characterized by the degeneration of articular cartilage, formation of osteophytes and alterations in the synovium. This process has a severe impact on the quality of life of the patients and the currently available treatments are unsatisfactory and often merely focused on pain relief. In our group we are working on the development of in situ cross-linkable hydrogel platforms that could be used for resurfacing the damaged articular cartilage using a minimally invasive arthroscopic procedure. Stable fixation of the gel at the joint surface, facilitating the ingrowth of local stem and progenitor cell populations and supporting intrinsic repair mechanisms are considered minimal design parameters. To achieve this, we are exploring the use of enzymatically cross-linkable natural polymer-tyramine conjugates.
Dextran-tyramine conjugates were prepared by activation of dextran-OH and subsequent reaction with tyramine. Hyaluronic acid-tyramine and protein-tyramine conjugates were prepared using DMTMM coupling. In situ crosslinking is achieved by mixing the polymer conjugates with the enzyme HRP and minute, non-toxic amounts of H_2O_2 as oxidizing agent. Support of cartilage formation was studied after mixing of the polymer conjugates with mesenchymal stem cells, chondrocytes or combinations of both prior to crosslinking. Cell ingrowth was studied by implanting the hydrogels in an ex-vivo cartilage defect while mechanically loading the explant in a bioreactor and cell migration in the hydrogels was evaluated by tracking the sprouting of fluorescently labelled cell-spheroids.
We prepared dextran-tyramine conjugates with a degree of substitution of 10 tyramine residues per 100 monosaccharide units. The conjugated hyaluronic acid-tyramine had a degree of substitution of 10% of the carboxylic acid groups, while for the proteins the substitution was dependent on the protein type.
Enzymatically crosslinked hydrogels, based on dextran and hyaluronic acid, with the addition of co-cross linkable proteins show excellent properties for application in the regeneration of damaged cartilage.

ADDITIVELY MANUFACTURED BIODEGRADABLE POROUS ZINC IMPLANTS FOR ORTHOPAEDIC APPLICATIONS

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As compared to magnesium (Mg) and iron (Fe), solid zinc (Zn)-based absorbable implants show better degradation rates. An ideal bone substitute should provide sufficient mechanical support, but pure Zn itself is not strong enough for load-bearing medical applications. Modern processing techniques, like additive manufacturing (AM), can improve mechanical strength of Zn. To better mimic the in vivo situation in the human body, we evaluated the degradation behavior of porous Zn implants in vitro under dynamic conditions. Our study applied selective laser melting (SLM) to build topographically ordered absorbable Zn implants with superior mechanical properties. Specimens were fabricated from pure Zn powder using SLM and diamond unit cell topological design. In vitro degradation was performed under both static and dynamic conditions in a custom-built set-up under cell culture conditions (37 Â°C, 20% O2 and 5% CO2) for up to 28 days. Mechanical properties of the porous structures were determined according to ISO 13314: 2011 at different immersion time points. Modified ISO 10993 standards were used to evaluate biocompatibility through direct cell seeding and indirect extract-based cytotoxicity tests (MTS assay, Promega) against identically designed porous titanium (Ti-6Al-4V) specimens as reference material. Twenty-four hours after cell seeding, its efficacy was evaluated by Live-Dead staining (Abcam) and further analyzed using dual channel fluorescent optical imaging (FOI) and subsequent flow cytometric quantification. Porous Zn implants were successfully produced by means of SLM with a yield strength and Young's modulus in the range of 3.9-9.6 MPa and 265-570 MPa, respectively. Dynamic flow significantly increased the degradation rate of AM porous Zn after 28 days. Results from Zn extracts were similar to Ti-6Al-4V with >95% of cellular activity at all tested time points, confirming level 0 cytotoxicity (i.e., This study clearly shows the great potential of AM porous Zn as a bone substituting material. Moreover, we demonstrate that complex topological design permits control of mechanical properties and degradation behavior.

LONGITUDINAL ANALYSIS OF EFFECTS OF MODIFIED BETA TRICALCIUM SCAFFOLD ON BONE REGENERATION USING A CRITICAL SIZE FRACTURE: HEALING MODEL IN MICE

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The large bone defects with high risk of delayed bone union and pseudoarthrosis remain significant clinical challenge. Aim of the present study was the investigation of the critical size fracture healing process in transgenic mice using a novel beta-TCP scaffold. The luciferase transgenic mice strains (BALB/C-Tg(NF-kappaB-RE-luc)-Xen) and FVB/N-Tg(Vegfr2-luc)-Xen were used. Critical size fracture on femur was performed and stabilized using external fixation (RISystem). The fracture was bridged with a synthetic scaffold with and without Strontium. In consequence, the expression levels of NF-kappaB and VEGFR2 could be monitored in a longitudinal fashion using the Xenogen imaging system for two months. Animals were euthanized, serial section of femur were prepared, and the fracture sites were histologically examined. Sr reduced inflammation in the early phase of healing (15th days), but it was increased in the late healing stage. The level of VEGFR2 activity increases in the Sr doped beta-TCP group at the 15th day, the luciferase activity starts to decrease in this group and show significantly less activity compared to other groups in the second half. In the group without scaffold a connective tissue formation were observed. In both, beta-TCP and beta-TCP+Sr, the connection of newly formed tissue within integrated canals in scaffold was visible. Tissue formation in beta-TCP+Sr group was significantly higher than in the beta-TCP group, whereas the percentage of osseous tissue in relation to the newly formed tissue was in beta-TCP scaffold much more than in beta-TCP+ Sr groups.This study presents the first data regarding VEGFR2 and NF-kappB and angiogenesis activity profiles during fracture healing. The collected longitudinal data reduces the number of experimental animals in the study. Addition of strontium in scaffolds influenced the inflammation in different stage of the healing. This effect might influence the healing process and may prove to be advantageous for osteoporosis fracture healing.

BIOCOMPATIBILITY AND ABSORPTION BEHAVIOR IN VITRO OF DIRECT PRINTED POROUS IRON IMPLANTS

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Direct metal printed (DMP) porous iron implants possess promising mechanical and corrosion properties for various clinical application. Nevertheless, there is a requirement for better co-relation between in vitro and in vivo corrosion and biocompatibility behaviour of such biomaterials. Our present study evaluates absorption of porous iron implants under both static and dynamic conditions. Furthermore, this study characterizes their cytocompatibility using fibroblastic, osteogenic, endothelial and macrophagic cell types.
In vitro degradation was performed statically and dynamically in a custom-built set-up placed under cell culture conditions (37^{o}C, 5% CO2 and 20% O2) for 28 days. The morphology and composition of the degradation products were analysed by scanning electron microscopy (SEM, JSM-IT100, JEOL). Iron implants before and after immersion were imaged by Î¼CT (Quantum FX, Perkin Elmer, USA). Biocompatibility was also evaluated under static and dynamic in vitro culture conditions using L929, MG-63, HUVEC and RAW 264.7 cell lines. According to ISO 10993, cytocompatibility was evaluated directly using live/dead staining (Live and Dead Cell Assay kit, Abcam) in dual channel fluorescent optical imaging (FOI) and additionally quantified by flow cytometry. Furthermore, cytotoxicity was indirectly quantified using ISO conform extracts in proliferation assays. Strut size of DMP porous iron implants was 420 microns, with a porosity of 64% ± 0.2% as measured by micro-CT. After 28 days of physiological degradation in vitro, dynamically tested samples were covered with brownish degradation products. They revealed a 5.7- fold higher weight loss than statically tested samples, without significant changes in medium pH. Mechanical properties (E = 1600-1800 MPa) of these additively manufactured implants were still within the range of the values reported for trabecular bone, even after 28 days of biodegradation. Less than 25% cytotoxicity at 85% of the investigated time points was measured with L929 cells, while MG-63 and HUVEC cells showed 75% and 60% viability, respectively, after 24 h, with a decreasing trend with longer incubations. Cytotoxicity was analysed by two-way ANOVA and post-hoc Tukey's multiple comparisons test. Under dynamic culture conditions, live-dead staining and flow cytometric quantification showed a 2.8-fold and 5.7-fold increase in L929 and MG-63 cell survival rates, respectively, as compared to static conditions.
Therefore, rationally designed and properly coated iron-based implants hold potential as a new generation of absorbable Orthopaedic implants.

THE IN VITRO EXPRESSION OF THE HYPOXIC INDUCIBLE FACTOR (HIF): ITS ROLE IN CLINICAL OSSEOINTEGRATION

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Hypoxic Inducible Factor and Hypoxic mimicking agents (HMA) trigger the initiation and promotion of angiogenic-osteogenic cascade events. However, there has been paucity of studies investigating how HIF could be over expressed under chronic hypoxic conditions akin to that seen in sickle cell disease patients to help form a template for tackling the matter of macrocellular avascular necrosis. Angiogenesis and osteogenesis are tightly coupled during bone development and regeneration, and the hypoxia-inducible factor-1 alpha (HIF-1) pathway has been identified as a key component in this process studies have shown. There are still no established experimental models showing how this knowledge can be used for the evaluation of bone implant integration and suggest ways of improving osseointegration in sickle cell disease patients with hip arthroplasty and thereby prevent increased implant loosening. The aim of this study is to help develop an in vitro experimental model which would mimic the in vivo pathologic state in the bone marrow of sickle cell disease patients. It also seeks to establish if the hypoxic inducible factor (HIF) could be over expressed in vitro and thus enhancing osseointegration. MG63 osteoblastic cells were cultured under normoxia and hypoxic conditions (20%; and 1% oxygen saturation) for 48 and 72 hours. Cobalt chloride was introduced to the samples in order to mimic true hypoxia. Cells cultured under normoxic conditions and without cobalt chloride was used as the control in this study. The expression of the hypoxic inducible factor was assessed using the reverse transcriptase qualitative polymerase chain reaction (RT-qPCR). There was increased expression of HIF1-alpha at 72hours as compared to 48hours under the various conditions. The level of expression of HIF increased from 48hrs (mean rank= 4.60) to 72hrs (mean rank =5.60) but this difference was not statistically significant, $X2(1) = 0.24$, $p = 0.625$. The mean rank fold change of HIF in hypoxic samples decreased compared to the normoxic samples but this difference was not statistically significant, $X2(1) = 0.54$, $p = 0.462$. Therefore, the expression of HIF is only increased with prolonged hypoxia as seen in the 72hours samples. The expression of HIF increased in samples with $CoCl_2$ (mean rank=5.17), compared with samples without $CoCl_2$ (mean rank 4.67), however this was not statistically significant, $X2(1) = 0.067$, $p=0.796$, p value > 0.05. The over expression of HIF was achieved within a few days (72hours) with the introduction of Cobalt Chloride, which is a mimetic for hypoxia similar to the in vivo environment in sickle cell disease patients. This is an in vitro model which could help investigate osseointergation in such pathologic bone conditions.

ACCURACY AND QUALITY OF EDUCATIONAL VIDEOS FOR ELBOW PHYSICAL EXAMINATION: WHAT SHOULD OUR STUDENTS WATCH?

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Driven by increasing emphasis on problem-based and self-directed learning, medical students and doctors in orthopedic specialty training rely increasingly on the internet as learning resource. As students or residents performance on physical examination may be less supervised in comparison to other clinical skills (for example surgical competence), online videos may provide a valuable source for education of physical examination skills. Cognitive psychological research has shown that videos can help viewers to understand techniques and manage the sequential steps of physical examination and approach to patients. YouTube is the largest open-access video platform available and provides access to thousands of educational videos on orthopedics-related topics. VuMedi, G9MD, and Orthobullets are examples of online platforms requiring user-registration with video content that is more directly focused on orthopedic topics. The objective of this study was to investigate the accuracy and quality of instructional videos on the physical examination of the elbow and identify factors influencing the educational usefulness.

A YouTube, VuMedi, Orthobullets, and G9MD search was performed on October 7, 2018 for videos on the physical examination of the elbow. We included both basic examination and disease specific tests. The included videos were rated for accuracy and quality by two independent authors using a modified version of a validated scoring system. Inter-rater reliability was analyzed using mean difference and intra-class correlation coefficient.

Twenty-three out of 126 videos were indicated as useful for educational purposes. Accuracy, quality and total scores were statistically significant higher for videos from specialized platforms compared to YouTube: 16.5 (95% CI 16 to 17) vs. 12.816 (95% CI 12.3 to 13.3) respectively. Video accuracy and quality were highly variable and did not correlate. The number of days online, views, and likes showed no or weak correlation with accuracy and quality. For the total score, our assessment tool showed excellent inter-rater reliability of 0.93 (95% CI 0.09-0.95) and a mean difference of 0.024 point between the two observers (p=0.871).

There is considerable variation in accuracy and quality of online available videos on the physical examination of the elbow. We indicated 23 educationally useful videos and provided an assessment method. This assessment method can be useful for both viewers to assess reliability of a video and educators interested in creating videos.

OSTEOARTHRITIC CHANGES IN CHONDROCYTES INFLUENCE THE RESPONSE TO MECHANICAL STIMULATION

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Osteoarthritis (OA) is the most common joint disease, which is characterized by a progressive loss of proteoglycans and the destruction of extracellular matrix (ECM), leading to a loss of cartilage integrity and joint function. During OA development, chondrocytes alter ECM synthesis and change their gene expression profile including upregulation of hypertrophic markers known from the growth plate. Although physiological mechanical loading can support cartilage formation and maintenance, mechanical overload represents one major risk factor for OA development. To date, little is known on how an OA-like hypertrophic chondrocyte phenotype alters the response of cartilage tissue to mechanical loading. The aim of this study was to investigate whether a hypertrophic phenotype change of chondrocytes affects the response to physiological mechanical loading and to reveal differences compared to normal control cartilage. Cartilage replacement tissue was generated using human articular chondrocytes (normal control cartilage, n=3-5) or human mesenchymal stromal cells which develop a hypertrophic phenotype similar to the one observed in OA (OA cartilage model, n=3-6). Cells were seeded in a collagen type I/III carrier and attached to a beta-TCP bone replacement phase, building an osteochondral unit for simulation of natural conditions. After 21 and 35 days of chondrogenic (re)differentiation, a single physiological mechanical compression episode (1 Hz, 25 %, 3 h) was applied, imitating three hours of normal walking in ten-minute intervals. Proteoglycan and collagen synthesis, gene expression and activation of signaling pathways were assessed. Cartilage replacement tissue of both groups had similar proteoglycan and collagen type II content as well as hardness properties. During (re)differentiation, both cell types showed a comparable upregulation of the chondrogenic marker genes COL2A1 and ACAN. As expected, hypertrophic marker genes (COL10A1, ALPL, MEF2C, IBSP) were only upregulated in the OA cartilage model. Mechanotransduction in both tissues was confirmed by load-induced activation of pERK1/2 signaling. While the 3 h loading episode significantly increased proteoglycan synthesis in normal control cartilage at day 35, the same protocol resulted in a suppression of proteoglycan and collagen synthesis in the OA cartilage model, which was accompanied by a downregulation of COL2A1 gene expression. In addition, hypertrophic marker genes COL10A1, ALPL and IBSP were significantly reduced after loading. Along lower load-induced SOX9 mRNA and protein stimulation in the OA cartilage tissue, a weaker induction of mechanosensitive BMP2, BMP6, FOS and FOSB gene expression was observed. While stable cartilage showed anabolic effects after physiological loading, the hypertrophic chondrocytes reacted with a reduced extracellular matrix synthesis. This could be explained by a lower mechanoinduction of the BMP signaling cascade and insufficient SOX9 stimulation.

Progressive OA development could thus be influenced by a reduced mechanocompetence of osteoarthritic chondrocytes.

ASSESSMENT OF GRAFT MATURITY AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION USING AUTOGRAFTS: A SYSTEMATIC REVIEW OF BIOPSY AND MRI STUDIES

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The purpose of this investigation was to evaluate systematically the literature concerning biopsy, MRI signal to noise quotient (SNQ) and clinical outcomes in graft-maturity assessment after autograft anterior cruciate ligament reconstruction (ACLR) and their possible relationships. Methods: The systematic review was reported and conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines. Studies through May 2019 evaluating methods of intra-articular ACL autograft maturity assessment were considered for inclusion. Eligible methods were histologic studies of biopsy specimens and conventional MRI studies reporting serial SNQ and/ or correlation with clinical parameters. Ten biopsy studies and 13 imaging studies, with a total of 706 patients, met the inclusion criteria. Biopsy studies show that graft remodeling undergoes an early healing phase, a phase of remodeling or proliferation and a ligamentization phase as an ongoing process even 1 year after surgery. Imaging studies showed an initial increase in SNQ, peaking at approximately 6 months, followed by a gradual decrease over time. There is no evident correlation between graft SNQ and knee stability outcome scores at the short- and long-term follow-up after ACLR. The remodeling of the graft is an ongoing process even 1 year after ACLR, based on human biopsy studies. MRI SNQ peaked at approximately 6 months, followed by a gradual decrease over time. Heterogeneity of the MRI methods and technical restrictions used in the current literature limit prediction of graft maturity and clinical and functional outcome measures by means of MRI graft SNQ after ACLR.

A PATIENT-SPECIFIC MICRO-TISSUE PLATFORM TO COMPARE BIOLOGICAL PROPERTIES IN VITRO TO PATIENT OUTCOME FOR ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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After anterior cruciate ligament (ACL) rupture, reconstructive surgery with a hamstring tendon autograft is often performed. Despite overall good results, ACL re-rupture occurs in up to 10% of the patient population, increasing to 30% of the cases for patients aged under 20 years. This can be related to tissue remodelling in the first months to years after surgery, which compromises the graft's mechanical strength. Resident graft fibroblasts secrete matrix metalloproteinases (MMPs), which break down the collagen I extracellular matrix. After necrosis of these fibroblasts, myofibroblasts repopulate the graft, and deposit more collagen III rather than collagen I. Eventually, the cellular and matrix properties converge towards those of the native ACL, but full restoration of the ACL properties is not achieved. It is unknown how inter-patient differences in tissue remodelling capacity contribute to ACL graft rupture risk. This research measured patient-specific tissue remodelling-related properties of human hamstring tendon-derived cells in an in vitro micro-tissue platform, in order to identify potential biological predictors for graft rupture.
Human hamstring tendon-derived cells were obtained from remnant autograft tissue after ACL reconstructions. These cells were seeded in collagen I gels on a micro-tissue platform to assess inter-patient cellular differences in tissue remodelling capacity. Remodelling was induced by removing the outermost micro-posts, and micro-tissue compaction over time was assessed using transmitted light microscopy. Protein expression of tendon marker tenomodulin and myofibroblast marker α-smooth muscle actin (αSMA) were measured using Western blot. Expression and activity of remodelling marker MMP2 were determined using gelatin zymography.
Cells were obtained from 12 patients (aged 12-51 years). Patient-specific variations in micro-tissue compaction speed or magnitude were observed. Up to 50-fold differences in αSMA expression were found between patients, although these did not correlate with faster or stronger compaction. Surprisingly, tenomodulin was only detected in samples obtained from two patients. Total MMP2 expression varied between patients, but no large differences in active fractions were found. No correlation of patient age with any of the remodelling-related factors was detected.
Remodelling-related biological differences between patient tendon-derived cells could be assessed with the presented micro-tissue platform, and did not correlate with age. This demonstrates the need to compare this biological variation in vitro - especially cells with extreme properties - to clinical outcome. Sample size is currently increased, and patient outcome will be determined. Combined with results obtained from the in vitro platform, this could lead to a predictive tool to identify patients at risk for graft rupture.

OSSEOINTEGRATION OF BCP-COATED VERSUS NON-COATED NON-DEGRADABLE POLYURETHANE FOCAL KNEE RESURFACING IMPLANTS: A CAPRINE PROOF-OF-CONCEPT STUDY

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Focal knee resurfacing implants (FKRIs) are typically intended to treat focal cartilage defects in middle-aged patients. All currently available FKRIs are (partly) composed of metal, which potentially leads to degeneration of the opposing articulating cartilage and hampers follow-up using magnetic resonance imaging (MRI). The purpose of this study was to investigate the in vivo osseointegration process of a novel non-degradable thermoplastic polycarbonate-urethane (TPU) osteochondral implant. Bi-layered implants measuring 6 mm in diameter, with a double-curvature to match the approximate curvature of the goat medial femoral condyle were fabricated. TPU implants were composed of an articulating Bionate® II 80A top layer, and a Bionate® 75D bottom layer (DSM Biomedical, Geleen, the Netherlands) which is intended to osseointegrate. A biphasic calcium phosphate coating formulation, optimized during a prior in vitro study, was applied to half of the TPU implants, while the other half was left uncoated. Bi-layered metal implants (articulating cobalt-chromium top layer and titanium bottom layer) were used as positive control implants. Eight implants per group were implanted bilaterally in the medial femoral condyle of the stifle joints in 12 Dutch milk goats. 18F-sodium fluoride (18F-NaF) positron emission tomography-computed tomography (PET-CT) scanning was performed at 3 and 12 weeks postoperatively, and the corrected maximum standard uptake values (cSUVmax) was calculated to assess the peri-implant bone metabolism. After sacrifice 12 weeks postoperatively, bone histomorphometric analysis was performed to assess the bone-to-implant contact area (BIC). Student's T-test was used in case of normal distribution and the Mann-Whitney-U-test was used in case of abnormal distribution for comparison of BIC and cSUVmax. The BIC value of 10.27 ± 4.50% (mean ± SD) for the BCP-coated TPU implants was significantly (P=0.03) higher than the 4.50 Â± 2.61% for the uncoated TPU implants. The uncoated TPU implants scored significantly (P=0.04) lower than the BIC of 12.81 ± 7.55% for the metal implants, whereas there was no significant difference between BCP-coated TPU implants and the metal implants (P=0.68). There was a strong correlation between the cSUVmax values and the BIC values at 12 weeks (Pearson’s R=0.74, P=0.001). The cSUVmax values significantly decreased between 3 and 12 weeks for the metal implants (p=0.04). BCP-coated TPU implants followed a similar trend but did not reach statistical significance (p=0.07). cSUVmax in the uncoated TPU implants did not show a significant difference between the time-points (p=0.31). Osseointegration of BCP-coated TPU implants did not significantly differ from metal implants. 18F-NaF PET-CT is a feasible modality to assess osseointegration patterns and showed a similar trend between the BCP-coated and metal implants. Hence, an implant fully composed of TPU may avoid the typical metal-related drawbacks of currently available FKRIs. Long-term follow-up studies are advocated to address the effects of the implant to the opposing cartilage, and are therefore warranted.

THE MODIFIED HEDGEHOG TECHNIQUE TO REATTACH CHONDRAL FRAGMENTS IN THE YOUNG ADULT KNEE: FOLLOW-UP WITH PROMS AND 7.0T MRI AT 3 MONTHS AND 1 YEAR AFTER SURGERY

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The modified Hedgehog technique was previously used to reattach pure chondral shear-off fragments in the pediatric knee. In the modified Hedgehog technique, the calcified side of chondral fragments is multiple times incised and trimmed obliquely for an interlocking fit in the defect site. Fibrin glue with or without sutures is subsequently applied to fix the fragment to the defect. This preliminary report further elucidates the potential of the technique by evaluation of its application in young adults using patient reported outcome measures (PROMs) and high-field Magnetic Resonance Imaging (MRI) as outcome measures. Three patients with a femoral cartilage defect (2 medial, 1 lateral), and a concomitant pure chondral corpus liberum were operatively treated by the modified Hedgehog technique. Age at surgery ranged from 20.6-21.2 years, defect size ranged from 3.8-6.0 cm2. Patients were evaluated at three months and one year after surgery by PROMs and 7.0T MRI. PROMs included the Internation Knee Documentation (IKDC), Knee Injury and Osteoarthritis Outcome Score (KOOS) and Visual Analog Scale (VAS) questionnaires. 7.0T MRI (Magnetom, Siemens Healthcare, Erlangen, Germany) using a 28-channel proton knee coil (QED, Electrodynamics LLC, Cleveland, OH) included a proton density weighted turbo spin-echo sequence with fat suppression to assess morphological tissue structure andgagCEST imaging to measure the biochemical tissue composition in terms of glycosaminoglycans (GAG). Twelve months after surgery all patients reported no pain and showed full range of motion. While PROMs at three months showed large variability between patients, one year after surgery the scores were consistently improved. Over time, morphological MRI visualized improvements in integration of the cartilage fragment with the surrounding cartilage, which was supported by biochemical MRI showing increased GAG values at the defect edges. Statistics were not applied to the results because of the small sample size. The modified Hedgehog technique in young adults with an acute onset caused by a pure chondral corpus liberum can be considered promising. The improved PROM results over time were supported by 7.0T MRI that visualized improvements in tissue structure and biochemical composition. Inclusion of more patients in future studies would allow statistical analysis and more conclusive results. The etiology of loosening and time between onset of symptoms and surgery for successful graft integration may differ between pediatric and young adult patients and is subject for future studies.

MORPHOMETRIC CHARACTERISTICS OF THE TROCHLEODYSPLASTIC KNEE: A LANDMARK-BASED 3D ANALYSIS

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Trochlear dysplasia is a specific morphotype of the knee, characterized by but not limited to a specific anatomy of the trochlea. The notch, posterior femur and tibial plateau also seem to be involved. In our study we conducted a semi-automated landmark-based 3D analysis on the distal femur, tibial plateau and patella.

The knee morphology of a study population (n=20), diagnosed with trochlear dysplasia and a history of recurrent patellar dislocation was compared to a gender- and age-matched control group (n=20). The arthro-CT scan-based 3D-models were isotropically scaled and landmark-based reference planes were created for quantification of the morphometry. Statistical analysis was performed to detect shape differences between the femur, tibia and patella as individual bone models (Mann-Whitney U test) and to detect differences in size agreement between femur and tibia (Pearson's correlation test).

The size of the femur did not differ significantly between the two groups, but the maximum size difference (scaling factor) over all cases was 35%. Significant differences were observed in the trochlear dysplasia (TD) versus control group for all conventional parameters. Morphometrical measurements showed also significant differences in the three directions (anteroposterior (AP), mediolateral (ML), proximodistal (PD)) for the distal femur, tibia and patella. Correlation tests between the width of the distal femur and the tibial plateau revealed that TD knees show less agreement between femur and tibia than the control knees; this was observed for the overall width (TD: r=0.172; p=0.494 - control group: r=0.636; p=0.003) and the medial compartment (TD: r=0.164; p=0.516 - control group: r=0.679; p=0.001), but not for the lateral compartment (TD: r=0.512; p=0.029 - control: r=0.683; p=0.001). In both groups the intercondylar eminence width was strongly correlated with the notch width (TD: r=0.791; p=0.001 - control: r=0.643; p=0.002).

The morphology of the trochleodysplastic knee differs significantly from the normal knee by means of an increased ratio of AP/ML width for both femur and tibia, a smaller femoral notch and a lack of correspondence in mediolateral width between the femur and tibia. More specifically, the medial femoral condyle shows no correlation with the medial tibial plateau.

ACTIVITY LEVEL AFTER LATERAL ANKLE LIGAMENT RECONSTRUCTION, USING GRACILIS AUTOGRAFT

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Limited information is published regarding the activity level after gracilis autograft reconstruction, and usually a knee-injury based score is used rather than a specific ankle PROM. The purpose of this study was to investigate the activity level and functional results after lateral ankle gracilis autograft reconstruction in patients with severe lateral ankle instability. The hypothesis was that patients would regain their pre-injury Tegner activity level or one level below and secondary to compare a specific ankle activity score, instability and function score. Finally, donor site and graft complications, clinical stability and range of motion were measured.

All 69 patients (50 women, 19 men) recorded at the hospital with severe instability who underwent reconstruction of the anterior talofibular and the calcaneofibular ligament with a gracilis autograft and were minimum 6 months post-operative, were invited to participate in the study. Outcomes measures included the Tegner Activity level (1-10), Ankle Activity Score (0-10) recorded as pre-injury and at follow up. The Karlsson Petterson Ankle Function Score (0-100) and Visual Analog Score (VAS)(0-10) recorded pre-operatively and at follow up. All pre-injury and pre-operative data were recalled retrospectively from memory. Identification of functional ankle instability (IDFAI)(0-37) was recorded at follow up. The clinical tests, Anterior drawer test (0-4), Talar tilt test (0-4) and Range of motion (ROM)(degrees) were compared to the unaffected side at follow up. A difference of 1 in the activity scores was chosen as a clinical relevant difference. Data was tested for normal distribution and for statistical significant difference with a students t-test. study design: Cross sectional clinical study with a retrospective questionnaire.

A total of 33 patients (27 women, 6 men), with a mean age on 45 years (range 19-68), were included in this study. Mean follow up was 3.7 years. Mean pre-operative Tegner score was 5.8 vs 5.6 at follow up (p

On average, the patients returned to their pre-injury activity level, with similar specific ankle activity scores to the Tegner. The majority had good functional results and few residual symptoms of functional instability. The response rate was low with few men responding; hence a prospective study is called for to establish the true effect of the surgical technique.