

# EORS 2023



27-29 SEPTEMBER | PORTO, PORTUGAL

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

Orally presented accepted abstracts will be published in ORTHOPAEDIC PROCEEDINGS: A supplement to The Bone & Joint Journal after the meeting.

**Abstracts****Arthritis Gene Therapy: From Concept to Clinical Trials**

Chris Evans

Mayo Clinic, Rochester, MN, USA

Intra-articular injection is a common way to deliver biologics to joints, but their effectiveness is limited by rapid clearance from the joint space [1]. This barrier can be overcome by genetically modifying cells within the joint such that they produce anti-arthritic gene products endogenously, thereby achieving sustained, therapeutic, intra-articular concentrations of the transgene products without re-dosing. A variety of non-viral and viral vectors have been subjected to preclinical testing to evaluate their suitability for delivering genes to joints [2]. The first transfer of a gene to a human joint used an *ex vivo* protocol involving retrovirally transduced, autologous, synovial fibroblasts [3]. Recent advances in vector technology allow *in vivo* delivery using adeno-associated virus (AAV). We have developed an AAV vector encoding the interleukin-1 receptor antagonist (AAV.IL-1Ra) for injection into joints with osteoarthritis (OA). It showed efficacy and safety in equine [4, 5] and rat [6] models of OA, leading to a recently-completed, investigator-initiated, Phase I, dose-escalation clinical trial in 9 subjects with mid-stage OA of the knee (ClinicalTrials.gov Identifier: NCT02790723). Three cohorts of three subjects with mild to moderate OA in the index knee were injected intra-articularly under ultrasound guidance with a low ( $10^{11}$  viral genomes) medium ( $10^{12}$  viral genomes) or high ( $10^{13}$  viral genomes) dose of AAV.IL-1Ra and followed for one year. The data confirm safety, with evidence of sustained intra-articular expression of IL-1Ra and a clinical response in certain subjects. Funding for a subsequent Phase Ib trial involving 50 subjects (ClinicalTrials.gov Identifier: NCT05835895), expected to start later this year, has been acquired. Progress in this area has stimulated commercial activity and there are now at least seven different companies developing gene therapies for OA and a number of clinical trials are in progress [7].

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## 27-29 SEPTEMBER | PORTO, PORTUGAL

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**Acknowledgement:** Clinical trial funded by US Department of Defense Clinical Trial Award W81XWH-16-1-0540.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Abolition of Sagittal T7-T10 Dynamics During Forced Ventilation in AIS Patients with Lenke 1A curves**Gonzalo Mariscal<sup>1</sup>, Jesús Burgos<sup>2</sup>, Luis Antón-Rodríguez<sup>3</sup>, Eduardo Hevia<sup>4</sup>, Carlos Barrios<sup>5</sup>

<sup>1</sup>School of Doctorate, Valencia Catholic University, Valencia, Spain; <sup>2</sup>Spine Surgery Unit, Hospital Viamed Fuensanta, Madrid, Spain; <sup>3</sup>Division of Paediatric Orthopaedics, Hospital Ramon y Cajal, Madrid, Spain; <sup>4</sup>Spine Surgery Unit, Hospital La Fraternidad-Muprespa, Madrid, Spain; <sup>5</sup>Institute for Research on Musculoskeletal Disorders, Valencia Catholic University, Valencia, Spain.

In healthy subjects, respiratory maximal volumes are highly dependent on the sagittal range of motion of the T7-T10 segment. In AIS, the abolition of T7-T10 dynamics related to the stiffness induced by the apex region in Lenke IA curves could harm ventilation during maximal breathing. The aim of this study was to analyze the dynamics of the thoracic spine during deep breathing in AIS patients and in healthy matched controls. This is a cross-sectional, case-control study. 20 AIS patients (18 girls, Cobb angle,  $54.7 \pm 7.9^\circ$ ; Risser  $1.35 \pm 1.2$ ) and 15 healthy volunteers (11 girls) matched in age (12.5 versus 15.8 yr. mean age) were included. In AIS curves, the apex was located at T8 (14) and T9 (6). Conventional sagittal radiographs of the whole spine were performed at maximal inspiration and exhalation. The ROM of each spinal thoracic functional segment (T1-T7, T7-T10, T10-T12) and the global T1-T12 ROM were measured. In healthy subjects, the mean T1-T12 ROM during forced breathing was  $16.7 \pm 3.8$ . AIS patients showed a T1-T12 ROM of  $1.1 \pm 1.5$  ( $p < 0.05$ ), indicating a sagittal stiffness of the thoracic spine. A wide T7-T10 ROM ( $15.3 \pm 3.0$ ) was found in healthy controls (91.6% of the T1-T12 ROM). AIS patients showed only  $0.4 \pm 1.4$  ROM at T7-T10 (36.4% of the T1-T12 ROM) ( $p < 0.001$ ). There was a significant positive correlation between the magnitude of T7-T10 kyphosis in maximal exhalation and both FVC (% of predicted FVC) and FEV1. In conclusion, Lenke 1A AIS patients show a restriction of the thoracic spine motion with an almost complete abolition of T7-T10 ROM, a crucial segment for deep breathing. T7-T10 stiffness could explain the ventilatory limitations found in AIS patients.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Osteoarthritis patients develop an autonomic dysfunction

Rebecca Sohn<sup>1</sup>, Tina Assar<sup>1</sup>, Sebastian Braun<sup>1</sup>, Marco Brenneis<sup>1</sup>, Isabelle Kaufhold<sup>1</sup>, Frank Zaucke<sup>1</sup>, Georg Pongratz<sup>2</sup>, Zsuzsa Jenei-Lanzl<sup>1</sup>

<sup>1</sup>Dr. Rolf M. Schwiete Research Unit for Osteoarthritis, Department of Orthopedics (Friedrichsheim), University Hospital Frankfurt, Goethe University Frankfurt/Main, 60528, Germany; <sup>2</sup>Center for Rheumatologic Rehabilitation, Asklepios Hospital Bad Abbach, Medical Faculty of the University of Regensburg, 93077, Bad Abbach, Germany

Osteoarthritis (OA) is the most common degenerative joint disorder. Its multifactorial etiology includes age, sex, joint overloading, genetic or nervous influences<sup>1</sup>. In particular, the autonomic nervous system is increasingly gaining in importance. Its two branches, the sympathetic (SNS) and parasympathetic nervous system, are well-balanced under healthy conditions<sup>2</sup>. OA patients seem to be prone to an autonomic imbalance and therefore, we analyzed their autonomic status.

More than 200 participants including patients with early and late stage knee OA (before and 1 year after knee replacement surgery) and healthy probands (age-matched) were analyzed. Heart rate variability was measured via electrocardiogram to assess long-term sympathetic (low-frequency=LF) and parasympathetic (high-frequency=HF, pRR50) activities or general variability (RMSSD, SDRR)<sup>4</sup>. Serum cortisol concentrations were measured by ELISA. Perceived chronic stress (PSQ) was assessed via questionnaire. Multivariate regression was performed for data analysis. LF/HF value of early OA was slightly increased compared to healthy controls but significantly higher compared to late OA patients before ( $p>0.05$ ) and after TKR ( $p>0.01$ ). HF in late OA patients before TKR was significantly decreased compared to patients after TKR ( $p>0.001$ ) or healthy controls ( $p>0.05$ ). Healthy probands exhibited the highest SDRR values, early OA patients had slightly lower levels and late OA patients before TKR displayed significantly reduced SDRR ( $p>0.001$ ). The same differences were observed in pRR50 and RMSSD. Serum cortisol concentrations and PSQ scores increased in late OA patients before TKR. At the time point of TKR, women with beta blocker medication had significantly higher age ( $71 \pm 9$  years) than those without ( $63 \pm 12$  years) ( $p>0.01$ ). An autonomic dysfunction with sympathetic dominance occurs in OA patients. The fact that beta blocker medication in women delayed the need of TKR indicates that SNS inhibition might counteract OA. Future therapeutic interventions for OA should consider a systemic approach with special regard on the ANS.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Results of Biphasic Calcium Phosphate Bone Graft with Submicron-Sized Needle-Shaped Surface Topography as Standalone Alternative to Autograft are Favorable in a Prospective, Multi-center, Randomized, Intra-patient Controlled Trial**

Kucko NW., PhD<sup>1</sup>, Sage K., MS, DO<sup>1</sup>; Delawi, D., MD, PhD<sup>2</sup>; Hoebink E., MD<sup>3</sup>; Kempen, DHR., MD, PhD<sup>4</sup>; Van Susante, J., MD, PhD<sup>5</sup>; de Bruijn, J., PhD<sup>1</sup>, Kruyt. M., MD, PhD<sup>6</sup>

<sup>1</sup>Kuros Biosciences BV, Bilthoven, Netherlands; <sup>2</sup>St. Antonius Ziekenhuis, Urecht, Netherlands; <sup>3</sup>Amphia, Breda, Netherlands; <sup>4</sup>OLVG Amsterdam, Netherlands  
<sup>5</sup>Rijnstate, Arnhem, Netherlands; <sup>6</sup>UMC Utrecht, Utrecht, Netherlands

Pseudoarthrosis after spinal fusion is an important complication leading to revision spine surgeries. Iliac Crest Bone Graft is considered the gold standard, but with limited availability and associated co-morbidities, spine surgeons often utilize alternative bone grafts.

Determine the non-inferiority of a novel submicron-sized needle-shaped surface biphasic calcium phosphate (BCP<math>\mu\text{m}</math>) as compared to autograft in instrumented posterolateral spinal fusion.

Adult patients indicated for instrumented posterolateral spinal fusion of one to six levels from T10-S2 were enrolled at five participating centers. After instrumentation and preparation of the bone bed, the randomized allocation side of the graft type was disclosed. One side was grafted with 10cc of autograft per level containing a minimum of 50% iliac crest bone. The other side was grafted with 10cc of BCP<math>\mu\text{m}</math> granules standalone (without autograft or bone marrow aspirate). In total, 71 levels were treated. Prospective follow-up included adverse events, Oswestry Disability Index (ODI), and a fine-cut Computerized Tomography (CT) at one year. Fusion was systematically scored as fused or not fused per level per side by two spine surgeons blinded for the procedure.

The first fifty patients enrolled are included in this analysis (mean age: 57 years; 60% female and 40% male). The diagnoses included deformity (56%), structural instability (28%), and instability from decompression (20%). The fusion rate determined by CT for BCP<math>\mu\text{m}</math> was 76.1%, which compared favorably to the autograft fusion rate of 43.7%. Statistical analysis through binomial modeling showed that the odds of fusion of BCP<math>\mu\text{m}</math> was 2.54 times higher than that of autograft. 14% of patients experienced a procedure or possible device-related severe adverse event and there were four reoperations. Oswestry Disability Index (ODI) score decreased from a mean of 46.0 ( $\pm 15.0$ ) to a mean of 31.7 ( $\pm 16.9$ ), and 52.4% of patients improved with at least 15-point decrease.

This data, aiming to determine non-inferiority of standalone BCP<math>\mu\text{m}</math> as compared to autograft for posterior spinal fusions, is promising. Ongoing studies to increase the power of the statistics with more patients are forthcoming.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Does Inflammatory and Metabolomic Activity on Normal and Osteoarthritic Chondrocytes differ between Mesenchymal Stem Cells (MSCs) derived from the Infrapatellar Fat, Synovium and Subcutaneous Tissues?**Levend Karaçoban<sup>1</sup>, Merve Gizer<sup>2</sup>, Bilge Basak Fidan<sup>3</sup>, Ozan Kaplan<sup>3</sup>, Mustafa Çelebier<sup>3</sup>, Petek Korkusuz<sup>4</sup>, Egemen Turhan<sup>5</sup>, Feza Korkusuz<sup>1</sup>

Hacettepe University <sup>1</sup>Faculty of Medicine, Department of Sports Medicine, <sup>2</sup>Graduate School of Health Sciences, Department of Stem Cell Sciences, <sup>3</sup>Faculty of Pharmacy, Department of Analytical Chemistry, <sup>4</sup>Faculty of Medicine, Department of Histology and Embryology, and <sup>5</sup>Faculty of Medicine, Department of Orthopedic Surgery, Ankara 06230, Turkey

Osteoarthritis (OA) is a disabling disease depriving the quality of life of patients. Mesenchymal stem cells (MSCs) are recently used to modify the inflammatory and degenerative cascade of the disease. Source of MSCs could change the progression and symptoms of OA due to their different metabolomic activities. We asked whether MSCs derived from the infrapatellar fat (IPF), synovium (Sy) and subcutaneous (SC) tissues will decrease inflammatory and degenerative markers of normal and OA chondrocytes and improve regeneration in culture. Tissues were obtained from three male patients undergoing arthroscopic knee surgery due to sports injuries after ethical board approval. TNF $\alpha$  concentration decreased in all MSC groups (Sy=156,6 $\pm$ 79, SC=42,1 $\pm$ 6 and IPF=35,5 $\pm$ 3 pg/ml; p=0,036) on day 14 in culture. On day seven (Sy=87,4 $\pm$ 43,7, SC=23 $\pm$ 8,9 and IPF=14,7 $\pm$ 3,3 pg/ml, p=0,043) and 14 (Sy=29,1 $\pm$ 11,2, SC=28,3 $\pm$ 18,5 and IPF=20,3 $\pm$ 16,2 pg/ml, p=0,043), MMP3 concentration decreased in all groups. COMP concentration changes however were not significant. Plot scores of tissues for PC2-13,4% were significantly different. Based on the results of liquid chromatography-mass spectrometry (LC-MS) metabolomics coupled with recent data processing strategies, clinically relevant seven metabolites (L-fructose, a-tocotrienol, coproporphyrin, nicotinamide, bilirubin, tauro-deoxycholic acid and galactose-sphingosine) were found statistically different (p<0.05 and fold change>1.5) ratios in tissue samples. Focusing on these metabolites as potential therapeutics could enhance MSC therapies.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Lipid inhibition in high glucose condition contributes osteoarthritis derived progenitor cells chondrogenesis**

Wenguang Liu<sup>1</sup>, Meng Feng<sup>2</sup>, Peng Xu<sup>3</sup>

<sup>1</sup>Honghui Hospital, Xi'an Jiaotong University, Xi'an, China; <sup>2</sup>Department of Orthopedics, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

More and more evidences showed that cartilage harbored local progenitor cells that could differentiate toward osteoblast, chondrocyte, and adipocyte. However, our previous results showed that osteoarthritis derived chondroprogenitor cells (OA-CPC) exhibited strong osteogenic potential even in chondrogenic condition [1]. How to promote their chondrogenic potential is the key for cartilage repair and regeneration in osteoarthritis. Recently, lipid availability was proved to determine skeletal progenitor fate [2]. Therefore, we aim to determine whether lipid inhibition under 3D culture condition could enhance OA-CPC chondrogenesis. Moreover, glucose concentration was also evaluated for chondrogenic capacity. Although there are many researches showed that lower glucose promotes chondrogenesis, in our results, we found that OA-CPC in high concentration of glucose (4.5g/L) with lipid inhibitor (GW1100) showed strongest chondrogenic potential, which could form largest cell pellet with strong proteoglycan staining, COL II expression and no COL I expression. Besides, *COL2A1* was increased and *COL10A1* was decreased significantly by GW1100 under high glucose condition in 2D culture. Interestingly, although the expression level of MMP13 was not changed by GW1100 at RNA and protein level, less MMP13 protein secreted out of cell nuclear. In summary, we estimated that higher glucose and lower lipid supplies benefit OA-CPC chondrogenesis and cartilage repair.

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**How could MSC-polarised macrophages promote cartilage repair?**

Alexandra Macmillan<sup>1</sup>, Hayat Muhammad<sup>1</sup>, Rawiya Al Hosni<sup>1</sup>, Mohammed Alkhayref<sup>1</sup>, Andrew Hotchen<sup>1</sup>, Eve Robertson-Waters<sup>1</sup>, Estelle Strangmark<sup>1</sup>, Ben Gompels<sup>1</sup>, Jia Hua Wang<sup>1</sup>, Steven McDonnell<sup>1</sup>, Wasim Khan<sup>1</sup>, Menna Clatworthy<sup>3,4</sup>, Mark Birch<sup>1</sup>, Andrew McCaskie<sup>1,2</sup>

<sup>1</sup>Division of Trauma and Orthopaedic Surgery, Department of Surgery, University of Cambridge, UK; <sup>2</sup>Wellcome-MRC Stem Cell Institute, University of Cambridge, UK; <sup>3</sup>Molecular Immunity Unit, University of Cambridge Department of Medicine, Cambridge, UK; <sup>4</sup>Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK

In relation to regenerative therapies in osteoarthritis and cartilage repair, mesenchymal stromal cells (MSCs) have immunomodulatory functions and influence macrophage behaviour. Macrophages exist as a spectrum of pro-(M1) and anti-(M2) inflammatory phenotypic subsets. In the context of cartilage repair, we investigated MSC-macrophage crosstalk, including specifically the priming of cartilage cells by macrophages to achieve a regenerative rather than fibrotic outcome. Human monocytes were isolated from blood cones and differentiated towards M1 and M2 macrophages. Monocytes (Mo), M1 and M2 macrophages were cultured directly and indirectly (trans-well system) with human bone marrow derived MSCs. MSCs were added during M1 polarisation and separately to already induced M1 cells. Outcomes (M1/M2 markers and ligands/receptors) were evaluated using RT-qPCR and flow cytometry. Influence on chondrogenesis was assessed by applying M1 and M2 macrophage conditioned media (CM) sequentially to cartilage derived cells (recapitulating an acute injury environment). RT-qPCR was used to evaluate chondrogenic/fibrogenic gene transcription. The ratio of M2 markers (CD206 or CD163) to M1 markers (CD38) increased when MSCs were added to Mo/M1 macrophages, regardless of culture system used (direct or indirect). Pro-inflammatory markers (including TNF $\beta$ ) decreased. CXCR2 expression by both M1 macrophages and MSCs decreased when MSCs were added to differentiated M1 macrophages in transwell. When adding initially M1 CM (for 12 hours) followed by M2 CM (for 12 hours) sequentially to chondrocytes, there was a significant increase of Aggrecan and Collagen type 2 gene expression and decrease in fibroblastic cell surface markers (PDPN/CD90). Mo/M1 macrophages cultured with MSCs, directly or indirectly, are shifted towards a more M2 phenotype. Indirect culture suggests this effect can occur via soluble signaling mediators. Sequential exposure of M1CM followed by M2CM to chondrocytes resulted in increased chondrogenic and reduced fibrotic gene expression, suggesting that an acute pro-inflammatory stimulus may prime chondrocytes before repair.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Prevalence and severity of intra-discal vacuum phenomenon in a normal aging population

Habash M<sup>1,2</sup>, Cawley D<sup>2,3</sup>, Devitt A<sup>3</sup>

<sup>1</sup>University Hospital Galway, Galway, Ireland. Affiliated to National University of Ireland, Galway (NUIG); <sup>2</sup>Mater Misericordiae University Hospital, Dublin, Ireland. Affiliated to University College Dublin, Ireland; <sup>3</sup>University Hospital Galway, Galway, Ireland. Affiliated to NUIG

Intra-Discal Vacuum Phenomenon (IDVP) represents an intradiscal nitrogen gas accumulation where a cavity opens in a supine position, lowering intra-discal pressure and generating a bubble. IDVP has been observed in up to 20% of elderly patients and reported in almost 50% of chronic LBP patients. With a highly accurate detection on CT, its significance lacks clarity and consideration within normative data. IDVP occurs with patterns of lumbar and/or lumbopelvic morphology and associated diagnoses. Over-60s population based sample of 2020 unrelated CT abdomen scans without acute spinal presentations, with sagittal reconstructions, inclusive of T12 to femoral heads, were analyzed for IDVP and pelvic incidence (PI). Subjects with diagnostic morphological associations of the lumbar spine, including previous fracture, autofusion, transitional vertebra and listhesis, were selected out and analyzed separately. Subjects were then equally grouped into low, medium and high PI. Prevalence of lumbar spine IDVP is 41.3%. 125 cases were excluded. 1603 subjects yielded 663 IDVP. This was increased in severity towards the lumbosacral junction (L1L2 9.4%, L2L3 10.9%, L3L4 13.7%, L4L5 19.9%, L5S1 28.5%) and those with low PI, while distribution was more even with high PI. 292 had positive diagnostic associations, which were more likely to occur at the level of isthmic spondylolisthesis, adjacent to a previous fracture or suprajacent to lumbosacral transitional vertebra ( $p < 0.05$ ).

This study has identified normative values for prevalence and severity of IDVP in a normal aging population. Morphological patterns that influence the pattern of IDVP such as pelvic incidence and diagnostic associations provide novel insights to the function of the aging spine.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Evaluation of clinical outcomes and accuracy in transpedicular screw fixation using intraoperative CT-guided navigation for lumbar spondylolisthesis**Gianluca Vadalà <sup>1,2</sup>, Giuseppe Francesco Papalia <sup>1,2</sup>, Fabrizio Russo <sup>1,2</sup>, Luca Ambrosio <sup>1,2</sup>, Domenico Franco <sup>1,2</sup>, Paolo Brigato <sup>1,2</sup>, Rocco Papalia <sup>1,2</sup>, Vincenzo Denaro <sup>1,2</sup>

<sup>1</sup>Operative Research Unit of Orthopaedic and Trauma Surgery, Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy; <sup>2</sup>Research Unit of Orthopaedic and Trauma Surgery, Department of Medicine and Surgery, Università Campus Bio-Medico di Roma, Rome, Italy

The use of intraoperative navigation and robotic surgery for minimally invasive lumbar fusion has been increasing over the past decade <sup>1,2</sup>. The aim of this study is to evaluate postoperative clinical outcomes, intraoperative parameters, and accuracy of pedicle screw insertion guided by intraoperative navigation in patients undergoing lumbar interbody fusion for spondylolisthesis. Patients who underwent posterior lumbar fusion interbody using intraoperative 3D navigation since December 2021 were included. Visual Analogue Scale (VAS), Oswestry Disability Index (ODI), and Short Form Health Survey-36 (SF-36) were assessed preoperatively and postoperatively at 1, 3, and 6 months. Screw placement accuracy, measured by Gertzbein and Robbins classification<sup>3</sup>, and facet joint infringement, measured by Yson classification<sup>4</sup>, were assessed by intraoperative Cone Beam CT scans performed at the end of instrumentation. Finally, operation time, intraoperative blood loss, hospital stay, and screw insertion time were evaluated. This study involved 50 patients with a mean age of 63.7 years. VAS decreased from 65.8±23 to 20±22 (p<.01). ODI decreased from 35.4%±15 to 11.8%±14 (p<.01). An increase of SF-36 from 51.5±14 to 76±13 (p<.01) was demonstrated. The accuracy of “perfect” and “clinically acceptable” pedicle screw fixation was 89.5% and 98.4%, respectively. Regarding facet violation, 96.8% of the screws were at grade 0. Finally, the average screw insertion time was 4.3±2 min, hospital stay was 4.2±0.8 days, operation time was 205±53 min, and blood loss was 169±107 ml. Finally, a statistically significant correlation of operation time with hospital stay, blood loss and placement time per screw was found. We demonstrated excellent results for accuracy of pedicle screw fixation and violation of facet joints. VAS, ODI and SF-36 showed statistically significant improvements from the control at one month after surgery.

Navigation with intraoperative 3D images represents an effective system to improve operative performance in the surgical treatment of spondylolisthesis.

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## 27-29 SEPTEMBER | PORTO, PORTUGAL

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**Calcium sulfate/hydroxyapatite mediated controlled co-delivery of BMP-2 and zoledronic acid enhances spinal fusion**

Xinggui Tian<sup>1,2</sup>, Corina Vater<sup>1,2</sup>, Deepak B. Raina<sup>3</sup>, Lisa Findeisen<sup>1,2</sup>, Lucas-Maximilian Matuszewski<sup>1,2</sup>, Magnus Tägil<sup>3</sup>, Lars Lidgren<sup>3</sup>, Klaus-Dieter Schaser<sup>1</sup>, Alexander C. Disch<sup>1</sup>, Stefan Zwillingenberger<sup>1, 2</sup>

<sup>1</sup>University Center of Orthopaedic, Trauma and Plastic Surgery, University Hospital Carl Gustav Carus at Technische Universität Dresden, 01307 Dresden, Germany; <sup>2</sup>Center for Translational Bone, Joint and Soft Tissue Research, University Hospital Carl Gustav Carus at Technische Universität Dresden, 01307 Dresden, Germany; <sup>3</sup>Lund University, Faculty of Medicine, Department of Clinical Sciences Lund, Orthopaedics, Lund 22185, Sweden

Although bone morphogenetic protein 2 (BMP-2) has been FDA-approved for spinal fusion for decades, its disadvantages of promoting osteoclast-based bone resorption and suboptimal carrier (absorbable collagen sponge) leading to premature release of the protein limit its clinical applications. Our recent study showed an excellent effect on bone regeneration when BMP-2 and zoledronic acid (ZA) were co-delivered based on a calcium sulphate/hydroxyapatite (CaS/HA) scaffold in a rat critical-size femoral defect model [1]. Therefore, the aim of this study was to evaluate whether local application of BMP-2 and ZA released from a CaS/HA scaffold is favorable for spinal fusion. We hypothesized that CaS/HA mediated controlled co-delivery of rhBMP-2 and ZA could show an improved effect in spinal fusion over BMP-2 alone. 120, 8-week-old male Wistar rats (protocol no. 25-5131/474/38) were randomly divided into six groups in this study (CaS/HA, CaS/HA + BMP-2, CaS/HA + systemic ZA, CaS/HA + local ZA, CaS/HA + BMP-2 + systemic ZA, CaS/HA + BMP-2 + local ZA). A posterolateral spinal fusion at L4 to L5 was performed bilaterally by implanting group-dependent scaffolds. At 3 weeks and 6 weeks, 10 animals per group were euthanized for  $\mu$ CT, histological staining, or mechanical testing.  $\mu$ CT and histological results showed that the CaS/HA + BMP-2 + local ZA group significantly promoted bone regeneration than other treated groups. Biomechanical testing showed breaking force in CaS/HA + BMP + local ZA group was significantly higher than other groups at 6 weeks. In conclusion, the CaS/HA-based biomaterial functionalized with bioactive molecules rhBMP-2 and ZA enhanced bone formation and concomitant spinal fusion outcome

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Depression influences postoperative pain and disability in patients undergoing lumbar spinal fusion: preliminary data of a prospective study

Giorgia Petrucci<sup>1</sup>, Giuseppe Francesco Papalia<sup>2</sup>, Fabrizio Russo<sup>2</sup>, Luca Ambrosio<sup>1</sup>, Rocco Papalia<sup>2</sup>, Gianluca Vadalà<sup>2</sup>, Vincenzo Denaro<sup>2</sup>

<sup>1</sup>Department of Medicine and Surgery, Università Campus Bio-Medico di Roma, Via Alvaro del Portillo, 21 – 00128 Roma, Italy. Research Unit of Orthopaedic and Trauma Surgery; <sup>2</sup>Fondazione Policlinico Universitario Campus-Medico, Via alvaro del Portillo, 200 – 00128 Roma, Italy, Operative Research Unit of Orthopaedic and Trauma Surgery

Chronic low back pain (CLBP) is the most common cause of disability worldwide, and lumbar spine fusion (LSF) is often chosen to treat pain caused by advanced degenerative disease when clinical treatment failed<sup>1</sup> certain cases, the post-surgical outcomes are not what was expected. Several studies highlight how important are. In psychological variables during the postoperative spine surgery period. The aim of this study is to assess the role of preoperative depression on postoperative clinical outcomes. We included patients who underwent LSF since December 2021. Preoperative depression was assessed administering Beck Depression Inventory questionnaire (BDI). And pain and disability were evaluated at 1, 3, and 6 months, administering respectively Visual Analogic Scale (VAS) and Oswestry Disability Index (ODI). As statistical analysis Mann-Whitney test was performed. We included 46 patients, 20 female (43,5%) and 26 male (56,5%) with an average age of 64,2. The population was divided in two groups, fixing the BDI cut-off point at 10. Patients with BDI < 10 points (N=28) had normal mental health status, instead patients with BDI > 10 points (N=16) had depressive disorders. At 3 months patients with healthy mental status reported statistically significant reduction of pain (U = 372,5, p = .006) and improvement of disability but without statistical significance (U = 318, p = 0,137). At 6 months patients without psychological disease reported statistically significant reduction of pain (U = 342, p = 0,039) and disability (U = 372,5, p = 0,006).

This study demonstrates the correlation between pre-existing depressive state and poorer clinical outcomes after spine surgery. These results are consistent with the literature. Therefore, during the surgical decision making it is crucial to take psychological variables into account in order to predict the results after surgery and inform patients on the potential influence of mental status.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Advancing Muscular Dystrophy Therapy Evaluation through Muscle-on-Chip Devices**

Juan M. Fernández-Costa<sup>1</sup>, Ainoa Tejedera-Villafranca<sup>1</sup>, María J Ugarte-Orozco<sup>1</sup>, Armando Cortés-Reséndiz<sup>1</sup>, and Javier Ramón-Azcón<sup>1,2</sup>

<sup>1</sup>Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain; <sup>2</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Duchenne muscular dystrophy (DMD) is a prevalent childhood neuromuscular disease characterized by progressive skeletal and cardiac muscle degeneration due to dystrophin protein deficiency. Despite ongoing drug development efforts, no cure exists, with limited success in preclinical studies. To expedite DMD drug development, we introduce an innovative organ-on-a-chip (OOC) platform. This microfluidic device sustains up to six 3D patient-derived skeletal muscle tissues, enabling real-time evaluation of anti-DMD treatments. Our in vitro model recreates myotube integrity loss, a hallmark of DMD, by encapsulating myogenic precursors in a fibrin-composite matrix using a PDMS casting mold. Continuous contractile regimes mimic sarcolemmal instability, monitored through tissue contractibility and Creatine Kinase (CK) levels—an established marker of muscle damage. We further enhance our platform with a nanoplasmonic CK biosensor, enabling rapid, label-free, and real-time sarcolemmal damage assessment. Combining these elements, our work demonstrates the potential of OOCs in accelerating drug development for DMD and similar neuromuscular disorders.

**27-29 SEPTEMBER | PORTO, PORTUGAL****3D Biomimetic Constructs Steer Stem Cell Commitment by Synergistic Modulation of Biophysical Cues and Growth Factor Signaling**Simão P. B. Teixeira<sup>1,2</sup>, Alberto Pardo<sup>1,2,3</sup>, Syeda M. Bakht<sup>1,2</sup>, Manuel Gomez-Florit<sup>1,2</sup>, Rui L. Reis<sup>1,2</sup>, Manuela E. Gomes<sup>1,2</sup>, Rui M. A. Domingues<sup>1,2</sup>

<sup>1</sup>3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark – Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco GMR, Portugal.

<sup>2</sup>ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal.

<sup>3</sup>Colloids and Polymers Physics Group, Particle Physics Department and Health Research Institute, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain.

Tendon diseases are prevalent health concerns for which current therapies present limited success, in part due to the intrinsically low regenerative ability of tendons. Therefore, tissue engineering presents a potential to improve this outcome. Here, we hypothesize that a concurrent control over both biophysical and biochemical stimuli will boost the tenogenic commitment of stem cells, thus promoting regeneration. To achieve this, we combine molecularly imprinted nanoparticles (MINPs), which act as artificial amplifiers for endogenous growth factor (GF) activity,<sup>1</sup> with bioinspired anisotropic hydrogels<sup>2</sup> to manufacture 3D tenogenic constructs.

MINPs were solid phase-imprinted using a TGF- $\beta$ 3 epitope as template and their affinity for the target was assessed by SPR and dot blot. Magnetically-responsive microfibers were produced by cryosectioning electrospun meshes containing iron oxide nanoparticles. The constructs were prepared by encapsulating adipose tissue-derived stem cells (ASCs), microfibers, and MINPs within gelatin hydrogels, while aligning the microfibers with an external magnetostatic field during gelation. This allows an effective modulation of hydrogel fibrillar topography, mimicking the native tissue's anisotropic architecture. Cell responses were analyzed by multiplex immunoassay, quantitative polymerase chain reaction, and immunocytochemistry.

MINPs showed an affinity for the template comparable to monoclonal antibodies. Encapsulated ASCs acquired an elongated shape and predominant orientation along the alignment direction. Cellular studies revealed that combining MINPs with aligned microfibers increased TGF- $\beta$  signaling via non-canonical Akt/ERK pathways and upregulated tendon-associated gene expression, contrasting with randomly oriented gels. Immunostaining of tendon-related proteins presented analogous outcomes, corroborating our hypothesis.

Our results thus demonstrate that microstructural cues and biological signals synergistically direct stem cell fate commitment, suggesting that this strategy holds potential for improving tendon healing and might be adaptable for other biological tissues. The proposed concept highlights the GF-sequestering ability of MINPs which

## 27-29 SEPTEMBER | PORTO, PORTUGAL

allows a cost-effective alternative to recombinant GF supplementation, potentially decreasing the translational costs of tissue engineering strategies.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****The potential of Magnesium-based micro cylinder for cartilage and bone regeneration utilizing an *in vitro* Osteoarthritis model**Helmholz H<sup>1</sup>, Mavila Chathoth B<sup>1</sup>, Angrisani N<sup>2</sup>, Reifenrath J<sup>2</sup>, Willumeit-Römer R<sup>1</sup>

<sup>1</sup>Helmholtz-Zentrum Hereon, Institute of Metallic Biomaterials, Geesthacht, Germany  
<sup>2</sup>Hannover Medical School, Clinic for Orthopedic Surgery, NIFE – Lower Saxony Center for Biomedical Engineering, Implant Research and Development, Hannover, Germany

Osteoarthritis (OA) is an inflammatory disease affecting the complete synovial joint including the cartilage layer and the subchondral bone plate. Due to the multifactorial causes and the not yet completely resolved molecular mechanisms, it lacks a gold standard treatment to mitigate OA. Hence, biomaterials capable of delaying or preventing OA are a promising alternative or supplement to antiphlogistic and surgical interventions. Magnesium (Mg) and its alloys are among the promising biomaterials with osteoinductive effects. This work investigated the impact of Mg micro cylinders (length ≈ of 1.0 mm and width of 0.5 mm) *in vitro*, in favoring joint regeneration together with preventing OA progression. Therefore, a mesenchymal stem cell line (SCP-1) was applied in order to assess the compatibility of the degradable material. Furthermore, an *in vitro* OA model utilizing SCP-1 cells based on the supplementation of the cytokines; IL-1 $\beta$ , TNF- $\alpha$  was established and disclosed the capability of Mg microparticles in differentiating SCP-1 cells into chondrogenic and osteogenic lineages proven through extracellular matrix staining and gene marker analysis. A concentration above 10 mM revealed a reduction in the cell viability by 50 %. An increase in the expression of collagens especially and proteoglycans (COL2A1, Aggrecan) as extracellular matrix proteins as well as an increase in osteogenic marker (ALP, BMP2) favoring the mineralization process were observed. The inflammatory condition reduced the viability and productivity of the applied stem cell line. However, the application of Mg microparticles induced a cell recovery and reduction of inflammation marker such as MMP1 and IL6. The cytocompatible and the ability of Mg microparticles in supporting bone and cartilage repair mechanisms *in vitro* even under inflammatory conditions make biodegradable Mg microparticles a suitable implant material to treat OA therapy.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Antimicrobial nanogels to prevent Orthopedic Device-Related Infections

B. Costa<sup>1,2,3</sup>, P. Alves<sup>1,2,3</sup>, D. Fonseca<sup>1,2,3</sup>, F. Campos<sup>1,2</sup>, Ana C. Monteiro<sup>1,2</sup>, R. Pereira<sup>1,2,4</sup>, F. Costa<sup>1,2</sup>, P. Gomes<sup>5</sup>, Guillermo Martínez-de-Tejada<sup>6,7</sup>, C. Monteiro<sup>1,2</sup>, M.C.L. Martins<sup>1,2,4</sup>

<sup>1</sup>i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; <sup>2</sup>INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Portugal; <sup>3</sup>FEUP – Faculdade de Engenharia, Universidade do Porto, Portugal; <sup>4</sup>ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal; <sup>5</sup>CIQ-UP – Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Portugal; <sup>6</sup>Department of Microbiology and Parasitology, University of Navarra, Spain; <sup>7</sup>Navarra Institute for Health Research (IdiSNA), Pamplona, Spain

Orthopedic Device-Related Infections (ODRIs) are a major medical challenge, particularly due to the involvement of biofilm-encased and multidrug-resistant bacteria [1]. Current treatments, based on antibiotic administration, have proven to be ineffective. Consequently, there is a need for antibiotic-free alternatives. Antimicrobial peptides (AMPs) are a promising solution due to their broad-spectrum of activity, high efficacy at very low concentrations, and low propensity to induce resistance [2]. We aim to develop a new AMP-based chitosan nanogel to be injected during orthopedic device implantation to prevent ODRIs. Chitosan was functionalized with norbornenes (NorChit) through the reaction with carbic anhydride and then, a cysteine-modified AMP, Dhvar5, a peptide with potent antibacterial activity, even against methicillin-resistant *Staphylococcus aureus* (MRSA), was covalently conjugated to NorChit (NorChit-Dhvar5), through a thiol-norbornene photoclick chemistry (UV= 365 nm) [3]. For NorChit-Dhvar5 nanogels production, the NorChit-Dhvar5 solution (0.15% w/v) and Milli-Q water were injected separately into microfluidic system. The nanogels were characterized regarding size, concentration, and shape, using Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA) and Dynamic light scattering (DLS). The nanogels antibacterial properties were assessed in Phosphate Buffer (PBS) for 6 h, against four relevant microorganisms (*Pseudomonas aeruginosa*, *S. aureus* and MRSA, and in Muller-Hinton Broth (MHB), 50% (v/v) in PBS, supplemented with human plasma (1% (v/v)), for 6 and 24 h against MRSA. The obtained NorChit-Dhvar5 nanogels, presented a round-shaped and ~100 nm. NorChit-Dhvar5 nanogels in a concentration of 10<sup>10</sup> nanogels/mL in PBS were capable of reducing the initial inoculum of *P. aeruginosa* by 99%, *S. aureus* by 99%, and MRSA by 90%. These results were corroborated by a 99% MRSA reduction, after 24 h in medium. Furthermore, NorChit-Dhvar5 nanogels do not demonstrate signs of cytotoxicity against MC3T3-E1 cells (a pre-osteoblast cell line)

**27-29 SEPTEMBER | PORTO, PORTUGAL**

after 14 days, having high potential to prevent antibiotic-resistant infection in the context of ODRIs.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Development of a Bone-On-A-Chip microfluidic device as a model of osteoporosis**

Lipreri M.V.<sup>1</sup>, Pasquarelli A.<sup>1</sup>, Scelfo D.<sup>2</sup>, Baldini N.<sup>1,2</sup>, Avnet S.<sup>1</sup>

<sup>1</sup>Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy.; <sup>2</sup>Biomedical Science and Technologies Lab, IRCSS Istituto Ortopedico Rizzoli, Bologna, Italy.

Osteoporosis is a progressive, chronic disease of bone metabolism, characterized by decreased bone mass and mineral density, predisposing individuals to an increased risk of fractures. The use of animal models, which is the gold standard for the screening of anti-osteoporosis drugs, raises numerous ethical concerns and is highly debated because the composition and structure of animal bones is very different from human bones. In addition, there is currently a poor translation of pre-clinical efficacy in animal models to human trials, meaning that there is a need for an alternative method of screening and evaluating new therapeutics for metabolic bone disorders, *in vitro*.

The aim of this project is to develop a 3D Bone-On-A-Chip that summarizes the spatial orientation and mutual influences of the key cellular components of bone tissue, in a citrate and hydroxyapatite-enriched 3D matrix, acting as a 3D model of osteoporosis. To this purpose, a polydimethylsiloxane microfluidic device was developed by CAD modelling, stereolithography and replica molding. The device is composed by two layers: (i) a bottom layer for a 3D culture of osteocytes embedded in an osteomimetic collagen-enriched matrigel matrix with citrate-doped hydroxyapatite nanocrystals, and (ii) a upper layer for a 2D perfused co-culture of osteoblasts and osteoclasts seeded on a microporous PET membrane.

Cell vitality was evaluated via live/dead assay. Bone deposition and bone resorption was analysed respectively with ALP, Alizarin RED and TRACP staining. Osteocytes dendrite expression was evaluated via immunofluorescence. Subsequently, the model was validated as drug screening platform inducing osteocytes apoptosis and administrating standard anti-osteoporotic drugs.

This device has the potential to substitute or minimize animal models in pre-clinical studies of osteoporosis, contributing to pave the way for a more precise and punctual personalized treatment.

## **Implants: don't let the bacteria grow!**

Wildemann, Britt

Experimental Trauma Surgery, Jena University Hospital, Germany

The Global Burden of Disease Study 2019 showed a 33.4% increase in fractures and a 65.3% increase in Years lived with disability (YLD) since 1990. Although the overall rate of fracture related infection (FRI) is low, it increases to 30% in complex fractures. In addition, the implantation of foreign materials, such as fracture stabilizing implants, decreases the number of bacteria needed to cause an infection. Then, when infections do occur, they are difficult to treat and often require multiple surgeries to heal. The bacteria can persist in the canaliculi of the bony tissue, in cells, in a biofilm on material or necrotic bone or in abscess communities. In the last decades, different approaches have been pursued to modify biomaterials as well as implant surface and to develop antimicrobial surfaces or local drug release strategies. This talk will give an introduction to the problem of bony and implant associated infections and presents the development and preclinical (as well as clinical) studies of two approaches for local drug delivery.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Customized coatings functionalized with metals to prevent prostheses infections caused by multidrug-resistant bacteria

Daniele Ghezzi<sup>1,2</sup>, Maria Sartori<sup>3</sup>, Marco Boi<sup>2</sup>, Matteo Montesissa<sup>4</sup>, Enrico Sassoni<sup>5</sup>, Milena Fini<sup>6</sup>, Nicola Baldini<sup>2,4</sup>, Martina Cappelletti<sup>1</sup>, Gabriela Graziani<sup>2,7</sup>

<sup>1</sup>University of Bologna, Department of Pharmacy and Biotechnology, via Irnerio 42, 40126, Bologna, Italy; <sup>2</sup>IRCCS Istituto Ortopedico Rizzoli, Biomedical Science and Technologies and Nanobiotechnology Lab, via di Barbiano 1/10, 40136, Bologna, Italy; <sup>3</sup>IRCCS Istituto Ortopedico Rizzoli, Surgical Sciences and Technologies, via di Barbiano 1/10, 40136, Bologna, Italy; <sup>4</sup>University of Bologna, Department of Biomedical and Neuromotor Sciences, via Massarenti 9, 40128, Bologna, Italy; <sup>5</sup>University of Bologna, Department of Civil, Chemical, Environmental and Materials Engineering, via Terracini 28, 40131, Bologna, Italy; <sup>6</sup>IRCCS Istituto Ortopedico Rizzoli, Scientific Direction, via di Barbiano 1/10, 40136, Bologna, Italy; <sup>7</sup>Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "G. Natta", Via Luigi Mancinelli 7, 20131, Milan, Italy

Prosthetic joint infections represent complications connected to the implantation of biomedical devices, they have high incidence, interfere with osseointegration, and lead to a high societal burden. The microbial biofilm, which is a complex structure of microbial cells firmly attached to a surface, is one of the main issues causing infections. Biofilm-forming bacteria are acquiring more and more resistances to common clinical treatments due to the abuse of antibiotics administration. Therefore, there is increasing need to develop alternative methods exerting antibacterial activities against multidrug-resistant biofilm-forming bacteria. In this context, metal-based coatings with antimicrobial activities have been investigated and are currently used in the clinical practice. However, traditional coatings exhibit some drawbacks related to the insufficient adhesion to the substrate, scarce uniformity and scarce control over the toxic metal release reducing their efficacy. Here, we propose the use of antimicrobial silver-based nanostructured thin films to discourage bacterial infections. Coatings are obtained by Ionized Jet Deposition, a plasma-assisted technique that permits to manufacture films of submicrometric thickness having a nanostructured surface texture, allow tuning silver release, and avoid delamination. To mitigate interference with osseointegration, here silver composites with bone apatite and hydroxyapatite were explored. The antibacterial efficacy of silver films was tested *in vitro* against gram-positive and gram-negative species to determine the optimal coatings characteristics by assessing reduction of bacterial viability, adhesion to substrate, and biofilm formation. Efficacy was tested in an *in vivo* rabbit model, using a multidrug-resistant strain of *Staphylococcus aureus* showing significant reduction of the bacterial load on the silver prosthesis both when coated with the metal only (>99% reduction) and when in combination with bone apatite (>86% reduction). These

**27-29 SEPTEMBER | PORTO, PORTUGAL**

studies indicate that IJD films are highly tunable and can be a promising route to overcome the main challenges in orthopedic prostheses.

27-29 SEPTEMBER | PORTO, PORTUGAL

## From novel coating strategy on biodegradable Mg based orthopaedic implant material towards clinical trial

Rachel W, Li<sup>1,2</sup>, Jizhou Zheng<sup>1</sup>, Paul N. Smith<sup>2</sup>, Xiaobo Chen<sup>3</sup>

<sup>1</sup>Australian National University, School of Medicine and Psychology, Canberra City, ACT 2601, Australia; <sup>2</sup>Department of Surgery, ACT Health, Canberra, ACT2601, Australia; <sup>3</sup>School of Engineering, RMIT University, Melbourne, Australia

Device-associated bacterial infections are a major and costly clinical challenge. This project aimed to develop a smart new biomaterial for implants that helps to protect against infection and inflammation, promote bone growth, and is biodegradable. Gallium (Ga) doped strontium-phosphate was coated on pure Magnesium (Mg) through a chemical conversion process. Mg was distributed in a graduated manner throughout the strontium-phosphate coating GaSrPO<sub>4</sub>, with a compact structure and a Ga-rich surface. We tested this sample for its biocompatibility, effects on bone remodeling and antibacterial activities including *Staphylococcus aureus*, *S. epidermidis* and *E. coli* - key strains causing infection and early failure of the surgical implantations in orthopaedics and trauma.

Ga was distributed in a gradient way throughout the entire strontium-phosphate coating with a compact structure and a gallium-rich surface. The GaSrPO<sub>4</sub> coating protected the underlying Mg from substantial degradation in minimal essential media at physiological conditions over 9 days. The liberated Ga ions from the coatings upon Mg specimens inhibited the growth of bacterial tested. The Ga dopants showed minimal interferences with the SrPO<sub>4</sub> based coating, which boosted osteoblasts and undermined osteoclasts in *in vitro* co-cultures model.

The results evidenced this new material may be further translated to preclinical trial in large animal model and towards clinical trial.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****The course of septic arthritis following anterior cruciate ligament reconstruction: Infectious agents, clinical presentation and treatment: A case-series of 158 patients.**Osama Omar<sup>1</sup>, Jesper Kraus-Schmitz<sup>3</sup>, Björn Barenius<sup>2</sup>, Karl Eriksson<sup>1</sup>, Anders Stålmán<sup>2</sup>

<sup>1</sup>Department of Orthopedics Södersjukhuset, Department of Clinical Science and Education, Södersjukhuset, Karolinska institute, Stockholm; <sup>2</sup>Capio artroclinic, Stockholm Sports Trauma Research Center, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm; <sup>3</sup>Department of Orthopedics, Lund University, Skåne university hospital, Malmö

Septic arthritis following anterior cruciate ligament reconstruction (ACLR) is a rare and serious complication. Previous studies have shown that septic arthritis is associated with inferior outcome of ACLR. Despite that, there is no standardized treatment protocol, and the course of the disease has mainly been studied within single institutions with a small number of patients.

The aim of the present study is to describe the course of septic arthritis following ACLR in a large nationwide cohort. The hypothesis was that the clinical presentation of septic arthritis following ACLR varies according to the infectious agent.

The present cohort represents patients with septic arthritis identified in a previous study that analyzed compensation claims reported to the Swedish national insurance company (Löf) in 2005-2014 (1). The diagnosis was confirmed by medical experts at Löf after review of medical records. We conducted a comprehensive analysis of the medical records as well as data available from the Swedish National Knee Ligament Registry (SNKLR) for the study group.

The study involved 158 patients who received compensation due to developing septic arthritis. 94 (59.9%) patients were infected with Coagulase negative staphylococci (CoNS), and 25 patients by Staphylococcus Aureus (S.Aureus) (15.9%). There was a significant difference between the groups regarding Maximum CRP ( $p < 0.001$ ), and duration between ACLR and first washout operation ( $p < 0.005$ ). S.aureus group had the highest maximum CRP (281) and the shortest duration between ACLR and first washout operation (12 days).

The Clinical presentation of septic arthritis following ACLR can vary according to the agent causing the infection, and low virulent agents are responsible for the majority of the infections. Clinicians need to be aware of these differences and consider them when making diagnosis or treatment decisions.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Visualization of nanoporous silica nanoparticle distribution for implant-directed magnetic drug targeting by $^{68}\text{Ga}$ -labeling and PET/CT

Heidi Harting<sup>1,3</sup>, Andras Polyak<sup>2,3</sup>, Nina Angrisani<sup>1,3</sup>, Timo Herrmann<sup>4</sup>, Nina Ehlert<sup>4</sup>, Jessica Meißner<sup>5</sup>, Michael Willmann<sup>2</sup>, Silav Al-Bazaz<sup>2</sup>, Tobias L Ross<sup>2</sup>, Jens P Bankstahl<sup>2</sup>, Janin Reifenrath<sup>1,3</sup>

<sup>1</sup>Hannover Medical School, Clinic for Orthopedic Surgery, Carl-Neuberg-Straße 1, 30625 Hannover, Germany; <sup>2</sup>Hannover Medical School, Department of Nuclear Medicine, Carl-Neuberg-Straße 1, 30625 Hannover, Germany; <sup>3</sup>NIFE – Lower Saxony Centre for Biomedical Engineering, Implant Research and Development, Stadtfeldamm 34, D-30625 Hannover, Germany; <sup>4</sup>Institute for Inorganic Chemistry, Leibniz University Hannover, Callinstraße 9, D-30167 Hannover, Germany; <sup>5</sup>Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Foundation, Buenteweg 17, D-30559 Hannover, Germany

In orthopedic surgery, implant infections are a serious issue and difficult to treat. The aim of this study was to use superparamagnetic nanoporous silica nanoparticles (MNPSNP) as candidates for directed drug delivery. Currently, short blood circulation half-life due to interactions with the host's immune system hinder nanoparticles in general from being clinically used. PEGylation is an approach to reduce these interactions and to enhance blood circulation time. The effect of PEGylation of the used  $^{68}\text{Ga}$ -labelled MNPSNP on the distribution and implant accumulation was examined by PET/CT imaging and gamma counting in an implant mouse model.

Female Balb/c mice (n=24) received a magnetic implant subcutaneously on the left and a titanium implant on the right hind leg. On day one, 12 of these mice received an additional clodronate<sup>®</sup>-injection for macrophage depletion. On the second postoperative day, mice were anaesthetized and MNPSNP (native or PEGylated) injected intravenously, followed by a dynamic PET-scan over 60 minutes, a CT- and a static PET-scan at 120 min. As control, 12 mice received only  $^{68}\text{Ga}$ -MNPSNP (native or PEGylated). Gamma counting of inner organs, urine, blood and implant area was performed as further final analysis.

Although PEGylation of the nanoparticles already resulted in lower liver uptakes, both variants of  $^{68}\text{Ga}$ -labeled MNPSNP accumulated in liver and spleen. Combination of PEGylation with clodronate<sup>®</sup>-injection led to a highly significant effect whereas clodronate<sup>®</sup>-injection alone could not reveal significant differences. In gamma counting, a significantly higher %I.D./g was found for the tissue surrounding the magnetic implants compared to the titanium control, although in a low range. PEGylation and/or clodronate<sup>®</sup>-injection revealed no significant differences regarding nanoparticle accumulation at the implantation site.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

PEGylation increases circulation time, but MNPSNP accumulation at the implant site was still insufficient for treatment of infections. Additional efforts have to further increase circulation time and local accumulation.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Is total hip arthroplasty fixation associated with osteoblasts activity in patients with osteonecrosis of the femoral head? A prospective case-control study**

Eduardo García-Rey<sup>1, 2</sup>, Fátima Pérez-Barragans<sup>1</sup>, Laura Saldaña<sup>2</sup>

<sup>1</sup> Orthopedic Surgery and Trauma Service, La Paz University Hospital, Madrid; <sup>2</sup> IdiPaz, La Paz University Hospital, Madrid

Total hip arthroplasty (THA) outcome in patients with osteonecrosis of the femoral head (ONFH) are excellent, however, there is controversy when compared with those in patients with osteoarthritis (OA). Reduced mineralization capacity of osteoblasts of the proximal femur in patients with ONFH could affect implant fixation.

We asked if THA fixation in patients with ONFH is worse than in those with OA.

We carried out a prospective comparative case (OA)-control (ONFH) study of patients undergoing THA at our hospital between 2017 and 2019. The minimum follow-up was 2 years. Inclusion criteria were patients with uncemented THA, younger than 70 years old, a Dorr femoral type C and idiopathic ONFH. We compared the clinical (Merlé D'Aubigné-Postel score) and radiological results related with implant positioning and fixation. Engh criteria and subsidence were assessed at the immediate postoperative, 12 weeks, 6 months, 12 months and yearly. Osteoblastic activity was determined by mineralization assay on primary cultures of osteoblasts isolated from trabecular bone samples collected from the intertrochanteric area obtained during surgery.

Group 1 (ONFH) included 18 patients and group 2 (OA), 22. Average age was 55.9 years old in group 1 and 61.3 in group 2. ( $p=0.08$ ). There were no differences related with sex, Dorr femoral type or femoral filling. The mean clinical outcome score was 17.1 in group 1 and 16.5 in group 2 ( $p=0.03$ ). There were no cases of dislocation, infection, or revision surgery in this series. There were 5 cases (28%) of femoral stem subsidence greater than 3mm within 6 first months in group 1 and 1 case (4.5%) in group 2 ( $p=0.05$ ).

Although there were no significant differences related to clinical results, bone fixation was slower, and a greater subsidence was observed in patients with ONFH. Greater femoral stem subsidence was associated with a lower capacity for mineral nodule formation in cultured osteoblasts. The surgical technique could influence THA outcome in patients with reduced mineralization capacity of osteoblasts.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Metal-On-Metal hip prosthesis: A retrospective study on 681 implants with a follow-up of between 12 And 22 years**Corrado Ciatti<sup>1</sup>, Fabrizio Quattrini<sup>1</sup>, Chiari Asti<sup>1</sup>, Pietro Maniscalco<sup>1</sup><sup>1</sup>Guglielmo da Saliceto Hospital, AUSL Piacenza, Italy

Previous scientific studies have highlighted how coupling is an important element affecting total hip arthroplasty's survival.

This study aims to evaluate whether metal-on-metal (MOM) coupling could be a statistically significant risk factor.

The data from the regional joint registry (Registro dell'Impiantologia Protesica Ortopedica, RIPO) was used for analysis. The data collection accuracy of this registry was 97.2% in 2017.

We retrospective evaluate all MOM total hip arthroplasties (THAs) implanted in our department between January 01st 2000 and December 31st 2011. We used a control group composed by all other prosthesis implanted in our Department in the same time lapse.

We registered 660 MOM THAs. Mean age of patients was 66.9 years. 603 patients have a >36mm head, while 78 a <36 mm one. Neck modularity was present in half of patients. 676 implants were cementless. We registered 69 revisions, especially due to aseptic mobilization (16 THAs), implant breakage (9 THAs) and periprosthetic fracture (6 THAs).

The MOM THAs overall Kaplan-Meier survival rate was 87.2 at 15 years, and the difference between MOM THAs and other implants two curves is statistically significant ( $p < 0.05$ ). Male sex is a significant risk factors. Further evaluations are in progress to establish the presence of any additional risk factors. We think weight and/or BMI may be included in this category.

Our study confirms the data currently present in the literature regarding a lower survival of metal-on-metal hip prostheses.

The male sex is a statistically significant risk factor ( $p < 0.05$ ), while age, head size and modularity of the prosthetic neck are not statistically significant ( $p > 0.05$ ).

Any new finds will be presented at the congress venue.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Fusionless all pedicle screws posterior deformity correction in AIS immature patients permits restauration of the normal vertebral morphology and removal of the instrumentation once bone maturity is reached**

Jesús Burgos<sup>1</sup>, Gonzalo Mariscal<sup>2</sup>, Luis Miguel Antón-Rodríguez<sup>3</sup>, Ignacio Sanpera<sup>4</sup>, Eduardo Hevia<sup>5</sup>, Vicente García<sup>6</sup>, Carlos Barrios<sup>7</sup>

<sup>1</sup>Spine Unit, Hospital Viamed Fuensanta, Madrid, Spain; <sup>2</sup>School of Doctorate, Valencia Catholic University, Valencia, Spain; <sup>3</sup>Pediatric Orthopedics, Ramon y Cajal Hospital, Madrid, Spain; <sup>4</sup>Pediatric Orthopedics, Hospital Son Espases, Palma de Mallorca, Spain; <sup>5</sup>Spine Unit, Hospital La Fraternidad-Muprespa, Madrid, Spain; <sup>6</sup>Sección de Cirugía de Columna, Hospital Universitario Araba, Vitoria, Spain; <sup>7</sup>Institute for Research on Musculoskeletal Disorders, Valencia Catholic University, Valencia, Spain.

The aim of this study was to report the restauration of the normal vertebral morphology and the absence of curve progression after removal the instrumentation in AIS patients that underwent posterior correction of the deformity by common all screws construct whitout fusion. A series of 36 AIS immature patients (Risser 3 or less) were include in the study. Instrumentation was removed once the maturity stage was complete (Risser 5). Curve correction was assessed at pre and postoperative, before instrumentation removal, just post removal, and more than two years after instrumentation removal. Epiphyseal vertebral growth modulation was assessed by a coronal wedging ratio (WR) at the apical level of the main curve (MC). The mean preoperative coronal Cobb was corrected from  $53.7^{\circ} \pm 7.5$  to  $5.5^{\circ} \pm 7.5^{\circ}$  (89.7%) at the immediate postop. After implants removal (31.0 $\pm$ 5.8 months) the MC was 13.1 $^{\circ}$ . T5–T12 kyphosis showed a significant improvement from 19.0 $^{\circ}$  before curve correction to 27.1 $^{\circ}$  after implants removal ( $p < 0.05$ ). Before surgery, WR was 0.71 $\pm$ 0.06, and after removal WR was 0.98 $\pm$ 0.08 ( $p < 0.001$ ). At the end of follow-up, the mean sagittal range of motion (ROM) of the T12-S1 segment was 51.2 $\pm$ 21.0 $^{\circ}$ . SRS-22 scores improved from 3.31 $\pm$ 0.25 preoperatively to 3.68 $\pm$ 0.25 at final assessment ( $p < 0.001$ ). In conclusion, fusionless posterior approach using a common all pedicle screws construct correct satisfactory scoliotic main curves and permits removal of the instrumentation once the bone maturity is reached. The final correction was highly satisfactory and an acceptable ROM of the previously lower instrumented segments was observed.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Transcriptomic analysis in trabecular bone of patients with idiopathic osteonecrosis of the femoral head**Saldaña L.<sup>1,2</sup>, N. Vilaboa<sup>1,2</sup>, E. García-Rey<sup>2,3</sup>

<sup>1</sup>Hospital Universitario La Paz-IdiPAZ, Madrid, Spain; <sup>2</sup>Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Spain; <sup>3</sup>Departamento de Cirugía Ortopédica y Traumatología, Hospital Universitario La Paz-IdiPAZ, Madrid, Spain

The pathophysiological basis of alterations in trabecular bone of patients with osteonecrosis of the femoral head (ONFH) remains unclear. ONFH has classically been considered a vascular disease with secondary changes in the subchondral bone. However, there is increasing evidence suggesting that ONFH could be a bone disease, since alterations in the functionality of bone tissue distant from the necrotic lesion have been observed. We comparatively studied the transcriptomic profile of trabecular bone obtained from the intertrochanteric region of patients with ONFH without an obvious aetiological factor, and patients with osteoarthritis (OA) undergoing total hip replacement in our Institution. To explore the biological processes that could be affected by ONFH, we compared the transcriptomic profile of trabecular bone from the intertrochanteric region and the femoral head of patients affected by this condition. Differential gene expression was studied using an Affymetrix microarray platform. Transcriptome analysis showed a differential signature in trabecular bone from the intertrochanteric region between patients with ONFH and those with OA. The gene ontology analyses of the genes overexpressed in bone tissue of patients with ONFH revealed a range of enriched biological processes related to cell adhesion and migration and angiogenesis. In contrast, most downregulated transcripts were involved in cell division. Trabecular bone in the intertrochanteric region and in the femoral head also exhibited a differential expression profile. Among the genes differentially expressed, we highlighted those related with cytokine production and immune response. This study identified a set of differently expressed genes in trabecular bone of patients with idiopathic ONFH, which might underlie the pathophysiology of this condition.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Biomechanical and radiographic assessment of an ALIF stand-alone device with integrated screws and angular-stable locking plate at L5/S1 and its association to lumbar Bone Mineral Density**

A. Jacob<sup>1</sup>, M. Heumann<sup>1</sup>, I. Zderic<sup>1</sup>, P. Varga<sup>1</sup>, J. Caspar<sup>1</sup>, S. Lauterborn<sup>1</sup>, D. Haschtmann<sup>2</sup>, T. Fekete<sup>2</sup>, B. Gueorguiev<sup>1</sup>, M. Loibl<sup>2</sup>

<sup>1</sup>Biomedical Department, AO Research Institute Davos, Davos, Switzerland;

<sup>2</sup>Department of Spine Surgery, Schulthess Clinic, Zurich, Switzerland

Stand-alone anterior lumbar interbody fusion (ALIF) provides the opportunity to avoid supplemental posterior fixation. This may reduce morbidity and complication rate, which is of special interest in patients with reduced bone mineral density (BMD). This study aims to assess immediate biomechanical stability and radiographic outcome of a stand-alone ALIF device with integrated screws in specimens of low BMD.

Eight human cadaveric spines (L4-sacrum) were instrumented with SynFix-LR™ (DePuy Synthes) at L5/S1. Quantitative computed tomography was used to measure BMD of L5 in AMIRA. Threshold values proposed by the American Society of Radiology 80 and 120 mg CaHa/mL were used to differentiate between Osteoporosis, Osteopenia, and normal BMD. Segmental lordosis, anterior and posterior disc height were analysed on pre- and postoperative radiographs (Fig 1). Specimens were tested intact and following instrumentation using a flexibility protocol consisting of three loading cycles to  $\pm 7.5$  Nm in flexion-extension, lateral bending, and axial rotation. The ranges of motion (ROM) of the index level were assessed using an optoelectronic system.

BMD ranged 58–181mg CaHA/mL. Comparison of pre- and postoperative radiographs revealed significant increase of L5/S1 segmental lordosis (mean 14.6°, SD 5.1,  $p < 0.001$ ) and anterior disc height (mean 5.8mm, SD 1.8,  $p < 0.001$ ), but not posterior disc height. ROM of 6 specimens was reduced compared to the intact state. Two specimens showed destructive failure in extension. Mean decrease was most distinct in axial rotation up to 83% followed by flexion-extension.

ALIF device with integrated screws at L5/S1 significantly increases segmental lordosis and anterior disc height without correlation to BMD. Primary stability in the immediate postoperative situation is mostly warranted in axial rotation. The risk of failure might be increased in extension for some patients with reduced lumbar BMD, therefore additional posterior stabilization could be considered.

**The challenges of stem cell therapy for the treatment of tendinopathy in the clinic - lessons from a large animal model**

Roger K.W. Smith

The Royal Veterinary College, London, UK

Stem cells represent an exciting biological therapy for the management of many musculoskeletal tissues that suffer degenerative disease and/or where the reparative process results in non-functional tissue ('failed healing'). The original hypothesis was that implanted cells would differentiate into the target tissue cell type and synthesise new matrix. However, there has been little evidence that this happens in live animals compared to the laboratory, and more recent theories have focussed on the immunomodulatory effects via the release of paracrine factors that can still improve the outcome, especially since inflammation is now considered one of the central processes that drive poor tendon healing. Because of the initial 'soft' regulatory environment for the use of stem cells in domestic mammals, bone and fat-derived stem cells quickly established themselves as a useful treatment for naturally occurring musculoskeletal diseases in the horse more than 20 years ago (Smith, Korda et al. 2003). Since the tendinopathy in the horse has many similarities to human tendinopathy, we propose that the following challenges and, the lessons learnt, in this journey are highly relevant to the development of stem cells therapies for human tendinopathy:

1. Source – while MSCs can be recovered from many tissues, the predominant sources for autologous MSCs have been bone and fat. Other sources, including blood, amnion, synovium, and dental pulp have also been commercialised for allogenic treatments.
2. Preparation – *ex vivo* culture requires transport from a licensed laboratory while 'minimally manipulated' preparations can be prepared patient-side. Cells also need a vehicle for transport and implantation.
3. Delivery – transport of cells from the laboratory to the clinic for autologous *ex vivo* culture techniques; implantation technique (usually by ultrasound-guided injection to minimise damage to the cells (or, more rarely, incorporated into a scaffold). They can also be delivered by regional perfusion via venous or arterial routes.
4. Retention – relatively poor although small numbers of cells do survive for at least 5 months. Immediate loss to the lungs if the cells are administered via vascular routes. Synovially administered cells do not engraft into tendon.
5. Adverse effects – very safe although needle tracts often visible (but do not seem to adversely affect the outcome). Allogenic cells require careful characterisation for MHC Class II antigens to avoid anaphylaxis or reduced efficacy.

## 27-29 SEPTEMBER | PORTO, PORTUGAL

6. Appropriate injuries to treat – requires a contained lesion when administered via intra-lesional injection. Intrasynovial tendon lesions are more often associated with surface defects and are therefore less appropriate for treatment. Earlier treatment appears to be more effective than delayed, when implantation by injection is more challenging.
7. Efficacy - beneficial effects shown at both tissue and whole animal (clinical outcome) level in naturally-occurring equine tendinopathy using bone marrow-derived autologous MSCs Recent (licenced) allogenic MSC treatment has shown equivalent efficacy while intra-synovial administration of MSCs is ineffective for open intra-synovial tendon lesions.
8. Regulatory hurdles – these have been lighter for veterinary treatments which has facilitated their development. There has been greater regulation of commercial allogenic MSC preparations which have required EMA marketing authorisation.

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**Influence of Mg-Cylinders on bone and cartilage in osteoarthritis**

Angrisani N<sup>1</sup>, Helmholtz H<sup>2</sup>, Windhagen H<sup>1</sup>, von der Ahe C<sup>1</sup>, Scheper V<sup>3</sup>, Willumeit-Römer R<sup>2</sup>, Mavila Chathoth B<sup>2</sup>, Reifenrath J<sup>1</sup>

<sup>1</sup>Hannover Medical School, Clinic for Orthopedic Surgery, NIFE – Lower Saxony Center for Biomedical Engineering, Implant Research and Development, Hannover, Germany; <sup>2</sup>Helmholtz Center Hereon Institute of Metallic Biomaterials, Geesthacht, Germany; <sup>3</sup>Hannover Medical School, Department of Otolaryngology, NIFE – Lower Saxony Center for Biomedical Engineering, Implant Research and Development, Hannover, Germany

There are no efficient treatment options for osteoarthritis (OA) that delay further progression (1). Besides osteoinduction, there is growing evidence of also anti-inflammatory, angiogenetic and neuroprotective effects of biodegradable magnesium-based biomaterials. Their use for the treatment of cartilage lesions in contrast is not well-evaluated yet.

Mg-cylinders were analysed in an *in vitro* and *in vivo* OA model. *In vitro*, SCP-1 stem cell line was analysed under inflammatory conditions and Mg-impact. *In vivo*, small Mg- and WE43 alloy-cylinders (1mm x 0,5mm) were implanted into the subchondral bone of the knee joint of 24 NZW rabbits after establishment of OA. As control, another 12 rabbits received only drill-holes.  $\mu$ CT-scan were performed and assessed for changes in bone volume and density. After euthanasia, cartilage was evaluated macroscopically and histologically after Safranin-O-staining. Furthermore, staining with CD271 directed antibody was performed to assess neuro-reactivity.

*In vitro*, an increased gene expression of extracellular matrix proteins as collagen II or aggrecan even under inflammatory conditions was observed under Mg-impact. *In vivo*,  $\mu$ CT evaluation revealed twice-elevated values for bone volume in femoral condyles with Mg-cylinders compared to controls while density remained unchanged. Cartilage showed no significant differences between the groups. Mg- and WE-samples showed significantly lower levels of CD271+ cells in the cartilage and bone of the operated joints than in non-operated joints, which was not the case in the Drilling-group. Furthermore, bone in operated knees of Drilling-group showed a strong trend to an increase in CD271+ cells compared to both Cylinder-groups. Counting of CD271+ vessels revealed that this difference was attributable to a higher amount of these vessels.

The *in vitro* results indicate a potential cartilage regenerative activity of the degradable Mg-based material. While so far there was no positive effect on the cartilage itself *in vivo*, implantation of Mg-cylinders seemed to reduce pain-mediating vessels.

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**Acknowledgements:** This work is funded by the German Research Foundation (DFG, project number 404534760). We thank Björn Wiese for production of the cylinders.

**Gluing osteochondral fragments: development of a novel dual adhesive preclinical model strategy**Alicja Bojan<sup>1,2</sup>, Philip Procter<sup>3,4</sup> Peyman Karami<sup>5</sup>, Dominique Pioletti<sup>5</sup>

<sup>1</sup>Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; <sup>2</sup>Department of Orthopaedics, Sahlgrenska University Hospital, Mölndal, Sweden; <sup>3</sup>Department of Engineering Sciences, Division of Applied Mat'l. Science, Uppsala University, Uppsala, Sweden; <sup>4</sup>Biomimetic Innovations Ltd, Shannon, Ireland; <sup>5</sup>Laboratory of Biomechanical Orthopedics, EPFL, Lausanne, 1015 Switzerland

The fixation of articular fractures, with many small osteochondral fragments, is a challenging unmet need where a bone adhesive would be a useful adjunct to standard treatments. Whilst there are no such adhesives in current clinical use, preclinical animal models have demonstrated good healing of bone in unloaded models using an adhesive based on phosphoserine modified calcium phosphate cement (PM-CPC). An ex-vivo human bone core model has shown that this adhesive bonds freshly harvested human bone (Bojan et al 2022). To confirm this adhesive is capable of supporting loaded osteochondral fragments a porcine model has been developed initially ex-vivo on the path to an in-vivo study. In this model bone cores, harvested from the medial knee condyle, are glued in place with the adhesive. In-vivo adjacent pairs of bone cores would be replaced with adhesive and a control with conventional pin fixation respectively. As osteochondral bone fragments have both bone and cartilage components, this suggested a dual adhesive strategy in which components designed for each tissue type are used. This concept has been explored in an ex-vivo porcine pilot study presented herewith. At the subchondral bone level, the PM-CPC was used. At the cartilage level, a second adhesive, a methacrylated phosphoserine containing hyaluronic acid (MePHa) hydrogel designed specifically for soft tissues (Karami et al 2021) was applied. This is a challenging model as both adhesives have to be used simultaneously in a wet field. The pilot showed that once the subchondral component is glued in place, the PM-CPC adhesive intruding into the cartilage gap can be removed before applying the cartilage adhesive. This enabled the MePHa adhesive to be injected between the cut cartilage edges and subsequently light-cured. This two-stage gluing method is demanding and an in-vivo pilot is necessary to perfect and prove the operative technique.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**A novel biomimetic bone adhesive: translation from small to large animal preclinical model on the path to first in human use**

Philip Procter<sup>1,2</sup>, Gry Hulsart-Billström<sup>3</sup>, Antoine Alves<sup>4</sup>, Michael Pujari-Palmer<sup>1</sup>, David Wenner<sup>1</sup>, Gerard Insley<sup>1,2</sup>, Håkan Engqvist<sup>1</sup>, Sune Larsson<sup>3</sup>, Benjamin Pippenger<sup>5</sup>, Dieter Bossard<sup>5</sup>

<sup>1</sup>Dept of Engineering Sciences, Division of Applied Mat'l. Science, Uppsala University, Uppsala, Sweden; <sup>2</sup>Biomimetic Innovations Ltd, Shannon, Ireland; <sup>3</sup>Dept of Surgical Sciences, Division of Orthopaedics, Uppsala University, Uppsala, Sweden; <sup>4</sup>NAMSA, Chasse-sur-Rhône, France; <sup>5</sup>School of Dental Medicine, University of Berne, Berne, Switzerland

Surgeons treating fractures with many small osteochondral fragments have often expressed the clinical need for an adhesive to join such fragments, as an adjunct to standard implants. If an adhesive would maintain alignment of the articular surfaces and subsequently heal it could result in improved clinical outcomes. However, there are no bone adhesives available for clinical indications and few pre-clinical models to assess safety and efficacy of adhesive biomaterial candidates. A bone adhesive candidate based on water,  $\alpha$ -TCP and an amino acid phosphoserine was evaluated *in-vivo* in a novel murine bone core model (preliminary results presented EORS 2019) in which excised bone cores were glued back in place and harvested @ 0, 3, 7, 14, 28 and 42 days. Adhesive pull-out strength was demonstrated 0-28 days, with a dip at 14 days increasing to 11.3N maximum. Histology 0-42 days showed the adhesive progressively remodelling to bone in both cancellous and cortical compartments with no signs of either undesirable inflammation or peripheral ectopic bone formation. These favourable results suggested translation to a large animal model. A porcine dental extraction socket model was subsequently developed where dental implants were affixed only with the adhesive. Biomechanical data was collected @ 1, 14, 28 and 56 days, and histology at 1,14,28 and 56 days. Adhesive strength assessed by implant pull-out force increased out to 28 days and maintained out to 56 days (282N maximum) with failure only occurring at the adhesive bone interface. Histology confirmed the adhesive's biocompatibility and osteoconductive behavior. Additionally, remodelling was demonstrated at the adhesive-bone interface with resorption by osteoclast-like cells and followed by new bone apposition and substitution by bone. Whilst the *in-vivo* dental implant data is encouraging, a large animal preclinical model is needed (under development) to confirm the adhesive is capable of healing, for example, loaded osteochondral bone fragments.

**References:** From failure to success: the *in-vivo* translation of a novel bone adhesive test model, Procter et al. Paper presented at EORS, Maastricht 2019, abstract <https://boneandjoint.org.uk/article/10.1302/1358-992X.2021.4.099>

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Peritenon and synovial Tppp3+ progenitor cells contribute to post-traumatic heterotopic bone formation**

Stefano Negri<sup>1,2</sup>, Ji-Hye Yea<sup>1</sup>, Mario Gomez-Salazar<sup>1</sup>, Sharon Onggo<sup>1</sup>, Zhao Li<sup>1</sup>, Neelima Thottappillil<sup>1</sup>, Masnsen Cherief<sup>1</sup>, Xin Xing<sup>1</sup>, Qizhi Qin<sup>1</sup>, Robert Joel Tower<sup>3</sup>, Chen-Ming Fan<sup>4</sup>, Benjamin Levi<sup>3</sup>, Aaron W. James<sup>1</sup>

<sup>1</sup>Department of Pathology, Johns Hopkins University, Baltimore, Maryland, USA;

<sup>2</sup>Department of Surgery, Orthopaedic Unit, Mater Salutis Hospital, Legnago, Verona, Italy;

<sup>3</sup>Center for Organogenesis and Trauma, Department of Surgery, University of Texas Southwestern, Dallas Texas, USA;

<sup>4</sup>Carnegie Institution for Science, Baltimore, Maryland, USA

Heterotopic ossification (HO) is defined as aberrant bone formation in extraskeletal locations<sup>1</sup>. In this process, local stromal cells of mesenchymal origin abnormally differentiate, resulting in pathologic cartilage and bone matrix deposition<sup>3</sup>. However, the specific cell type and mechanisms beyond this process are not well understood, in part due to the heterogeneity of progenitor cells involved. Here, a combination of single cell RNA sequencing (scrRNA-Seq) and lineage tracing, defined the extent to which synovial / tendon sheath progenitor cells contribute to HO. For this purpose, a Tppp3 (tubulin polymerization-promoting protein family member 3) inducible reporter model was used, in combination with either Scx (Scleraxis) or Pdgfra (Platelet derived growth factor receptor alpha) reporter animals. Both arthroplasty-induced and tendon injury-mouse experimental HO models were utilized<sup>3,4</sup>. ScrRNA-Seq of tendon-induced traumatic HO suggested that Tppp3 is a progenitor cell marker for either osteochondral or tendon or cells. After HO induction, Tppp3 reporter+ cell population expanded in number and contributed to cartilage and bone formation in tendon and joint-associated HO. Using double reporter animals, we found that both Pdgfra+Tppp3+ and Pdgfra+Tppp3- progenitor cells produced HO-associated cartilage. Finally, the examination of human samples showed a significant population of TPPP3+ cells overlapping with osteogenic markers in areas of HO. Overall, these results provide novel observations that peritenon and synovial progenitor cells undergo abnormal osteochondral differentiation and contribute to heterotopic bone formation after trauma.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Advanced tendon repair strategies – from EVs to mRNA-based approaches**Andreas Traweger<sup>1,2</sup>

<sup>1</sup>Institute of Tendon and Bone Regeneration, Paracelsus Medical University, Salzburg, Austria; <sup>2</sup>Austrian Cluster for Tissue Regeneration, Vienna, Austria

Approximately 30% of general practice consultations for musculoskeletal pain are related to tendon disorders, causing substantial personal suffering and enormous related healthcare costs. Treatments are often prone to long rehabilitation times, incomplete functional recovery, and secondary complications following surgical repair. Overall, due to their hypocellular and hypovascular nature, the regenerative capacity of tendons is very poor and intrinsically a disorganized scar tissue with inferior biomechanical properties forms after injury. Therefore, advanced therapeutic modalities need to be developed to enable functional tissue regeneration within a degenerative environment, moving beyond pure mechanical repair and overcoming the natural biological limits of tendon healing.

Our recent studies have focused on developing biologically augmented treatment strategies for tendon injuries, aiming at restoring a physiological microenvironment and boosting endogenous tissue repair. Along these lines, we have demonstrated that the local application of mesenchymal stromal cell-derived small extracellular vesicles (sEVs) has the potential to improve rotator cuff tendon repair by modulating local inflammation and reduce fibrotic scarring. In another approach, we investigated if the local delivery of the tendon ECM protein SPARC, which we previously demonstrated to be essential for tendon maturation and tissue homeostasis, has the potential to enhance tendon healing. Finally, I will present results demonstrating the utility of nanoparticle-delivered, chemically modified mRNAs (cmRNA) to improve tendon repair.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Extracellular vesicles from Tie2-overexpressing nucleus pulposus progenitors for intervertebral disc regeneration: an in vitro study

Luca Ambrosio<sup>1,2,3</sup>, Jordy Schol<sup>3</sup>, Gianluca Vadalà<sup>1,2</sup>, Rocco Papalia<sup>1,2</sup>, Daisuke Sakai<sup>3</sup>, Vincenzo Denaro<sup>2</sup>

<sup>1</sup>Laboratory for Regenerative Orthopaedics, Research Unit of Orthopaedic and Trauma Surgery, Department of Medicine and Surgery, Università Campus Bio-Medico di Roma, Rome, Italy; <sup>2</sup>Operative Research Unit of Orthopaedic and Trauma Surgery, Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy; <sup>3</sup>Department of Orthopaedic Surgery, Tokai University School of Medicine, Japan

Despite promising results in attempting intervertebral disc regeneration, intradiscal cell transplantation is affected by several drawbacks, including poor viability in the harsh disc environment, low cost-effectiveness, and immunogenic/tumorigenic concerns<sup>1</sup>. Recently, the development of cell-free approaches is gaining increasing interest in the field, with a particular regard towards extracellular vesicles (EVs)<sup>2</sup>. Nucleus pulposus cell (NPC) progenitors characterized by Tie2 expression have shown a higher chondrogenic differentiation potential compared to MSCs<sup>3</sup>. The aim of this study was to investigate the putative regenerative effects of EVs isolated from Tie2-overexpressing NPC progenitors on degenerative NPCs.

NPCs were isolated from young donors and underwent an optimized culture protocol to maximize Tie2 expression (NPCs<sup>Tie2+</sup>) or a standard protocol (NPCs<sup>STD</sup>). Following EV characterization, NPC isolated from patients affected by intervertebral disc degeneration (IDD) were treated with either NPCs<sup>Tie2+</sup>-EVs or NPCs<sup>STD</sup>-EVs. Cell proliferation and viability were assessed with the CCK-8 assay. Cell apoptosis and necrosis were evaluated with the Annexin V/PI assay. Cell senescence was investigated with  $\beta$ -galactosidase staining. EV uptake was assessed with PKH26 staining of EVs under confocal microscopy.

Treatment with EVs isolated from young NPC donors significantly increased degenerative NPC viability, especially in samples treated with NPCs<sup>Tie2+</sup>-EVs. Likewise, NPCs<sup>Tie2+</sup>-EVs significantly reduced cell senescence and did not show to exert necrotic nor apoptotic effects on recipient cells. Furthermore, EV uptake was successfully observed in all treated cells.

NPCs<sup>Tie2+</sup>-EVs demonstrated to significantly enhance degenerative NPC viability, senescence and apoptosis. The use of committed progenitors naturally residing in the nucleus pulposus may optimize EV regenerative properties and constitute the basis for a new therapy for IDD.

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**Mesenchymal Stem Cells-Derived Extracellular Vesicle Mimetics as Osteoinductive Mediators: An In Vitro Investigation**Antoine Karoichan<sup>1</sup>, Maryam Tabrizian<sup>1,2</sup>

<sup>1</sup>Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec; <sup>2</sup>Department of Biomedical Engineering, McGill University, Montreal, Quebec

Mesenchymal stem cells-derived extracellular vesicles (MSC-EVs) have great promise in the field of orthopaedic nanomedicine due to their regenerative, as well as immunomodulatory and anti-inflammatory properties.<sup>1</sup> Researchers are interested in harnessing these biologically sourced nanovesicles as powerful therapeutic tools with intrinsic bioactivity to help treat various orthopaedic diseases and defects. Recently, a new class of EV mimetics has emerged known as nanoghosts (NGs). These vesicles are derived from the plasma membrane of ghost cells, thus inheriting the surface functionalities and characteristics of the parent cell while at the same time allowing for a more standardized and reproducible production and significantly greater yield when compared to EVs.<sup>2</sup> This study aims to investigate and compare the osteoinductive potential of MSC-EVs and MSC-NGs *in vitro* as novel tools in the field of bone tissue engineering and nanomedicine. To carry out this investigation, MSC-EVs were isolated from serum-free MSC conditioned media through differential ultracentrifugation. The remaining cells were treated with hypotonic buffer to produce MSC-ghosts that were then homogenized and serially extruded through 400 and 200 nm polycarbonate membranes to form the MSC-NGs. The concentration, size distribution, zeta potential, and protein content of the isolated nanoparticles were assessed. Afterwards, MSCs were treated with either MSC-EVs or MSC-NGs under osteogenic conditions, and their differentiation was assessed through secreted ALP assay, qPCR, and Alizarin Red mineralization staining. Isolation of MSC-EVs and MSC-NGs was successful, with relatively similar mean diameter size and colloidal stability. No effect on MSC viability and metabolic activity was observed with either treatment. Both MSC-EV and MSC-NG groups had enhanced osteogenic outcomes compared to the control; however, a trend was observed that suggests MSC-NGs as better osteoinductive mediators compared to MSC-EVs.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Reactivation of the Nucleus Pulpous cells by platelet derivatives as a new tool in intervertebral disc regeneration**

<sup>1</sup>Nardini M., <sup>2</sup>Gentili C., <sup>1</sup>Muraglia A., <sup>3</sup>Zanirato A., <sup>4</sup>Ferrari P., <sup>3</sup>Formica M., <sup>5</sup>Cancedda R. and <sup>1</sup>Mastrogiacomo M.

<sup>1</sup>Department of Internal Medicine and Medical specialities (DIMI), University of Genova, viale Benedetto XV 10, 16132 Genova, Italy; <sup>2</sup>Regenerative Medicine Laboratory, Department of Experimental Medicine (DIMES), University of Genova, via Leon Battista Alberti 2, 16132 Genova, Italy; <sup>3</sup>Ospedale Policlinico San Martino IRCCS, largo Rosanna Benzi 10, 16132 Genova, Italy; <sup>4</sup>Department of Civil, Chemical and Environmental Engineering, University of Genoa, via Opera Pia, 15, 16145 Genoa, Italy; <sup>5</sup>Research Center for Biologically Inspired Engineering in Vascular Medicine and Longevity, University of Genoa, via Montallegro, 1, 16145 Genoa, Italy; <sup>5</sup>Emeritus Professor, Università degli Studi di Genova, Genova, Italy

Degenerative disc disease, associated to low back pain, afflicts more than 50% of humans, and represents a major healthcare problem, especially for the pathology initiation. Current treatments range from conservative strategies to more invasive surgical techniques, such as disc removal and vertebral fusion. In the Intervertebral Disease (IVD) the nucleus pulposus (NP) degeneration is a key factor for the pathology initiation. Several tissue engineering approaches aiming to restore the appropriate NP cell (NPCs) and matrix content, were attempted by using adult stromal cells either from bone marrow or adipose tissue, chondrocytes, notochordal cells and more recently also pluripotent stem cells. However, none was fully satisfactory since the NP acid and a-vascularized environment appeared aversive to the implanted heterologous cells. Several studies demonstrated the efficacy of platelet derivatives such as platelet rich plasma (PRP) in promoting the regeneration of connective tissues. We investigated the efficacy of PRP on NPCs proliferation and differentiation with the goal to propose the direct stimulation of resident cells (stimulation of endogenous cells – less invasive surgical procedure) or the implantation of NPCs expanded in vitro in the presence of PRP as therapeutic agents in IVD degeneration.

NPCs were isolated from small fragments of NP explants, cultivated in medium supplemented with PRP or FCS (standard condition control) and characterized by FACS analysis for the expression of the typical mesenchymal stem cells markers CD34, CD44, CD45, CD73, CD90 and CD105. NPCs cultured in PL showed a phenotypic profile like the cells cultured in FCS. However, compared to NPCs expanded in the presence of FCS, NPCs expanded in PRP showed a much better proliferation and differentiation capacity. NPCs differentiation was evaluated by the cell ability to produce an organized metachromatic cartilaginous matrix, confirmed by the positive immunohistochemical staining for chondrogenic markers.

**Effect of extracellular vesicles derived from human macrophages on osteosarcoma cells**

Sara Bagur-Cardona<sup>1</sup>, Karim Perez-Romero<sup>2</sup>, Kristiyan Stiliyanov<sup>1</sup>, Javier Calvo<sup>1,3</sup>, Antoni Gayà<sup>1,3</sup>, Gwendolyn Barceló-Coblijn<sup>2</sup>, Ramon Maria Rodriguez<sup>2</sup>, Manuel Gomez-Florit<sup>1</sup>

<sup>1</sup>Group of Cell Therapy and Tissue Engineering, Health Research Institute of the Balearic Islands (IdISBa), 07010 Palma, Spain; <sup>2</sup>Group of Lipids in Human Pathology, Health Research Institute of the Balearic Islands (IdISBa), 07010, Palma, Spain; <sup>3</sup>Fundació Banc de Sang i Teixits de les Illes Balears (FBSTIB), Carrer de Rosselló i Cazador, 20, 07004 Palma, Spain.

Macrophages (M $\phi$ ) are immune cells that play a crucial role in both innate and adaptive immunity as they are involved in a wide range of physiological and pathological processes. Depending on the microenvironment and signals present, M $\phi$  can polarize into either M1 or M2 phenotypes, with M1 macrophages exhibiting pro-inflammatory and cytotoxic effects, while M2 macrophages having immunosuppressive and tissue repair properties. Macrophages have been shown to play key roles in the development and progression or inhibition of various diseases, including cancer. For example, macrophages can stimulate tumor progression by promoting immunosuppression, angiogenesis, invasion, and metastasis. This work aimed to investigate the effect of extracellular vesicles (EVs)-derived from polarized macrophages on an osteosarcoma cell line. Monocytes were extracted from buffy coats and cultured in RPMI medium with platelet lysate or M-CSF. After 6 days of seeding, M $\phi$  were differentiated into M1 and M2 with INF- $\gamma$ /LPS and IL-4/IL-13, respectively. The medium with M1 or M2 derived EVs was collected and EVs were isolated by differential centrifugation and size exclusion chromatography and its morphology and size were characterized with SEM and NTA, respectively. The presence of typical EVs markers (CD9, CD63) was assessed by Western Blot. Finally, EVs from M1 or M2-polarized M $\phi$  were added onto osteosarcoma cell cultures and their effect on cell viability and cell cycle, proliferation, and gene expression was assessed. The EVs showed the typical shape, size and surface markers of EVs. Overall, we observed that osteosarcoma cells responded differentially to EVs isolated from the M1 and M2-polarized M $\phi$ . In summary, the use of M $\phi$ -derived EVs for the treatment of osteosarcoma and other cancers deserves further study as it could benefit from interesting traits of EVs such as low immunogenicity, nontoxicity, and ability to pass through tissue barriers.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **What happened to Orthopaedic Residency and Research during the pandemic** Jorge Mineiro

Hospital CUF Descobertas, Portugal

In March 2020, COVID-19 was declared a pandemic by the World Health Organization. The pandemic imposed drastic changes in our social and professional routine. Professionally at all levels our hospital tasks were changed and prioritized. Surgeons and residents were deployed on rotations to fields other than their expertise in orthopaedics. Health-care education received major changes in these challenging times, and students did face difficulties in receiving education, as well as training due to limited clinical and surgical exposure.

In response to the WHO regulations, most of the teaching centres and hospitals worldwide have adopted the web-based teaching and learning model to continue the education and training of orthopaedic residents. These results brought significant changes to the training experience in orthopaedic surgery in combination with the fact that clinical duty hours and case volume were substantially reduced.

In what concerns orthopaedic journal publications, the Covid-19 pandemic resulted in a decline in the annual publication rate for the first time in over 20 years. Although not uniform, the reduction was most likely due to multifactorial causes.

Regarding the appraisal at the end of training, at the Orthopaedic European Board Examination we were able to verify that the outcome at the written part 1 exam was good, equivalent to the outcome prior to the pandemic. However the oral viva was much worse, probably due to the fact that residents skipped much of the clinical and surgical teaching and exposure during 2020 and 2021. At the end of training, theoretical/factual knowledge was good but poor from the clinical practical experience.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Does the extent of bone defects affect the time to reach full weight-bearing after treatment with the Masquelet technique?**J. Frese<sup>1</sup>, AP Schulz<sup>2,3</sup>, B. Kowald<sup>2</sup>, U.-J. Gerlach<sup>1</sup>, K.-H. Frosch<sup>2,4</sup>, R. Schoop<sup>1</sup>

<sup>1</sup> Department of Septic Bone and Joint Surgery, BG Hospital Hamburg, Bergedorfer Straße 10, 21033, Hamburg, Germany; <sup>2</sup> Department of Trauma Surgery, Orthopaedics and Sports Traumatology, BG Hospital Hamburg, Bergedorfer Str. 10, 21033, Hamburg, Germany; <sup>3</sup> University Lübeck, Medical Faculty, Ratzeburger Allee 160, 23562 Lübeck, Germany; <sup>4</sup> Department of Trauma and Orthopaedic Surgery, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246, Hamburg, Germany

In a consecutive retrospective analysis of 190 patients treated with the Masquelet technique at the BG Klinikum Hamburg from January 2012 to January 2022, defect-specific features such as the extent and morphology of the defect were recorded, and their influence on the time to reach full weight-bearing of the affected limb was investigated.

A total of 217 defects were treated in 190 patients using the Masquelet technique. 70% of all defects were located in the tibia, followed by 22% in the femur and only about 7% in the upper extremity. The average length of all defects was 58 mm (+/- 31 mm), with the largest defect measuring 180 mm and the smallest measuring 20 mm. 89% of the patients achieved full weight-bearing at the end of therapy. The average time from initiation of therapy to reaching safe full weight-bearing was 589 days. There was a significant correlation between defect length and time to reach full weight-bearing ( $p = 0.0134$ ). These results could serve as a basis for creating a score for prognostics and evaluation of bone healing after treatment with the Masquelet technique. Additionally, the results could help guide indications for secondary stabilization using internal fixation.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Training with a novel Digitally Enhanced Hands-on Surgical Training (DEHST) enhances the performance during intramedullary nail distal interlocking**

Torsten Pastor<sup>1,2</sup>, Emanuele Cattaneo<sup>2</sup>, Tatjana Pastor MD<sup>1,3</sup>, Boyko Gueorguiev<sup>1</sup>, Markus Windolf<sup>1</sup>, Jan Buschbaum<sup>1</sup>

<sup>1</sup> AO Research Institute Davos, Davos, Switzerland; <sup>2</sup> Department of Orthopaedic and Trauma Surgery, Lucerne Cantonal Hospital, Lucerne, Switzerland; <sup>3</sup> Department of Hand surgery, Bern University Hospital, University of Bern, Bern, Switzerland

Freehand distal interlocking of intramedullary nails remains a challenging task. If not performed correctly it can be a time consuming and radiation expensive procedure. Recently, the AO Research Institute developed a new training device for Digitally Enhanced Hands-on Surgical Training (DEHST) that features practical skills training augmented with digital technologies, potentially improving surgical skills needed for distal interlocking. Aim of the study: To evaluate whether training with DEHST enhances the performance of novices without surgical experience in free-hand distal nail interlocking compared to a non-trained group of novices.

20 novices were assigned in two groups and performed distal interlocking of a tibia nail in an artificial bone model. Group 1: DEHST trained novices (virtual locking of five nail holes during one hour of training). Group 2: untrained novices without DEHST training. Time, number of x-rays, nail hole roundness, critical events and success rates were compared between the groups.

Time to complete the task (sec.) and x-ray exposure ( $\mu\text{Gcm}^2$ ) were significantly lower in Group 1 414.7 (290–615) and 17.8 (9.8–26.4) compared to Group 2 623.4 (339–1215) and 32.6 (16.1–55.3);  $p=0.041$  and  $0.003$ . Perfect circle roundness (%) was 95.0 (91.1–98.0) in Group 1 and 80.8 (70.1–88.9) in Group 2;  $p<0.001$ . In Group 1 90% of the participants achieved successful completion of the task (hit the nail with the drill), whereas only 60% of the participants in group 2 achieved this;  $p=0.121$ .

Training with DEHST significantly enhances the performance of novices without surgical experience in distal interlocking of intramedullary nails. Besides radiation exposure and operation time the complication rate during the operation can be significantly reduced.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Application of silver nanoparticles for improving motor recovery after spinal cord injury via reduction of pro-inflammatory M1 macrophages**Jie Lin<sup>1,2</sup>, Pei Kai Chen<sup>1</sup>, Zhi Jia Tan<sup>1</sup>, Yi Sun<sup>2,3</sup>, Wai Kit Tam<sup>2</sup>, Di Ao<sup>2</sup>, Wei Shen<sup>2</sup>, Victor Leung<sup>2</sup>, Kenneth Man Chee Cheung<sup>1,2</sup>, Michael Kai Tsun To<sup>1,2</sup>

<sup>1</sup>Department of Pediatric Orthopedics, The University of Hong Kong-Shenzhen Hospital (HKU-SZH), Shenzhen, Guangdong 518053, China; <sup>2</sup> Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR; <sup>3</sup> Department of Sports Medicine, Peking University-Shenzhen Hospital, Shenzhen, Guangdong, 518034, China

Silver nanoparticles (AgNPs) possess anti-inflammatory activities and have been widely deployed for promoting tissue repair. Here we explored the efficacy of AgNPs on functional recovery after spinal cord injury (SCI). Our data indicated that, in a SCI rat model, local AgNPs delivery could significantly recover locomotor function and exert neuroprotection through reducing of pro-inflammatory M1 survival. Furthermore, in comparison with Raw 264.7-derived M0 and M2, a higher level of AgNPs uptake and more pronounced cytotoxicity were detected in M1. RNA-seq analysis revealed the apoptotic genes in M1 were upregulated by AgNPs, whereas in M0 and M2, pro-apoptotic genes were downregulated and PI3k-Akt pathway signaling pathway was upregulated. Moreover, AgNPs treatment preferentially reduced cell viability of human monocyte-derived M1 comparing to M2, supporting its effect on M1 in human. Overall, our findings reveal AgNPs could suppress M1 activity and imply its therapeutic potential in promoting post-SCI motor recovery.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **A Novel Technique of Multiple Cantilever K-Wiring for Fracture Fixation in Severely Comminuted Metaphyseal Fractures**

Sushmit Singh<sup>1</sup>, Sanjay Dhar<sup>2</sup>, Sachin Kale<sup>2</sup>

<sup>1</sup>Warrington and Halton hospitals NHS Trust; <sup>2</sup>D Y Patil University School Of Medicine, Navi Mumbai

The management of comminuted metaphyseal fractures is a technical challenge and satisfactory outcomes of such fixations often remain elusive. The small articular fragments and bone loss often make it difficult for standard fixation implants for proper fixation. We developed a novel technique to achieve anatomical reduction in multiple cases of comminuted metaphyseal fractures at different sites by employing the cantilever mechanism with the help of multiple thin Kirschner wires augmented by standard fixation implants.

We performed a retrospective study of 10 patients with different metaphyseal fractures complicated by comminution and loss of bone stock. All patients were treated with the help of cantilever mechanism using multiple Kirschner wires augmented by compression plates. All the patients were operated by the same surgeon between November 2020 to March 2021 and followed up till March 2023. Surgical outcomes were evaluated according to the clinical and radiological criteria.

A total of 10 patients were included in the study. Since we only included patients with highly unstable and comminuted fractures which were difficult to fix with traditional methods, the number of patients in the study were less. All 10 patients showed satisfactory clinical and radiological union at the end of the study with good range of motion. One of the patient in the study had post-operative wound complication which was managed conservatively with regular dressings and oral antibiotics.

Comminuted metaphyseal fractures might differ in pattern and presentation with every patient and there can be no standard treatment for all. The cantilever technique of fracture fixation is based on the principle of cantilever mechanism used in bridges and helps achieve good anatomical reduction and fixation. It provides a decent alternative when standard modes of fixation don't give desired result owing to comminuted nature of fractures and deficiency of bone stock.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Primary Bone Tumors and Metastases: Clinical Approach**

Vânia Oliveira, Orthopaedic Surgeon, Musculo-Skeletal Tumors Unit

Centro Hospitalar Universitário de Santo António, ICBAS-UP, Porto, Portugal

Primary bone tumors are rare, complex and highly heterogeneous. Its diagnostic and treatment are a challenge for the multidisciplinary team. Developments on tumor biomarkers, immunohistochemistry, histology, molecular, bioinformatics, and genetics are fundamental for an early diagnosis and identification of prognostic factors. The personalized medicine allows an effective patient tailored treatment. The bone biopsy is essential for diagnosis. Treatment may include systemic therapy and local therapy. Frequently, a limb salvage surgery includes wide resection and reconstruction with endoprosthesis, biological or composites. The risk for local recurrence and distant metastases depends on the primary tumor and treatment response.

Cancer patients are living longer and bone metastases are increasing. Bone is the third most frequently location for distant lesions. Bone metastases are associated to pain, pathological fractures, functional impairment, and neurological deficits. It impacts survival and patient quality of life. The treatment of metastatic disease is a challenge due to its complexity and heterogeneity, vascularization, reduced size and limited access. It requires a multidisciplinary treatment and depending on different factors it is palliative or curative-like treatment. For multiple bone metastases it is important to relief pain and increases function in order to provide the best quality of life and expect to prolong survival. Advances in nanotechnology, bioinformatics, and genomics, will increase biomarkers for early detection, prognosis, and targeted treatment effectiveness. We are taking the leap forward in precision medicine and personalized care.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Design and Development of a custom platform to grow and characterize osteosarcoma spheroids

Maria Veronica Liperi<sup>1</sup>, Margherita Cortini<sup>1</sup>, Nicola Baldini<sup>1,2</sup>, Sofia Avnet<sup>1</sup>

<sup>1</sup>Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum, Università di Bologna, 40127 Bologna, Italy; <sup>2</sup>Biomedical Science and Technology and Nanobiotechnology Laboratory, Istituto di Ricovero e Cura a Carattere Scientifico, IRCCS Istituto Ortopedico Rizzoli, 40136 Bologna, Italy.

Osteosarcoma is a highly malignant primary tumor of bone tissue<sup>1</sup>. The 5-year survival rate of patients with metastasis is below 20% and this scenario is unchanged in the last two decades, despite great efforts in pre-clinical and clinical research<sup>2</sup>. Traditional preclinical models of osteosarcoma do not consider the whole complexity of its microenvironment, leading to poor correlation between *in vitro/in vivo* results and clinical outcomes. Spheroids are a promising *in vitro* model to mimic osteosarcoma and perform drug-screening tests, as they (i) reproduce the microarchitecture of the tumor, (ii) are characterized by hypoxic regions and necrotic core as the *in vivo* tumor, (iii) and recapitulate the chemo-resistance phenomena<sup>3</sup>. However, to date, the spheroid model is scarcely used in osteosarcoma research.

Our aim is to develop a customized culture dish to grow and characterize spheroids and to perform advanced drug-screening tests. The resulting platform must be adapted to automated image acquisition systems, to overcome the drawbacks of commercial spheroids platforms.

To this purpose, we designed and developed a micro-patterned culture dish by casting agarose on a 3D printed mold from a CAD design. We successfully obtained viable and reproducible homotypic osteosarcoma spheroids, with two different cells lines from osteosarcoma (i.e., 143b and MG-63). Using the platform, we performed viability assays and live fluorescent stainings (e.g., Calcein AM) with low reagent consumption. Moreover, the culture dish was validated as drug screening platform, administering Doxorubicin at different doses, and evaluating its effect on OS spheroids, in terms of morphology and viability. This platform can be considered an attractive alternative to the highly expensive commercial spheroid platforms to obtain homogeneous and reproducible spheroids in a high-throughput and cost effective mode.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Novel CB65-loaded liposome formulation as a chemotherapeutic candidate for osteosarcoma

Başak Işıl Zorba<sup>1</sup>, Özge Boyacıoğlu<sup>1,2</sup>, Tuğba Çağlayan<sup>1</sup>, Tuba Reçber<sup>3</sup>, İpek Eroğlu<sup>4</sup>, Emirhan Nemutlu<sup>3</sup>, Petek Korkusuz<sup>5</sup>

<sup>1</sup>Hacettepe University, Graduate School of Science and Engineering, Department of Bioengineering, 06800 Beytepe, Ankara, Turkey; <sup>2</sup>Atılım University, Faculty of Medicine, Department of Medical Biochemistry, 06830 Gölbaşı, Ankara, Turkey; <sup>3</sup>Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100 Sıhhiye, Ankara, Turkey; <sup>4</sup>Hacettepe University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, 06100 Sıhhiye, Ankara, Turkey; <sup>5</sup>Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, 06100 Sıhhiye, Ankara, Turkey

Osteosarcoma is common in children and adolescents [1] with high mortality due to rapid progression [2]. Therapeutic approaches for osteosarcoma are limited and may cause side effects. Cannabinoid ligands exert antiproliferative, apoptotic effect in cancer cells via CB1/2 or TRPV1 receptors [3]. In this study, we hypothesized that synthetic specific CB2R agonist CB65 might have an antiproliferative and apoptotic effect on osteosarcoma cell lines in vitro. If so, this agent might be a chemotherapeutic candidate for osteosarcoma, with prolonged release, increased stability and bioavailability when loaded into a liposomal system. We first determined CB2 receptor expression in MG63 and Saos-2 osteosarcoma cells by qRT-PCR and FCM. CB65 reduced proliferation in osteosarcoma cells by WST-1 and RTCA. IC50 for MG63 and Saos-2 cells were calculated as  $1.11 \times 10^{-11}$  and  $4.95 \times 10^{-11}$  M, respectively. The antiproliferative effect of CB65 on osteosarcoma cells was inhibited by CB2 antagonist AM630. IC50 of CB65 induced late apoptosis of MG63 and Saos-2 cells at 24 and 48 hours, respectively by FCM. CB65 was loaded into the liposomal system by thin film hydration method and particle size, polydispersity index, and zeta potentials were  $141.7 \pm 0.6$  nm,  $0.451 \pm 0.026$ , and  $-10.9 \pm 0.3$  mV, respectively. The CB65-loaded liposomal formulation reduced MG63 and Saos-2 cell proliferation by RTCA. IC50 of CB65 and CB65-loaded liposomal formulation induced late apoptosis of MG63 and Saos-2 cells at 24 and 48 hours, respectively, by FCM. Scratch width was higher in CB65 and CB65-loaded liposome-treated cells compared to control. In this study, the real-time antiproliferative and apoptotic effect of synthetic specific CB2 agonist CB65 in osteosarcoma cell lines was demonstrated for the first time, and the real time therapeutic window was determined. The CB65-loaded liposomal formulation presents a potential treatment option that can be translated to clinic following its validation within animal models and production under GMP conditions.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Novel antimicrobial coating on titanium with stable non-antibiotic quaternary ammonium compounds to prevent implant-associated infection**

Martijn Riool<sup>1,2</sup>, Rui Li<sup>3</sup>, Laure van Hofwegen<sup>1</sup>, Leonie de Boer<sup>1</sup>, Jacobus A. Loontjens<sup>3</sup>, Sebastian A.J. Zaat<sup>1</sup>

<sup>1</sup>Dept. of Medical Microbiology and Infection Prevention, Amsterdam institute for Infection and Immunity, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; <sup>2</sup>Dept. of Trauma Surgery, University Hospital Regensburg, Regensburg, Germany; <sup>3</sup>Dept. of Polymer Chemistry, Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands

Infection of implanted medical devices (biomaterials), like titanium orthopaedic implants, can have disastrous consequences, including removal of the device. These so-called biomaterial-associated infections (BAI) are mainly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. To prevent biofilm formation using a non-antibiotic based strategy, we aimed to develop a novel permanently fixed antimicrobial coating for titanium devices based on stable immobilized quaternary ammonium compounds (QACs).

Medical grade titanium implants were dip-coated in subsequent solutions of hyperbranched polymer, polyethyleneimine and 10 mM sodium iodide, and ethanol. The QAC-coating was characterized using water contact angle measurements, scanning electron microscopy, FTIR, AFM and XPS. The antimicrobial activity of the coating was evaluated against *S. aureus* strain JAR060131 and *S. epidermidis* strain ATCC 12228 using the JIS Z 2801:2000 surface microbicidal assay. Lastly, we assessed the *in vivo* antimicrobial activity in a mouse subcutaneous implant infection model with *S. aureus* administered locally on the QAC-coated implants prior to implantation to mimic contamination during surgery.

Detailed material characterization of the titanium samples showed the presence of a homogenous and stable coating layer at the titanium surface. Moreover, the coating successfully killed *S. aureus* and *S. epidermidis in vitro*. The QAC-coating strongly reduced *S. aureus* colonization of the implant surface as well as of the surrounding tissue, with no apparent macroscopic signs of toxicity or inflammation in the peri-implant tissue at 1 and 4 days after implantation.

An antimicrobial coating with stable quaternary ammonium compounds on titanium has been developed which holds promise to prevent BAI. Non-antibiotic-based antimicrobial coatings have great significance in guiding the design of novel antimicrobial coatings in the present, post-antibiotic era.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

coatings with stable non-antibiotic Quaternary Ammonium Compounds and photosensitizer technology'.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Spatial transcriptomics of fracture healing

Esther Wehrle<sup>1,2</sup>

<sup>1</sup>AO Research Institute Davos, Davos Platz, Switzerland; <sup>2</sup>Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

Fracture healing is a spatially controlled process involving crosstalk of multiple tissues. To precisely capture and understand molecular mechanism underlying impaired healing, there is a need to integrate spatially-resolved molecular analyses into preclinical fracture healing models. I will present our recent data obtained by spatial transcriptomics of musculoskeletal samples from fracture healing studies in mice. Subsequently, I will show how spatial transcriptomics can be integrated into multimodal approaches in preclinical fracture healing models. In combination with established *in vivo* imaging and emerging omics techniques, spatially-resolved analyses have the potential to elucidate the molecular mechanisms underlying impaired healing with optimization of treatments.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Clinically relevant performance evaluation of automatic segmentation**

Jonas Grammens<sup>1</sup>

<sup>1</sup>University of Antwerp, Vision Lab

Accurate 3D models of the patient-specific anatomy are crucial for all biomechanical in-silico simulations. Segmentation of medical images into 3D models is a highly time-consuming task, which can be automated by computer vision algorithms. The performance of these algorithms in computer science is typically reported as a score, without incorporating any spatial or anatomical information. Next to a brief introduction into statistical shape analysis, a framework will be presented to evaluate segmentation performance using domain knowledge.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Non-linear applications of statistical shape analysis in orthopedic research**

Audenaert Emmanuel<sup>1</sup>

<sup>1</sup>University of Ghent

Non-linear methods in statistical shape analysis have become increasingly important in orthopedic research as they allow for more accurate and robust analysis of complex shape data such as articulated joints, bony defects and cartilage loss. These methods involve the use of non-linear transformations to describe shapes, rather than the traditional linear approaches, and have been shown to improve the precision and sensitivity of shape analysis in a variety of applications. In orthopedic research, non-linear methods have been used to study a range of topics, including the analysis of bone shape and structure in relation to osteoarthritis, the assessment of joint deformities and their impact on joint function, and the prediction of patient outcomes following surgical interventions. Overall, the use of non-linear methods in statistical shape analysis has the potential to advance our understanding of the relationship between shape and function in the musculoskeletal system and improve the diagnosis and treatment of orthopedic conditions.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Using Shape Modeling Techniques for Markerless Motion Analysis**

Kate Duquesne<sup>1</sup>

<sup>1</sup>University of Ghent, BioMics, Biomechanics, Insilico medicine, and Computational studies

For many years, marker-based systems have been used for motion analysis. However, the emergence of new technologies, such as 4D scanners provide exciting new opportunities for motion analysis. In 4D scanners, the subjects are measured as a dense mesh, which enables the use of shape analysis techniques. In this talk, we will explore how the combination of the rising new motion analysis methods and shape modelling may change the way we think about movement and its analysis.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Personalized Statistical Modeling of Meniscal Kinematics**

Aline Van Oevelen<sup>1</sup>

<sup>1</sup>University of Antwerp, Belgium

Intra-articular cartilage pressure distribution in the knee joint is critical in the understanding of osteoarthritis. Combining personalized statistical modeling of the morphological characteristics with discrete element modeling enables patient-specific predictions of the pressure on the tibial plateau. However, modeling of the meniscus during gait is complicated by the dynamic nature of the structure. Nevertheless, the position of the meniscus has a substantial impact on intra-articular stress distribution. Therefore, the focus of this presentation will be on how modeling of meniscal movement during knee flexion improves insight in general meniscal kinematics for the use in tibiofemoral stress distribution calculations.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Patient-Specific Contact Mechanics in Syndesmotic Ankle Lesions**

Matthias Peiffer<sup>1</sup>

<sup>1</sup>Foot and Ankle Research Innovation and Research Laboratory (FARIL), Boston, USA

Syndesmotic ankle lesions involve disruption of the osseous tibiofibular mortise configuration as well as ligamentous structures stabilizing the ankle joint. Incomplete diagnosis and maltreatment of these injuries is frequent, resulting in chronic pain and progressive instability thus promoting development of ankle osteoarthritis in the long term. Although the pathogenesis is not fully understood, abnormal mechanics has been implicated as a principal determinant of ankle joint degeneration after syndesmotic ankle lesions. Therefore, the focus of this presentation will be on our recent development of a computationally efficient algorithm to calculate the contact pressure distribution in patients with a syndesmotic ankle lesion, enabling us to stratify the risk of OA development in the long term and thereby guiding patient treatment.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Geometrics Deep Learning Applications in Orthopedic Research**

Ide Van den Borre<sup>1</sup>

<sup>1</sup>University of Ghent, Group for Artificial Intelligence and Sparse Modeling

Geometric deep learning is a relatively new field that combines the principles of deep learning with techniques from geometry and topology to analyze data with complex structures, such as graphs and manifolds. In orthopedic research, geometric deep learning has been applied to a variety of tasks, including the analysis of imaging data to detect and classify abnormalities, the prediction of patient outcomes following surgical interventions, and the identification of risk factors for degenerative joint disease. This review aims to summarize the current state of the field and highlight the key findings and applications of geometric deep learning in orthopedic research. The review also discusses the potential benefits and limitations of these approaches and identifies areas for future research. Overall, the use of geometric deep learning in orthopedic research has the potential to greatly advance our understanding of the musculoskeletal system and improve patient care.

**Accelerations Recorded by Low-Frequency Wearable Sensors as Effective Discriminators of Knee and Hip Osteoarthritis**

Arash Ghaffari<sup>1</sup>, Pernille Damborg Clasen<sup>1</sup>, Rikke Vindberg Boel<sup>1</sup>, Andreas Kappel<sup>1</sup>, Thomas Jakobsen<sup>1</sup>, Søren Kold<sup>1</sup>, Ole Rahbek<sup>1</sup>

<sup>1</sup> Interdisciplinary Orthopaedics, Aalborg University Hospital, Aalborg, Denmark

Wearable inertial sensors can detect abnormal gait associated with knee or hip osteoarthritis (OA). However, few studies have compared sensor-derived gait parameters between patients with hip and knee OA or evaluated the efficacy of sensors suitable for remote monitoring in distinguishing between the two. Hence, our study seeks to examine the differences in accelerations captured by low-frequency wearable sensors in patients with knee and hip OA and classify their gait patterns.

We included patients with unilateral hip and knee OA. Gait analysis was conducted using an accelerometer ipsilateral with the affected joint on the lateral distal thighs. Statistical parametric mapping (SPM) was used to compare acceleration signals. The k-Nearest Neighbor (k-NN) algorithm was trained on 80% of the signals' Fourier coefficients and validated on the remaining 20% using 10-fold cross-validation to classify the gait patterns into hip and knee OA.

We included 42 hip OA patients (19 females, age 70 [63-78], BMI of 28.3 [24.8-30.9]) and 59 knee OA patients (31 females, age 68 [62-74], BMI of 29.7 [26.3-32.6]). The SPM results indicated that one cluster (12-20%) along the vertical axis had accelerations exceeding the critical threshold of 2.956 ( $p=0.024$ ). For the anteroposterior axis, three clusters were observed exceeding the threshold of 3.031 at 5-19% ( $p = 0.0001$ ), 39-54% ( $p=0.00005$ ), and 88-96% ( $p = 0.01$ ). Regarding the mediolateral axis, four clusters were identified exceeding the threshold of 2.875 at 0-9% ( $p = 0.02$ ), 14-20% ( $p=0.04$ ), 28-68% ( $p < 0.00001$ ), and 84-100% ( $p = 0.004$ ). The k-NN model achieved an AUC of 0.79, an accuracy of 80%, and a precision of 85%.

In conclusion, the Fourier coefficients of the signals recorded by wearable sensors can effectively discriminate the gait patterns of knee and hip OA. In addition, the most remarkable differences in the time domain were observed along the mediolateral axis.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Can Kinetics and Kinematics of Single Leg Forward and Crossover Triple Hop Tests Determine Recreational Male Athletes Return to Sports after ACL Reconstruction?**

Ömer Faruk İliceşinar<sup>1</sup>, Mehmet İmir<sup>2</sup>, Berat Can Cengiz<sup>2</sup>, Senih Gürses<sup>2</sup>, Yigitcan Menderes<sup>1</sup>, Egemen Turhan<sup>3</sup>, Gürhan Dönmez<sup>1</sup>, [Feza Korkusuz](#)<sup>1</sup>

<sup>1</sup>Hacettepe University Medical School, Department of Sport Medicine; <sup>2</sup>Middle East Technical University, Department of Engineering Sciences; <sup>3</sup>Hacettepe University Medical School, Department of Orthopedic Surgery and Traumatology, Ankara, Turkey

Hop tests are used to determine return to sports after ACL reconstruction. They mostly measure distance and symmetry but do not assess kinematics and kinetics. Recently, biomechanical evaluations have been incorporated into these functional jump tests for the better assessment of return to sport. We assessed the sagittal plane range of motion (ROM) of the knee, the deviation axis of rotation (DAOR), and the vertical ground reaction force (vGRF) normalized to body weight in nine healthy participants during the single leg (SLH) and crossover hop tests (COHT).

Participants' leg lengths were measured. Jumping distances were marked in the test area as being 4/5 of the leg length. Four sensors were placed on the thighs, the legs and the feet. These body parts were handled as a single rigid body. Eight 480 Hz cameras were used to capture the movements of these rigid bodies. vGRF at landing were measured using a force plate (Bertec, Inc, USA). The ROM of the knee joint and the DAOR were obtained from kinematic data.

Participants' joint kinematics metrics were similar in within-subjects statistical tests for SLH and COHT. We therefore asked whether the repeated vGRF normalized to body weight will be similar in both legs during these jumps. Joint kinematics metrics however were different in between subjects indicating the existence of a personalized jumping strategy. These hop tests can be recorded at the beginning of the training season for each individual, which can establish a comparative evaluation database for prospective lower extremity injury recovery and return to sport after ACL injury.

**Effect of blood flow restriction on the painful knee after knee arthroplasty in patients without mechanical failure: preliminary results of a prospective cohort study.**

Lenka Stroobant<sup>1</sup>, Ewoud Jacobs<sup>2</sup>, Nele Arnout<sup>1</sup>, Stefaan Van Onsem<sup>3</sup>, Arne Burssens<sup>1</sup>, Jan Victor<sup>1</sup>

<sup>1</sup>Department Orthopaedic Surgery, University Hospital Ghent, Ghent, Belgium;

<sup>2</sup>Department of Rehabilitation Sciences, Ghent University Faculty of Medicine and Health Sciences, Ghent, Belgium; <sup>3</sup>Department Orthopaedic Surgery, AZ Alma, Eeklo, Belgium

7-20 % of the patients with a total knee arthroplasty (TKA) are dissatisfied without an indication for revision. Therapeutic options for this patient population with mostly a lack of quadriceps strength are limited. The purpose of this study is to evaluate the effect of six weeks low load resistance training with blood flow restriction (BFR) on the clinical outcome in these unhappy TKA patients.

Thirty-one unhappy TKA patients (of the scheduled fifty patients) without mechanical failure were included in this prospective study since 2022. The patients participate in a supervised resistance training combined with BFR, two times a week during nine weeks. Patients were evaluated by the Knee Osteoarthritis Outcome Score (KOOS), Knee Society Score: satisfaction (KSSs) and the Pain Catastrophizing Scale (PCS). Functionality was tested using the Six Minute Walk Test (6MWT) and the 30-Second Chair Stand Test (30CST). Follow-up took place at six weeks, three months and six months after the start.

Six weeks training with BFR provided statistically significant improvements in all the KOOS subscales compared to the baseline, especially for symptoms (55.1 ( $\pm$ 15.4) versus 48.0 ( $\pm$ 16.5);  $p < 0.001$ ), activities in daily living (50.3 ( $\pm$ 21.1) versus 43.7 ( $\pm$ 17.2);  $p < 0.001$ ) and quality of life (24.6 ( $\pm$ 18.5) versus 17.3 ( $\pm$ 13.0);  $p < 0.001$ ). The PCS reduced from 27.4 ( $\pm$ 11.0) to 23.2 ( $\pm$ 11.4) at six weeks ( $p < 0.01$ ), whereas the KSSs increased from 11.8 ( $\pm$ 6.5) to 14.9 ( $\pm$ 7.6) ( $p = 0.021$ ). Both the 6MWT and the 30CST improved statistically significant from respectively 319.7 ( $\pm$ 15.0) to 341.6m ( $\pm$ 106.5) ( $p < 0.01$ ) and 8.6 ( $\pm$ 3.9) to 9.3 times ( $\pm$ 4.5) ( $p < 0.01$ ).

Blood flow restriction appears to enhance the quality of life and functional performance of unhappy TKA patients. Based on these preliminary results, BFR seems to be a promising and valuable alternative for these TKA patients with limited therapeutic options.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Lateral rim variable angle locked plating versus tension band wiring of simple and complex patella fractures – a biomechanical study**

Ivan Zderic<sup>1</sup>, Stephen Warner<sup>2</sup>, Karl Stoffel<sup>3</sup>, William Woodburn<sup>4</sup>, Richard Castle<sup>4</sup>, Jessica Penman<sup>4</sup>, Eladio Saura-Sanchez<sup>5</sup>, David L. Helfet<sup>6</sup>, Boyko Gueorguiev<sup>1</sup>, Christoph Sommer<sup>7</sup>

<sup>1</sup>AO Research Institute Davos, Davos, Switzerland; <sup>2</sup>University of Texas Health Science Center, Houston, TX, United States; <sup>3</sup>University Hospital Basel, Basel, Switzerland  
<sup>4</sup>DePuy Synthes, West Chester, PA, United States; <sup>5</sup>University Hospital of Elche, Elche, Spain; <sup>6</sup>New York Presbyterian Hospital, New York Weill Cornell Center, NYC, USA;  
<sup>7</sup>Cantonal Hospital Graubünden, Chur, Switzerland

Treatment of both simple and complex patella fractures is a challenging clinical problem. The aim of this study was to investigate the biomechanical performance of recently developed lateral rim variable angle locking plates versus tension band wiring used for fixation of simple and complex patella fractures.

Twelve pairs of human anatomical knees were used to simulate either two-part transverse simple AO/OTA 34C1 or five-part complex AO/OTA 34C3 patella fractures by means of osteotomies, with each fracture model created in six pairs. The complex fracture pattern was characterized by a medial and a lateral proximal fragment, together with an inferomedial, an inferolateral, and an inferior fragment mimicking comminution around the distal patellar pole. The specimens with simple fractures were pairwise assigned for fixation with either tension band wiring through two parallel cannulated screws, or a lateral rim variable angle locking plate. The knees with complex fractures were pairwise treated with either tension band wiring through two parallel cannulated screws plus circumferential cerclage wiring, or a lateral rim variable angle locking plate.

Each specimen was tested over 5000 cycles by pulling on the quadriceps tendon, simulating active knee extension and passive knee flexion within the range of 90° flexion to full knee extension. Interfragmentary movements were captured via motion tracking.

For both fracture types, the longitudinal and shear articular displacements measured between the proximal and distal fragments at the central patella aspect between 1000 and 5000 cycles, together with the relative rotations of these fragments around the mediolateral axis were all significantly smaller following the lateral rim variable angle locked plating compared with tension band wiring,  $p < 0.01$ .

Lateral rim locked plating of both simple and complex patella fractures provides superior construct stability versus tension band wiring under dynamic loading.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Conception, implementation and pre-clinical testing of 3D-printed custom-made orthopaedic implants in complex knee & hip revision arthroplasty – Challenges in treatment of peri-prosthetic fractures and severe bone defect situations**Thomas M. Grupp<sup>1</sup><sup>1</sup>Aesculap AG Research & Development, Tuttlingen, Germany

In severe cases of total knee & hip arthroplasty, where off-the-shelf implants are not suitable (i.e., in cases with extended bone defects or periprosthetic fractures), 3D-printed custom-made knee & hip revision implants out of titanium or cobalt-chromium alloy represent one of the few remaining clinical treatment options. Design verification and validation of such custom-made implants is very challenging. Therefore, a methodology was developed to support surgeons and engineers in their decision on whether a developed design is suitable for the specific case. A novel method for the pre-clinical testing of 3D-printed custom-made knee implants has been established, which relies on the biomechanical test and finite element analysis (FEA) of a comparable clinically established reference implant. The method comprises different steps, such as identification of the main potential failure mechanism, reproduction of the biomechanical test of the reference implant via FEA, identification of the maximum value of the corresponding FEA quantity of interest at the required load level, definition of this value as the acceptance criterion for the FEA of the custom-made implant, reproduction of the biomechanical test with the custom-made implant via FEA, decision making for realization or re-design based on the acceptance criterion is fulfilled or not. Exemplary cases of custom-made knee & hip implants were evaluated with this new methodology. The FEA acceptance criterion derived from the reference implants was fulfilled in both custom-made implants and subsequent biomechanical tests verified the FEA results. The suggested method allows a quantitative evaluation of the biomechanical properties of custom-made knee & hip implant without performing physical bench testing. This represents an important contribution to achieve a sustainable patient treatment in complex revision total knee & hip arthroplasty with custom-made 3D printed implants in a safe and timely manner.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **3D printing technology in orthopaedic solutions: materials, implants, and instruments**

Berna Richter<sup>1</sup>

<sup>1</sup>Aesculap AG Research & Development, Tuttlingen, Germany

An overview about 3D printing technology in orthopaedic applications will be given based on examples. The process from early prototypes to certified implants coming from serial production will be demonstrated also considering relevant surrounding conditions. Today's focus is mostly on orthopaedic implants, but there is a high potential for new implant-related surgical instrument solutions taking into account upcoming clinical demands and user needs accessible by actual 3D printing technologies.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Production and Characterization of Coated Porous Titanium Implants by Laser Powder Bed Fusion (L-PBF) Technology**

Fatma Nur Depboylu<sup>1</sup>

<sup>1</sup>Hacettepe University, Turkey

Production of porous titanium bone implants is a highly promising research and application area due to providing high osseointegration and achieving the desired mechanical properties. Production of controlled porosity in titanium implants is possible with laser powder bed fusion (L- PBF) technology. The main topics of this presentation includes the L-PBF process parameter optimization to manufacture thin walls of porous titanium structures with almost full density and good mechanical properties as well as good dimensional accuracy. Moreover, the cleaning and coating process of these structures to further increase osseointegration and then in-vitro biocompatibility will be covered.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **3D-printed absorbable metallic medical devices and their application potential** Holger Jahr<sup>1</sup>

<sup>1</sup>RWTH Aachen University, Germany

AM specifically allows for cost-efficient production of patient-specific Orthopaedic medical devices with unusual designs and properties. A porous design allows to adjust the stiffness of metallic implants to that of the host bone. Beyond traditional metals, like titanium alloys, this talk will review the present state-of-the-art of directly printed absorbable metal families. Physicochemical, mechanical and biological properties of standardized design prototypes from all currently available metal families will be compared and their clinical application potential discussed. The impact of *in vitro* test environments on comparative corrosion behavior, post manufacturing aspects, and the recent *status quo* in biocompatibility testing and present knowledge gaps will be addressed.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Additive manufacturing of biomimetic nanostructured materials for bone modelling and regeneration**Gabriela Graziani<sup>1</sup><sup>1</sup>Rizzoli Orthopaedic Institute, Bologna, Italy

Functionalization of biomimetic nanomaterials allows to reproduce the composition of native bone, permitting better regeneration, while nanoscale surface morphologies provide cues for cell adhesion, proliferation and differentiation. Functionalization of 3D printed and bioprinted constructs, by plasma-assisted deposition of calcium phosphates-based (CaP) nanostructured coatings and by nanoparticles, respectively, will be presented. Stoichiometric and ion doped CaP- based nanocoatings, including green materials (mussel seashells and cuttlefish bone), will be introduced to guide tissue regeneration. We will show interactions between biomimetic surfaces and MSCs to address bone regeneration and SAOS-2 cells for bone tumor models. Our results show that combining AM and nanostructured biomimetic films permits to reproduce the architecture and the mechanical and compositional characteristics of bone. Stability behavior of the coatings, as well as MSCs behavior strongly depend on the starting CaP material, with more soluble CaPs and ion-doped ones showing better biological behavior. Green materials appear promising, as biomimetic films can be successfully obtained upon conversion of the marine precursors into hydroxyapatite. Last-not-least, nanoparticles-loaded scaffolds could be bioprinting without loss of cell viability, but ink characteristics depend on ion-doping as demonstrated for SAOS-2 cells over 14 days of culture. Biomimetic nanomaterials for functionalization in AM is a promising approach for bone modelling and regeneration.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Biofunctionalized 3D Bioprinted Vascular-Like Structures: An Advanced Approach to Promote Endothelial Tissue Regeneration**

Varvara Platania<sup>1</sup>, Nikoleta Natalia Tavernaraki<sup>1</sup>, Ioanna Gontika<sup>2</sup>, Eirini Fragiadaki<sup>2</sup>, Nikoleta Triantopoulou<sup>3</sup>, Helen A Papadaki<sup>2</sup>, Kalliopi Alpantaki<sup>4</sup>, Marina Vidaki<sup>3,5</sup>, Maria Chatzinikolaidou<sup>1,6</sup>

<sup>1</sup>Department of Materials Science and Technology, University of Crete, Heraklion, Greece; <sup>2</sup>Haemopoiesis Research Laboratory, Faculty of Medicine, University of Crete, & Public Umbilical Cord Blood Bank of Crete, University Hospital of Heraklion, Heraklion, Greece; <sup>3</sup>Department of Basic Science, Faculty of Medicine, University of Crete, Heraklion, Greece; <sup>4</sup>Department of Orthopaedics and Trauma, Venizeleion General Hospital of Heraklion, Heraklion, Greece; <sup>5</sup>Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece; <sup>6</sup>Institute of Electronic Structure and Laser, Foundation for Research and Technology Hellas, Heraklion, Greece

Biofabrication is a popular technique to produce personalized constructs for tissue engineering. In this study we combined laponite (Lap), gellan gum (GG) with platelet-rich plasma (PRP) aiming to enhance the endothelial regeneration through the synergistic effects of their individual properties. Laponite has the ability to form porous three-dimensional networks mimicking the extracellular matrix structure, and PRP delivery of growth factors stimulates the endothelial cell proliferation and migration, offering a composite bioink for cell growth and support. The sustained release of these growth factors from the GG-laponite-PRP composite material over time provides a continuous source of stimulation for the cells, leading to more effective tissue engineering strategies for endothelial tissue regeneration. Four blend compositions comprising 1% w/v GG and 0.5 or 1% w/v Lap and 25% v/v PRP were combined with Wharton jelly mesenchymal stem cells (WJ-MSCs) and bioprinted into vessel-like structures with an inner diameter of 3 mm and a wall thickness of 1 mm. Stress/strain analysis revealed the elastomeric properties of the hydrogels with Young modulus values of 10 MPa. Increasing the Lap concentration led to a non-significant decrease of swelling ratio from 93 to 91%. Live/dead assay revealed cell viability of at least 76%, with the 0.5%Lap-GG viability exceeding 99% on day 21. Gradual increase of glycosaminoglycans accumulation and collagen production indicate promotion of ECM formation. The expression and membranous localization of PECAM-1 from day 7 and the granular intracellular localization of vWF after 2 weeks demonstrate in vitro endothelial functionality. In vivo subcutaneous implantation indicated the absence of any adverse immunological reactions. The results reveal the expression of both vWF and PECAM-1 by WJ-MSCs entrapped in all four construct compositions with significantly higher expression of vWF in the presence of PRP.

27-29 SEPTEMBER | PORTO, PORTUGAL

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Mechanically evolutive 3D-printed scaffolds for bone regeneration**

B. Charbonnier<sup>1</sup>, L. Guyon<sup>1</sup>, N. Touya<sup>1</sup>, M. Dutilleul<sup>1</sup>, J. Véziers<sup>1</sup>, P. Maitre<sup>1</sup>, O. Gauthier<sup>1</sup>, P. Corre<sup>1</sup>, P. Weiss<sup>1</sup>

<sup>1</sup>Nantes Université, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000 Nantes, France; <sup>2</sup>Nantes Université, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F- 44000 Nantes, France

Developments in the field of additive manufacturing have allowed significant improvements in the design and production of scaffolds with biologically relevant features to treat bone defects. Unfortunately, the workflow to generate personalized scaffolds is source of inaccuracies leading to a poor fit between the implant and patients' bone defects. In addition, scaffolds are often brittle and fragile, uneasing their handling by surgeons, with significant risks of fracture during their insertion in the defect <sup>a</sup>. Consequently, we developed organo-mineral cementitious scaffolds displaying evolutive mechanical properties which are currently being evaluated to treat maxillofacial bone deformities in veterinary clinics. Treatment of dog patients was approved by ethic and welfare committees (CERVO-2022-14-V). To date, 8 puppies with cleft palate/lip deformities received the following treatment. Two weeks prior surgery, CT-scan of patient's skull was performed to allow for surgical planning and scaffold designing. Organo-mineral printable pastes were formulated by mixing an inorganic cement precursor ( $\alpha$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>) to a self-reticulating hydrogel (silanized hyaluronic acid) supplemented with a viscosifier (hydroxymethylpropylcellulose). Scaffolds were produced by robocasting of these pastes. Surgical interventions included the reconstruction of soft tissues, and the insertion of the scaffold soaked with autologous bone marrow. Bone formation was monitored 3 and 6 months after reconstruction, and a biopsy at 6 months was performed for more detailed analyses. Scaffolds displayed great handling properties and were inserted within bone defects without significant issue with a relevant bone edges/scaffold contact. Osteointegration of the scaffolds was observed after 3 months, and regeneration of the defect at 6 months seemed quite promising. Preliminary results have demonstrated a potential of the set-up strategy to treat cleft lip/palate deformities in real, spontaneous clinical setting. Translation of these innovative scaffolds to orthopedics is planned for a near future.

**Geometric control of bone tissue growth and organisation**S.J.P. Callens<sup>1</sup>, R. Burdis<sup>1</sup>, M. Cihova<sup>1</sup>, J.A. Kim<sup>1</sup>, Q.Y. Lau<sup>1</sup>, M. M. Stevens<sup>1</sup>

<sup>1</sup>Department of Materials, Department of Bioengineering, and Institute of Biomedical Engineering, Imperial College London, SW7 2AZ, UK

Cells typically respond to a variety of geometrical cues in their environment, ranging from nanoscale surface topography to mesoscale surface curvature<sup>1</sup>. The ability to control cellular organisation and fate by engineering the shape of the extracellular milieu offers exciting opportunities within tissue engineering. Despite great progress, however, many questions regarding geometry-driven tissue growth remain unanswered.

Here, we combine mathematical surface design, high-resolution microfabrication, in vitro cell culture, and image-based characterization to study spatiotemporal cell patterning and bone tissue formation in geometrically complex environments. Using concepts from differential geometry, we rationally designed a library of complex mesostructured substrates ( $10^1$ - $10^3$   $\mu\text{m}$ ). These substrates were accurately fabricated using a combination of two-photon polymerisation and replica moulding, followed by surface functionalisation. Subsequently, different cell types (preosteoblasts, fibroblasts, mesenchymal stromal cells) were cultured on the substrates for varying times and under varying osteogenic conditions. Using imaging-based methods, such as fluorescent confocal microscopy and second harmonic generation imaging, as well as quantitative image processing, we were able to study early-stage spatiotemporal cell patterning and late-stage extracellular matrix organisation. Our results demonstrate clear geometry-dependent cell patterning, with cells generally avoiding convex regions in favour of concave domains. Moreover, the formation of multicellular bridges and collective curvature-dependent cell orientation could be observed. At longer time points, we found clear and robust geometry-driven orientation of the collagenous extracellular matrix, which became apparent with second harmonic generation imaging after  $\sim 2$  weeks of culture.

Our results highlight a key role for geometry as a cue to guide spatiotemporal cell and tissue organisation, which is relevant for scaffold design in tissue engineering applications. Our ongoing work aims at understanding the underlying principles of geometry-driven tissue growth, with a focus on the interactions between substrate geometry and mechanical forces.

**References:** <sup>1</sup>Callens et al., *Nature Communications*, 14(1), 2023

27-29 SEPTEMBER | PORTO, PORTUGAL

## Three Dimensional, Porous Composite Scaffolds with High Calcium Phosphate Content and Addition of Inorganic Ions for Bone Regeneration Applications

Martyna Nikody<sup>1,2</sup>, Jiaping Li<sup>1,2</sup>, David Koper<sup>1,2,3</sup>, Elizabeth Rosado Balmayor<sup>4</sup>, Pamela Habibovic<sup>1</sup>, Lorenzo Moroni<sup>2</sup>

<sup>1</sup>Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands;

<sup>2</sup>Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands;

<sup>3</sup>Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Center, Maastricht, the Netherlands; <sup>4</sup>Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany

Critical-sized bone defects remain challenging in the clinical setting. Autologous bone grafting remains preferred by clinicians. However, the use of autologous tissue is associated with donor-site morbidity and limited accessibility to the graft tissue (1). Advances in the development of synthetic bone substitutes focus on improving their osteoinductive properties. Whereas osteoinductivity has been demonstrated with ceramics, it is still a challenge in case of polymeric composites. One of the approaches to improve the regenerative properties of biomaterials, without changing their synthetic character, is the addition of inorganic ions with known osteogenic and angiogenic properties (2). We have previously reported that the use of a bioactive composite with high ceramic content composed of poly(ethyleneoxide terephthalate)/poly(butylene terephthalate) (1000PEOT70PBT30, PolyActive, PA) and 50% beta-tricalcium phosphate ( $\beta$ -TCP) with the addition of zinc in a form of a coating of the TCP particles can enhance the osteogenic differentiation of human mesenchymal stromal cells (hMSCs) (3). To further support the regenerative properties of these scaffolds, inorganic ions with known angiogenic properties, copper or cobalt, were added to the coating solution.

$\beta$ -TCP particles were immersed in a zinc and copper or zinc and cobalt solution with a concentration of 15 or 45 mM. 3D porous scaffolds composed of 1000PEOT70PBT30 and pure or coated  $\beta$ -TCP were additively manufactured by 3D fibre deposition. The osteogenic and angiogenic properties of the fabricated scaffolds were tested *in vitro* through culture with hMSCs and human umbilical vein endothelial cells, respectively. The materials were further evaluated through ectopic implantation in an *in vivo* mini-pig model. The early expression of relevant osteogenic gene markers (collagen-1, osteocalcin) of hMSCs was upregulated in the presence of lower concentration of inorganic ions. Further analysis will focus on the evaluation of ectopic bone formation and vascularisation of these scaffolds after implantation in a mini-pig ectopic intramuscular model.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Multiscale assessment of Achilles Tendon mechanics and mechanobiology

Hanna Isaksson<sup>1</sup>, Maria Pierantoni<sup>1</sup>, Isabella Silva Barreto<sup>1</sup>, Malin Hammerman<sup>1,2</sup>, Pernilla Eliasson<sup>1-2</sup>

<sup>1</sup>Lund University, Sweden; <sup>2</sup>Linköping University, Sweden; <sup>3</sup>Gothenburg University, Sweden.

Achilles tendon mechanical properties depend on a complex hierarchical design, with collagen being the smallest load-bearing unit. At the nanoscale, collagen molecules are organized into fibrils, which at the microscale are assembled into fibers, followed by larger structures such as sub-tendons or fascicles. Degree of *in vivo* loading affects the collagen content, and organization and consequently the tissue's mechanical response. We aim to unravel how composition, structural organization, and mechanical response are affected by degree of *in vivo* loading at each length scale. The presentation will outline the results to date about the use of high-resolution synchrotron-based tissue characterisation methods on several length scales in combination with *in situ* mechanical tests. We use a rat model, where the tendons are subjected to varying loading *in vivo* [1]. To characterize the tissue microstructure, phase-contrast enhanced synchrotron micro-tomography is performed [2]. The 3D fiber organization in fully loaded tendons is highly aligned, whereas the fibers in unloaded tendons are significantly more heterogeneously arranged and crimped (Fig 1). To characterize the collagen fibril response, Small Angle X-ray Scattering is performed [3]. Two types of fibril organizations are found; a single population oriented towards the main load direction and two fibril subpopulations with clearly distinct orientations. Scattering during loading showed that the fibrils in unloaded tendons did not strain as much in fully loaded (Fig 1). *In situ* loading concurrently with high resolution synchrotron experiments show the complex tendon response to *in situ* load and its relation to *in vivo* loading and tendon hierarchical structure. Unloading seems to alter the organization of the fibrils and fibers, e.g. increased crimping and more pronounced sub-tendon twists.

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**An ex vivo study investigating the degenerative impact of MMPs-2, -3, and -7 on the biomechanical properties of the pericellular matrix in articular cartilage****Benjamin Baumann<sup>1</sup>, Marina Danalache<sup>1</sup>**<sup>1</sup>Laboratory of Cell Biology, Department of Orthopedic Surgery, University of Tübingen, Germany

Matrix metalloproteinase enzymes (MMPs) play a crucial role in the remodeling of articular cartilage, contributing also to osteoarthritis (OA) progression. The pericellular matrix (PCM) is a specialized space surrounding each chondrocyte, containing collagen type VI and perlecan. It acts as a transducer of biomechanical and biochemical signals for the chondrocyte [1]. This study investigates the impact of MMP-2, -3, and -7 on the integrity and biomechanical characteristics of the PCM.

Human articular cartilage explants (n=10 patients, ethical-nr.:674/2016BO2) were incubated with activated MMP-2, -3, or -7 as well as combinations of these enzymes. The structural degradative effect on the PCM was assessed by immunolabelling of the PCM's main components: collagen type VI and perlecan. Biomechanical properties of the PCM in form of the elastic moduli (EM) were determined by means of atomic force microscopy (AFM), using a spherical cantilever tip (2.5µm).

MMPs disrupted the PCM-integrity, resulting in altered collagen type VI and perlecan structure and dispersed pericellular arrangement. A total of 3600 AFM-measurements revealed that incubation with single MMPs resulted in decreased PCM stiffness (p<0.001) when compared to the untreated group. The overall EM were reduced by ~36% for all the 3 individual enzymes. The enzyme combinations altered the biomechanical properties at a comparable level (~36%, p<0.001), except for MMP-2/-7 (p=0.202).

MMP-induced changes in the PCM composition have a significant impact on the biomechanical properties of the PCM, similar to those observed in early OA [2]. Each individual MMP was shown to be highly capable of selectively degrading the PCM microenvironment. The combination of MMP-2 and -7 showed a lower potency in reducing the PCM stiffness, suggesting a possible interplay between the two enzymes. Our study showed that MMP-2, -3, and -7 play a direct role in the functional and structural remodeling of the PCM.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Mechanical regulation of matrix remodeling in tendon wound healing

Alina M. Dintheer<sup>1,2</sup>, Patrick K. Jaeger<sup>1,2</sup>, Amro A. Hussien<sup>1,2</sup>, Jess G. Snedeker<sup>1,2</sup>

<sup>1</sup>University Hospital Balgrist, University of Zürich, Switzerland; <sup>2</sup>Institute for Biomechanics, ETH Zürich, Switzerland

Extracellular matrix (ECM) mechanical cues guide healing in tendons [1],[2]. Yet, the molecular mechanisms orchestrating the healing processes remain elusive. Appropriate tissue tension is essential for tendon homeostasis and tissue health [3]. By mapping the attainment of tensional homeostasis, we aim to understand how ECM tension regulates healing. We hypothesize that diseased tendon returns to homeostasis only after the cells reach a mechanically gated exit from wound healing. We engineered a 3D mechano-culture system to create tendon-like constructs by embedding patient-derived tendon cells into a collagen I hydrogel. Casting the hydrogel between posts anchored in silicone allowed adjusting the post stiffness. Under this static mechanical stimulation, cells remodel the (unorganized) collagen representing wound healing mechanisms. We quantified tissue-level forces using post deflection measurements. Secreted ECM was visualized by metabolic labelling with non-canonical amino acids, click chemistry and confocal microscopy [4]. We blocked cell-mediated actin-myosin contractility using a ROCK inhibitor (Y27632) to explore the involvement of the Rho/ROCK pathway in tension regulation [5],[6].

Tissue tension forces reached the same homeostatic level at day 21 independent of post compliance ( $p = 0.9456$ ). While minimal matrix was synthesized in early phases of tissue formation (d3-d5), cell-deposited ECM was present in later stages (d7-d9). More ECM was deposited by tendon constructs cultured on compliant (1Nm) compared to rigid posts ( $p = 0.0017$ ). Matrix synthesized by constructs cultured on compliant posts was less aligned (greater fiber dispersion,  $p = 0.0021$ ). ROCK inhibition significantly decreased tissue-level tensional forces ( $p < 0.0001$ ).

Our results indicate that tendon cells balance matrix remodeling and synthesis during tissue repair to reach an intrinsically defined “mechanostat setpoint” guiding tension-mediated exit from wound healing towards homeostasis. We are identifying specific molecular mechanosensors governing tension-regulated healing in tendon and investigate the Rho/ROCK system as their possible downstream pathway.

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**Chondrocyte-Specific Knockout of Piezo Ion Channels Protects Against Post-Traumatic Osteoarthritis**

Erica V. Ely<sup>1,2</sup>, Kelsey H. Collins<sup>1,2</sup>, Kristin Lenz<sup>1,2</sup>, Sophie Paradi<sup>1,2</sup>, Wolfgang Liedtke<sup>4</sup>, Yong Chen<sup>3</sup>, Farshid Guilak<sup>1,2</sup>

<sup>1</sup>Washington University in St. Louis, MO USA; <sup>2</sup>Shriners Hospitals for Children, St. Louis, MO USA; <sup>3</sup>Duke University, Durham, NC USA; <sup>4</sup>Regeneron Pharmaceuticals, Tarrytown NY USA

Osteoarthritis (OA) is the leading cause of pain and disability worldwide<sup>1</sup> and is characterized by the degenerative changes of articular cartilage. Joint loading is required for cartilage maintenance<sup>2</sup>; however, hyper-physiologic loading is a risk factor for OA<sup>2,3</sup>. Mechanosensitive ion channels Piezo1 and Piezo2 synergistically transduce hyper-physiologic compression of chondrocytes, leading to chondrocyte death and onset of OA<sup>4-8</sup>. This injury response is inhibited by Piezo channel loss of function<sup>5</sup>, however the mechanistic role of Piezo channels *in vivo* is unknown. We examined the **hypothesis** that deletion of *Piezo* in chondrocytes will protect mice from joint damage and pain-related behaviors following a surgical destabilization of the medial meniscus (DMM), investigating a key mechanistic and mechanobiological role of these channels in the pathogenesis of OA.

Aggrecan-Cre *Piezo1* and *Piezo1/2* knockout mice ((*Agc*)1-CRE<sup>ERT2</sup>; *Piezo1*<sup>fl/fl</sup> *Piezo2*<sup>fl/fl</sup>) were generated and given a 5-day Tamoxifen regimen at 12-weeks of age (n=6-12/group/sex). Cre-negative mice served as controls. At 16-weeks, mice received DMM surgery on the left knee. 12-weeks following DMM prior to sacrifice, activity and hyperalgesia were measured using spontaneous running wheels and a small animal algometer. Structural changes in bone, cartilage, and synovium were characterized using microCT, histology, and Modified Mankin Score criteria.

Knockout of *Piezo1/2* channels was chondroprotective in both sexes following DMM surgery as demonstrated by reduced Modified Mankin Score compared to control animals. *Piezo1* KO was chondroprotective in only female mice, indicating a sexually dimorphic response. *Piezo1* and *Piezo1/2* KO was protective against pain in male mice, while females displayed no differences compared to controls. No changes were observed in bone morphology.

Chondrocyte-specific *Piezo1/2* knockout protects the knee joint from structural damage, hyperalgesia and functional deficits in a surgical model of PTOA in male and female mice, illustrating the importance of Piezo channels in response to injury *in vivo*. Future work aims to interrogate potential sexually dimorphic responses to cartilage damage and investigating *Piezo2* KO mice.

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# EORS 2023

31st Annual Meeting of the  
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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****The Role of Mechanosensory Neurons in Musculoskeletal Diseases**

Shang Ma<sup>1</sup>, Adrienne Dubin<sup>2,3</sup>, Luis Romero<sup>4</sup>, Meaghan Loud<sup>2,3</sup>, Alexandra Salazar<sup>2</sup>, Sarah Chu<sup>5</sup>, Nikola Klier<sup>5</sup>, Sameer Masri<sup>5</sup>, Yunxiao Zhang<sup>2,3</sup>, Yu Wang<sup>2,3</sup>, Alex Chesler<sup>6</sup>, Katherine Wilkinson<sup>5</sup>, Valeria Vásquez<sup>4</sup>, Kara Marshall<sup>7</sup>, Ardem Patapoutian<sup>2,3</sup>

<sup>1</sup>UT Southwestern; <sup>2</sup>The Scripps Research Institute; <sup>3</sup>Howard Hughes Medical Institute, <sup>4</sup>University of Tennessee Health Science Center; <sup>5</sup>San Jose State University, <sup>6</sup>National Institute of Health; <sup>7</sup>Baylor College of Medicine

Distal arthrogyposis (DA) is a collection of rare developmental disorders characterized by congenital joint contractures. Most arthrogyposis mutations are in muscle- and joint-related genes, and the anatomical defects originate cell-autonomously within the musculoskeletal tissues. However, gain-of-function (GOF) mutations in PIEZO2, a principal mechanosensor in somatosensation, cause DA subtype 5 via unknown mechanisms. We show that expression of a GOF PIEZO2 mutation in proprioceptive sensory neurons mainly innervating muscle spindles and tendons is sufficient to induce DA5-like phenotypes in mice. Overactive PIEZO2 causes anatomical defects via increased activity within the peripheral nervous system during postnatal development. Surprisingly, overactive PIEZO2 is likely to cause joint abnormalities via increased exocytosis from sensory neuron endings without involving motor circuitry. This reveals a role for somatosensory neurons: excessive mechanosensation within these neurons disrupts musculoskeletal development. We also present proof-of-concept that Botox injection or dietary treatment can counteract the effect of overactive PIEZO2 function to evade DA-like phenotypes in mice when applied during a developmental critical period. These approaches might have clinical applications. Beyond this, our findings call attention to the importance of considering sensory mechanotransduction when diagnosing and treating other musculoskeletal disorders.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Age-related mechanisms of altered tendon structure and function

Alayna E. Loisel<sup>1</sup>

<sup>1</sup>University of Rochester Medical Center, Rochester, NY USA

During aging, tendons demonstrate substantial disruptions in homeostasis, leading to impairments in structure-function. Impaired tendon function contributes to substantial declines in quality of life during aging. Aged tendons are more likely to undergo spontaneous rupture, and the healing response following injury is impaired in aged tendons. Thus, there is a need to develop strategies to maintain tendon homeostasis and healing capacity through the lifespan. Tendon cell density sharply declines by ~12 months of age in mice, and this low cell density is retained in geriatric tendons. Our data suggests that this decline in cellularity initiates a degenerative cascade due to insufficient production of the extracellular matrix (ECM) components needed to maintain tendon homeostasis. Thus, preventing this decline in tendon cellularity has great potential for maintaining tendon health. Single cell RNA sequencing analysis identifies two changes in the aged tendon cell environment. First, aged tendons primarily lose tenocytes that are associated with ECM biosynthesis functions. Second, the tenocytes that remain in aged tendons have disruptions in proteostasis and an increased pro-inflammatory phenotype, with these changes collectively termed 'programmatic skewing'. To determine which of these changes drives homeostatic disruption, we developed a model of tenocyte depletion in young animals. This model decreases tendon cellularity to that of an aged tendon, including decreased biosynthetic tenocyte function, while age-related programmatic skewing is absent. Loss of biosynthetic tenocyte function in young tendons was sufficient to induce homeostatic disruption comparable to natural aging, including deficits in ECM organization, composition, and material quality, suggesting loss of biosynthetic tenocytes as an initiator of tendon degeneration. In contrast, our data suggest that programmatic skewing underpins impaired healing in aged tendons. Indeed, despite similar declines in the tenocyte environment, middle-aged and young-depleted tendons mount a physiological healing response characterized by robust ECM synthesis and remodeling, while aged tendons heal with insufficient ECM.

**Immunomodulatory potential of magnetically-assisted cell sheet under hypoxic environments upon magnetic stimulation**

Adriana Vinhas<sup>1,2</sup>, Márcia T. Rodrigues<sup>1,2</sup>, Ana I. Gonçalves<sup>1,2</sup>, Manuela E. Gomes<sup>1,2</sup>

<sup>1</sup>3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal; <sup>2</sup>ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

Common tendon injuries impair healing, leading to debilitation and an increased re-rupture risk. The impact of oxygen-sensing pathways on repair mechanisms, vital in regulating inflammation and fibrosis, remains unclear despite their relevance in tendon pathologies. Recent studies show that pulsed electromagnetic field (PEMF) reduce inflammation in human tendon cells (hTDCs) and in hypoxia-induced inflammation. We investigated the hypoxia's impact (1% and 2% oxygen tension) using magnetic cell sheet constructs (IL-1b-magCSs) primed with IL-1b. IL-1b-magCSs were exposed to low OT (1h, 4h,6h) in a hypoxic chamber. To confirm the role of PEMF (5Hz, 4mT, 50% duty cycle) on hypoxia modulation, IL-1b-magCSs, previously exposed to OT, were 1h-stimulated with PEMF. Our results show a significant increase in *HIF-1 $\alpha$*  and *HIF-2 $\alpha$*  expression on IL-1b-magCSs after exposure to 2%-OT at all time points, compared to 1%- OT and normoxia. TNF $\alpha$ , IL-6, and IL-8 expression increased after 6 hours of 1%-OT exposure. PEMF stimulation of hypoxic IL-1b-magCSs led to decreased pro-inflammatory genes and increased anti-inflammatory (IL-4,IL-10) expression compared to unstimulated magCSs. IFN-g, TNF-a, and IL-6 release increased after 6 hours, regardless of %-OT, while IL-10 levels tended to rise after PEMF stimulation at 2%-OT. Also, *NFkB* expression was increased on IL-1b-magCSs exposed to 4 h and 6 h of 2%-OT, suggesting a link between NFkB and the production of pro-inflammatory factors. Moreover, PEMF stimulation showed a significantly decreased NFkB level in IL-1b-magCSs.

Overall, low OT enhances expression of hypoxia-associated genes and inflammatory markers in IL-1b- magCSs with the involvement of NFkB. PEMF modulates the response of magCSs, previously conditioned to hypoxia and to inflammatory triggers, favouring expression of anti-inflammatory genes and proteins, supporting PEMF impact in pro-regenerative tendon strategies.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **TRP-channels in tendons: first insights, novel questions**

Christine Lehner<sup>1,3</sup>, Bruno Benedetti<sup>2,3</sup>, Herbert Tempfer<sup>1,3</sup>, Andreas Traweger<sup>1,3</sup>

<sup>1</sup>Institute of Tendon & Bone Regeneration, Paracelsus Medical University, 5020 Salzburg, Austria; <sup>2</sup>Institute of Experimental Neuroregeneration, Paracelsus Medical University, 5020 Salzburg, Austria; <sup>3</sup>Austrian Cluster for Tissue Regeneration, 1200 Vienna, Austria

Tendinopathy is a disease associated with pain and tendon degeneration, leading to a decreased range of motion and an increased risk of tendon rupture. The etiology of this frequent disease is still unknown. In other musculoskeletal tissues like cartilage and intervertebral discs, transient receptor potential channels (TRP- channels) were shown to play a major role in the progression of degeneration. Due to their responsiveness to a wide range of stimuli like temperature, pH, osmolarity and mechanical load, they are potentially relevant factors in tendon degeneration as well. We therefore hypothesize that TRP- channels are expressed in tendon cells and respond to degeneration inducing stimuli.

By immunohistochemistry, qRT-PCR and western blot analyses, we found three TRP channel members, belonging to the vanilloid (TRPV), and ankyrin (TRPA) subfamily, respectively, to be expressed in healthy human tendon tissue as well as in rodent tendon, with expression being located to cells within the dense tendon proper, as well as to endotenon resident cells. In vitro-inflammatory and ex vivo-mechanical stimulation led to a significant upregulation of TRPA1 expression in tendon cells, which correlates well with the fact that TRPA1 is considered as mechanosensitive channel being sensitized by inflammatory mediators.

This is the first description of TRP- channels in human and rodent tendon. As these channels are pharmacologically targetable by both agonists and antagonists, they may represent a promising target for novel treatments of tendinopathy.

**Rapamycin has a limited effect on tendon healing in a rodent model**

Neil Marr<sup>1</sup>, Danae E. Zamboulis<sup>1</sup>, Ross E. Beaumont<sup>2</sup>, Zofia J. Tatarczyk<sup>1</sup>, Richard Meeson<sup>2</sup>, Chavaunne T. Thorpe<sup>1</sup>

<sup>1</sup>Comparative Biomedical Sciences, Royal Veterinary College, London; <sup>2</sup>Clinical Sciences and Services, Royal Veterinary College, London

Tendon injuries occur frequently in athletes and the general population, with inferior healing leading to deposition of fibrotic scar tissue. New treatments are essential to limit fibrosis and enable tendon regeneration post-injury. In this study, we tested the hypothesis that rapamycin improves tendon repair and limits fibrosis by inhibiting the mTOR pathway.

The left hindlimb of female adult Wistar rats was injured by needle puncture and animals were either given daily injections of rapamycin (2mg/kg) or vehicle. Animals were euthanized 1 week or 3 weeks post-injury (n=6/group). Left and right Achilles tendons were harvested, with the right limbs acting as controls. Tendon sections were stained with haematoxylin & eosin, and scored by 2 blinded scorers, assessing alterations in cellularity, cell morphology, vascularity, extracellular matrix (ECM) organization and peritendinous fibrosis. Immunohistochemistry was performed for the tendon pan-vascular marker CD146 and the autophagy marker LC3.

Injury resulted in significantly altered ECM organization, cell morphology and cellularity in both rapamycin and vehicle-treated groups, but no alterations in vascularity compared to uninjured tendons. Rapamycin had a limited effect on tendon repair, with a significant reduction in peritendinous fibrosis 3 weeks after injury (p=0.028) but no change in cell morphology, cellularity or ECM organization compared to vehicle treated tendons at either 1 week or 3 weeks post injury. CD146 labelling was increased at the site of injury, but there was no apparent difference in CD146 or LC3 labelling in rapamycin and vehicle treated tendons.

The decrease in peritendinous fibrosis post-injury observed in rapamycin treated tendons indicates rapamycin as a potential therapy for tendon adhesions. However, the lack of improvement of other morphological parameters in response to rapamycin treatment indicates that rapamycin is not an effective therapy for injuries to the tendon core.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Establishing Novel Markers of Tendon Cell Populations

Zamboulis D.E.<sup>1</sup>, Ali F.S.S.<sup>1</sup>, Thorpe C.T.<sup>1</sup>

<sup>1</sup>Department of Comparative Biomedical Sciences, Royal Veterinary College, London, NW1 0TU, UK

Energy storing tendons such as the human Achilles and equine superficial digital flexor tendon (SDFT) are prone to age-related injury. Tendons have poor healing capacity and a lack of effective treatments can lead to ongoing pain, reduced function and re-injury. It is therefore important to identify the mechanisms underpinning age-related tendinous changes in order to develop more effective treatments. Our recent single cell sequencing data has shown that tendon cell populations have extensive heterogeneity and cells housed in the tendon interfascicular matrix (IFM) are preferentially affected by ageing. There is, however, a lack of established surface markers for cell populations in tendon, limiting the capacity to isolate distinct cell populations and study their contribution to age-related tendon degeneration. Here, we investigate the presence of the cell surface proteins MET proto-oncogene (MET), integrin subunit alpha 10 (ITGA10), fibroblast activation protein alpha (FAP) and platelet derived growth factor receptor alpha (PDGFRA) in the equine SDFT cell populations and their co-localisation with known markers.

Using Western blot we validated the specificity of selected antibodies in equine tissue before performing immunohistochemistry to establish the location of the respective proteins in the SDFT. We subsequently used double labelling immunofluorescence with the established mural cell marker desmin (DES) to distinguish between tenocyte and mural cell populations.

In situ, MET, ITGA10, and FAP presence was found in cells throughout the tendon whereas PDGFRA was present in cells within the IFM. Double labelling immunofluorescence with the mural cell marker DES showed lack of co-localisation between PDGFRA and DES suggesting PDGFRA is labelling an IFM cell population distinct from those associated with blood vessels.

PDGFRA is a promising target for the specific cell sorting of IFM-localised tenocytes, enabling their isolation and subsequent characterisation.

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**Biomechanics and Design of Intramedullary Nails**Boyko Gueorguiev<sup>1</sup>, Peter Varga<sup>1</sup><sup>1</sup>AO Research Institute Davos, Switzerland

Intramedullary nails (IMNs) are the current gold standard for treatment of long bone diaphyseal and selected metaphyseal fractures. Their design has undergone many revisions to improve fixation techniques, conform to the bone shape with appropriate anatomic fit, reduce operative time and radiation exposure, and extend the indication of the same implant for treatment of different fracture types with minimal soft tissue irritation.

The IMNs are made of either titanium alloy or stainless steel and work as load-sharing internal splints along the long bone, usually accommodating locking elements – screws and blades, often featuring angular stability and offering different configurations for multiplanar fixation – to secure secondary fracture healing with callus formation in a relative-stability environment. Bone cement augmentation of the locking elements can modulate the construct stiffness, increase the surface area at the bone-implant interface, and prevent cut-through of the locking elements.

The functional requirements of IMNs are related to maintaining fracture reduction in terms of length, alignment and rotation to enhance fracture healing. The load distribution during patient's activities is along the entire bone-nail interface, with nail length and anatomic fit being important factors to avoid stress risers.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Machine learning can predict difficulty in anterior approach total hip arthroplasty, to improve patient safety and surgical training**

Hariharan Subbiah Ponniah<sup>1</sup>, Thomas Edwards<sup>1</sup>, Jonathan Lex<sup>2</sup>, Ross Davidson<sup>1</sup>, Mustafa Al-Zubaidy<sup>1</sup>, Irrum Afzal<sup>3</sup>, Richard Field<sup>3</sup>, Alexander Liddle<sup>1</sup>, Justin Cobb<sup>1</sup>, Kartik Logishetty<sup>1</sup>

<sup>1</sup>Imperial College London, London, United Kingdom; <sup>2</sup>University of Toronto, Toronto, Canada; <sup>3</sup>South West London Elective Orthopaedic Centre, London, United Kingdom

Anterior approach total hip arthroplasty (AA-THA) has a steep learning curve, with higher complication rates in initial cases. Proper surgical case selection during the learning curve can reduce early risk. This study aims to identify patient and radiographic factors associated with AA-THA difficulty using Machine Learning (ML). Consecutive primary AA-THA patients from two centres, operated by two expert surgeons, were enrolled (excluding patients with prior hip surgery and first 100 cases per surgeon). K- means prototype clustering – an unsupervised ML algorithm – was used with two variables - operative duration and surgical complications within 6 weeks - to cluster operations into difficult or standard groups.

Radiographic measurements (neck shaft angle, offset, LCEA, inter-teardrop distance, Tonnis grade) were measured by two independent observers. These factors, alongside patient factors (BMI, age, sex, laterality) were employed in a multivariate logistic regression analysis and used for k-means clustering. Significant continuous variables were investigated for predictive accuracy using Receiver Operator Characteristics (ROC).

Out of 328 THAs analyzed, 130 (40%) were classified as difficult and 198 (60%) as standard. Difficult group had a mean operative time of 106mins (range 99-116) with 2 complications, while standard group had a mean operative time of 77mins (range 69-86) with 0 complications. Decreasing inter-teardrop distance (odds ratio [OR] 0.97, 95% confidence interval [CI] 0.95-0.99,  $p = 0.03$ ) and right-sided operations (OR 1.73, 95% CI 1.10- 2.72,  $p = 0.02$ ) were associated with operative difficulty. However, ROC analysis showed poor predictive accuracy for these factors alone, with area under the curve of 0.56. Inter-observer reliability was reported as excellent (ICC >0.7).

Right-sided hips (for right-hand dominant surgeons) and decreasing inter-teardrop distance were associated with case difficulty in AA-THA. These data could guide case selection during the learning phase. A larger dataset with more complications may reveal further factors.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Thoracolumbar Spine Patient-Specific Finite Element Model Repository with Online User-Interface Platform**

Morteza Rasouligandomani<sup>1</sup>, Francis Chemorion<sup>1,2,3</sup>, Marc-Antonio Bisotti<sup>2,4</sup>, Jérôme Noailly<sup>1</sup>, Miguel A. González Ballester<sup>1,5</sup>

<sup>1</sup>DTIC department, University Pompeu Fabra, Barcelona, Spain; <sup>2</sup>In-silico trials Technologies; <sup>3</sup>Barcelona Supercomputing Center; <sup>4</sup>Promeditec; <sup>5</sup>ICREA, Barcelona, Spain

Adult Spine Deformity (ASD) is a degenerative condition of the adult spine leading to altered spine curvatures and mechanical balance. Computational approaches, like Finite Element (FE) Models have been proposed to explore the etiology or the treatment of ASD, through biomechanical simulations. However, while the personalization of the models is a cornerstone, personalized FE models are cumbersome to generate. To cover this need, we share a virtual cohort of 16807 thoracolumbar spine FE models with different spine morphologies, presented in an online user-interface platform (SpineView). To generate these models, EOS images are used, and 3D surface spine models are reconstructed [1]. Then, a Statistical Shape Model (SSM), is built, to further adapt a FE structured mesh template [2] for both the bone and the soft tissues of the spine, through mesh morphing. Eventually, the SSM deformation fields allow the personalization of the mean structured FE model, leading to generate FE meshes of thoracolumbar spines with different morphologies. Models can be selectively viewed and downloaded through SpineView, according to personalized user requests of specific morphologies characterized by the geometrical parameters: Pelvic Incidence; Pelvic Tilt; Sacral Slope; Lumbar Lordosis; Global Tilt; Cobb Angle; and GAP score [3]. Data quality is assessed using visual aids, correlation analyses, heatmaps, network graphs, Anova and t-tests, and kernel density plots to compare spinopelvic parameter distributions and identify similarities and differences. Mesh quality and ranges of motion have been assessed to evaluate the quality of the FE models. This functional repository is unique to generate virtual patient cohorts in ASD.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Validated finite element simulations predict overloading failure of osteosynthesis plates

Dominic Mischler<sup>1</sup>, Markus Windolf<sup>1</sup>, Boyko Gueorguiev<sup>1</sup>, Peter Varga<sup>1</sup>

<sup>1</sup>AO Research Institute Davos, Switzerland

Osteosynthesis aims to maintain fracture reduction until bone healing occurs, which is not achieved in case of mechanical fixation failure. One form of failure is plastic plate bending due to overloading, occurring in up to 17% of midshaft fracture cases and often necessitating reoperation [2,3]. This study aimed to replicate in-vivo conditions in a cadaveric experiment and to validate a finite element (FE) simulation to predict plastic plate bending.

Six cadaveric bones were used to replicate an established ovine tibial osteotomy model with locking plates [4] in-vitro with two implant materials (titanium, steel) and three fracture gap sizes (30, 60, 80 mm). The constructs were tested monotonically until plastic plate deformation under axial compression. Specimen-specific FE models were created from CT images. Implant material properties were determined using uniaxial tensile testing of dog bone shaped samples. The experimental tests were replicated in the simulations. Stiffness, yield, and maximum loads were compared between the experiment and FE models.

Implant material properties (Young's modulus and yield stress) for steel and titanium were 184 GPa and 875 MPa, and 105 GPa and 761 MPa, respectively. Yield and maximum loads of constructs ranged between 469-491 N and 652- 683 N, and 759-995 N and 1252-1600 N for steel and titanium fixations, respectively. FE models accurately and quantitatively correctly predicted experimental results for stiffness ( $R^2=0.96$ ), yield ( $R^2=0.97$ ), and ultimate load ( $R^2=0.97$ ).

FE simulations accurately predicted plastic plate bending in osteosynthesis constructs. Construct behavior was predominantly driven by the implant itself, highlighting the importance of modelling correct material properties of metal. The validated FE models could predict subject-specific load bearing capacity of osteosyntheses *in vivo* in preclinical or clinical studies.

**References:** 1. Perren et al., Acta Chir. Orthop. Traum. Cech., 82, 2015; 2. Alzahrani et al., J Orthop Traumatol, 19:8, 2018; 3. Henderson et al., J Orthop Trauma, 1:S8-14, 2011; 4. Windolf et al., Medicina, 7:858, 2022.

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**Fatigue life prediction of 3D-printed porous titanium implants using validated finite element analyses**

Antoine Vautrin<sup>1</sup>, Jensen Aw<sup>2</sup>, Ed Attenborough<sup>2</sup>, Peter Varga<sup>1</sup>

<sup>1</sup>AO Research Institute Davos, Switzerland; <sup>2</sup>Attenborough Dental, United Kingdom

Although 3D-printed porous dental implants may possess improved osseointegration potential, they must exhibit appropriate fatigue strength [1]. Finite element analysis (FEA) has the potential to predict the fatigue life of implants and accelerate their development [2]. This work aimed at developing and validating an FEA-based tool to predict the fatigue behavior of porous dental implants.

Test samples mimicking dental implants were designed as 4.5 mm-diameter cylinders with a fully porous section around bone level. Three porosity levels (50%, 60% and 70%) and two unit cell types (Schwarz Primitive (SP) and Schwarz W (SW)) were combined to generate six designs that were split between calibration (60SP, 70SP, 60SW, 70SW) and validation (50SP, 50SW) sets.

Twenty-eight samples per design were additively manufactured from titanium powder (Ti6Al4V). The samples were tested under bending compression loading (ISO 14801) monotonically (N=4/design) to determine ultimate load ( $F_{ult}$ ) (Instron 5866) and cyclically at six load levels between 50% and 10% of  $F_{ult}$  (N=4/design/load level) (DYNA5dent). Failure force results were fitted to  $F/F_{ult} = a(N_f)^b$  (Eq1) with  $N_f$  being the number of cycles to failure, to identify parameters  $a$  and  $b$ . The endurance limit ( $F_e$ ) was evaluated at  $N_f = 5M$  cycles. Finite element models were built to predict the yield load ( $F_{yield}$ ) of each design. Combining a linear correlation between FEA-based  $F_{yield}$  and experimental  $F_{ult}$  with equation Eq1 enabled FEA-based prediction of  $F_e$ .

For all designs,  $F_e$  was comprised between 10% (all four samples surviving) and 15% (at least one failure) of  $F_{ult}$ . The FEA-based tool predicted  $F_e$  values of 11.7% and 12.0% of  $F_{ult}$  for the validation sets of 50SP and 50SW, respectively. Thus, the developed FEA-based workflow could accurately predict endurance limit for different implant designs and therefore could be used in future to aid the development of novel porous implants.

**References:** 1. Wally et al, Metals, 5(4):1902-1920,2015; 2. Wang et al, Proc Inst Mech Eng H, 233(2):170-180, 2019.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Does Lower Extremity Skeletal Muscle Mass Determine Osteoarthritis Risk?

Yasemin Polat Özer<sup>1</sup>, Didem Karaduman<sup>1</sup>, Yigitcan Karanfil<sup>2</sup>, Emine Çiftçi<sup>2</sup>, Cafer Balci<sup>1</sup>,  
Burcu Balam Doğu<sup>1</sup>, Meltem Gülhan Halil<sup>1</sup>, Mustafa Cankurtaran<sup>1</sup>, Feza Korkusuz<sup>2</sup>

<sup>1</sup>Hacettepe University Medical Faculty (1); Department of Geriatrics and (2) Department of Sports Medicine, Ankara, Turkey

Osteoarthritis (OA) of the knee joint is a complex peripheral joint disorder with multiple risk factors. We aimed to examine the relationship between the grade of knee OA and anterior thigh length (ATL).

A total of 64 geriatric patients who had no total hip or knee replacement with a BMI of  $\geq 30$  were evaluated. Patients' OA severity was determined by two independent experts from bilateral standing knee radiographs according to the Kellgren-Lawrence (KL) grade. Joint cartilage structure was assessed using ultrasonography (US). The ATL, the gastrocnemius medialis (GC), the rectus femoris (RF) and the rectus abdominis (RA) skeletal muscle thicknesses as well as the RF cross-sectional area (CSA) were measured with US. Sarcopenia was diagnosed using the handgrip strength (HGS), 5x sit-to-stand test (5xSST) and bioelectrical impedance analysis.

The median (IQR) age of participants was 72 (65-88) years. Seventy-one per cent of the patients (n=46) were female. They were divided into the sarcopenic obese (31.3 %) and the non-sarcopenic obese (68.8%) groups. KL grade of all patients correlated negatively with the ATL (mm) and the thickness of GC (mm) ( $r = -0,517$ ,  $p < 0.001$  and  $r = -0.456$ ,  $p < 0.001$ , respectively). In the sarcopenic obese and the non-sarcopenic obese groups, KL grade of the all patients was negatively correlated with ATL (mm) and thickness of GC (mm) ( $r = -0,986$ ,  $p < 0.001$ ;  $r = -0.456$ ,  $p = 0.05$  and  $r = -0,812$ ,  $p = 0.002$ ;  $r = -0,427$ ,  $p = 0.006$ ). KL grade negatively correlated with the RF thickness in the sarcopenic obese group ( $r = -0,928$ ,  $p = 0.008$ ).

In conclusion, OA risk may decrease as the lower extremity skeletal muscle mass increases.

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**Characterization of clinical data for in moderate osteoarthritis with support vector machines and regulatory network models**

Maria Segarra-Queral<sup>1</sup>, Mar Galofré<sup>1</sup>, Laura Tio<sup>2</sup>, Jordi Monfort<sup>2,3</sup>, Joan Carlos Monllau<sup>3,4</sup>, Gemma Piella<sup>1</sup> and Jérôme Noailly<sup>1</sup>

<sup>1</sup>BCN MedTech, Universitat Pompeu Fabra, Barcelona, Spain; <sup>2</sup>IMIM, Barcelona, Spain; <sup>3</sup>Rheumatology Department, Hospital del Mar, Barcelona, Spain; <sup>4</sup>Orthopedic Surgery and Traumatology, Department, Hospital del Mar, Barcelona, Spain

Knee osteoarthritis (KOA) diagnosis is based on symptoms, assessed through questionnaires such as the WOMAC. However, the inconsistency of pain recording and the discrepancy between joint phenotype and symptoms highlight the need for objective biomarkers in KOA diagnosis. To this end, we study relationships among clinical and molecular data in a cohort of women (n=51) with Kellgren-Lawrence grade 2-3 KOA through Support Vector Machine (SVM) [1] and a regulation network model (RNM) [2]. Clinical descriptors (i.e., pain catastrophism (CA); depression (DE); functionality (FU); joint pain (JP); rigidity (RI); sensitization (SE); synovitis (SY)) are used to classify patients. A Youden's test is performed for each classifier to determine optimal binarization thresholds for the descriptors. Thresholds are tested against patient stratification according to baseline WOMAC data from the Osteoarthritis Initiative, and the mean accuracy is 0.97. For our cohort, the data used as SVM inputs are KOA descriptors, synovial fluid (SL) proteomic measurements (n=25), and transcription factors (TF) activation obtained from RNM [2] stimulated with the SL measurements. The relative weights ( $\bar{w}$ ) after classification reflect input importance (Figure 1). The performance of each classifier is evaluated through AUC-ROC analysis. The best classifier with clinical data is CA (AUC = 0.9), highly influenced by FU and DE, suggesting that kinesophobia is involved in pain perception. With SL input, leptin strongly influences every classifier, suggesting the importance of low-grade inflammation. When TF are used, the mean AUC is limited to 0.608, which can be related to the pleomorphic behaviour of osteoarthritic chondrocytes. Nevertheless, FU has an AUC of 0.7 with strong importance of FOXO downregulation. Though larger and longitudinal cohorts are needed, this unique combination of SVM and RNM shall help to map objectively KOA descriptors.

**References:** [1] Vapnik et al. Mach. Learn, 20:273-297, 1995. DOI: 10.1007/BF00994018; [2] Segarra-Queral et al. Front. Bioeng. Biotechnol.11, 2023. DOI: 10.3389/FBIOE.2023.1006066

# EORS 2023

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**Subchondral microchannel network changes in early osteoarthritis**

Shahed Taheri<sup>1</sup>, T Yoshida<sup>1</sup>, Kai Oliver Böker<sup>1</sup>, Robert Hermann Foerster<sup>1</sup>, Lina Jochim<sup>1</sup>, Anna Lena Flux<sup>2</sup>, Birgit Grosskopf<sup>2</sup>, Thelonius Hawellek<sup>1</sup>, Wolfgang Lehmann<sup>1</sup>, Arndt Friedrich Schilling<sup>1</sup>

<sup>1</sup>Department of Trauma Surgery, Orthopaedic Surgery and Plastic Surgery, University Medical Center Göttingen, Göttingen, Germany; <sup>2</sup>University of Göttingen Johann-Friedrich-Blumenbach-Institute for Zoology & Anthropology, Department of Historical Anthropology and Human Ecology, Göttingen, Germany.

Articular cartilage (AC) and subchondral bone (SB) are intimately intertwined, forming a complex unit called the AC-SB interface. Our recent studies have shown that cartilage and bone marrow are connected by a three-dimensional network of microchannels (i.e. cartilage-bone marrow microchannel connector; CMMC), which differ microarchitecturally in number, size and morphology depending on the maturation stage of the bone<sup>1</sup> and the region of the joint<sup>2,3</sup>. However, the pathological significance of CMMC is largely unknown. Here, we quantitatively assessed how CMMC microarchitecture relates to cartilage condition and regional differences in early idiopathic osteoarthritis (OA).

Two groups of cadaveric female human femoral heads (intact cartilage vs early cartilage lesions) were identified and biopsy-based high-resolution micro-CT imaging was used. Subchondral bone (SB) thickness, CMMC number, maximum and minimum CMMC size, and CMMC morphology were quantified and compared between the two groups. The effect of joint region and cartilage condition on each dependent variable was examined.

The number and morphology of CMMCs were influenced by the region of the joint, but not by the cartilage condition. On the other hand, the minimum and maximum CMMC size was modified by both joint location and cartilage condition. The smallest CMMCs were consistently found in the load bearing region (LBR) of the joint. Compared to healthy subjects, the size of the microchannels was increased in early OA, most notably in the non-load bearing region (NLBR) and the peripheral rim (PR) of the femoral head. In addition, subchondral bone thinning was observed in early OA as a localized event associated with areas of partial chondral defect.

Our data suggest an enlargement of the SB microchannel network and a collective structural deterioration of the SB in early idiopathic OA.

**References:** <sup>1</sup>Taheri et al. (2019). *IJMS*; <sup>2</sup>Taheri et al. (2021). *Calcif Tissue Int*; <sup>3</sup>Taheri et al. (2021). *Osteologie*

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Spinal Fusion – a Clinical Challenge: Surgical and Biological Options**

Stefan Zwingenberger<sup>1, 2</sup>

<sup>1</sup>University Center of Orthopaedic, Trauma and Plastic Surgery, University Hospital Carl Gustav Carus at Technische Universität Dresden, 01307 Dresden, Germany;

<sup>2</sup>Center for Translational Bone, Joint and Soft Tissue Research, University Hospital Carl Gustav Carus at Technische Universität Dresden, 01307 Dresden, Germany;

Spinal diseases such as unstable fractures, infections, primary or secondary tumors or deformities require surgical stabilization with implants. The long-term success of this treatment is only ensured by a solid bony fusion. The size of the bony defect, the often poor bone quality and metabolic diseases increase the risk of non-union and make the case a great burden for the patient and a challenge for the surgeon. The goal of spinal fusion can only be achieved if the implants used offer sufficient mechanical stability and the local biological regeneration potential is large enough to form sufficient bone. The lecture will present challenging clinical cases. In addition, implant materials and new surgical techniques are discussed. Local therapeutic effects are achieved through the release of osteopromotive or anti-resorbative drugs, growth factors and antibiotics. By influencing biological pathways, basic orthopedic research has strong potential to further positively change future spinal surgery.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Mesenchymal Stem Cells Maintain Articular Stabilization and Promote Endogenous Cartilage Repair after High Tibial Osteotomy: a Second-look Arthroscopy Study**Meng Feng<sup>1</sup>, Sheng Dai<sup>1</sup>, Jianlong Ni<sup>1</sup>, Genwen Mao<sup>1</sup>, Xiaoqian Dang<sup>1</sup>, Zhibin Shi<sup>1</sup>

<sup>1</sup> Division of Sports Medicine, Department of Orthopedics, the Second Affiliated Hospital of Xi'an Jiaotong University

Varus malalignment increases the susceptibility of cartilage to mechanical overloading, which stimulates catabolic metabolism to break down the extracellular matrix and lead to osteoarthritis (OA). The altered mechanical axis from the hip, knee to ankle leads to knee joint pain and ensuing cartilage wear and deterioration, which impact millions of the aged population. Stabilization of the remaining damaged cartilage, and prevention of further deterioration, could provide immense clinical utility and prolong joint function. Our previous work showed that high tibial osteotomy (HTO) could shift the mechanical stress from an imbalanced status to a neutral alignment. However, the underlying mechanisms of endogenous cartilage stabilization after HTO remain unclear. We hypothesize that cartilage-resident mesenchymal stem cells (MSCs) dampen damaged cartilage injury and promote endogenous repair in a varus malaligned knee. The goal of this study is to further examine whether HTO-mediated off-loading would affect human cartilage-resident MSCs' anabolic and catabolic metabolism.

This study was approved by IACUC at Xi'an Jiaotong University. Patients with medial compartment OA (52.75±6.85 yrs, left knee 18, right knee 20) underwent open-wedge HTO by the same surgeons at one single academic sports medicine center. Clinical data was documented by the Epic HIS between the dates of April 2019 and April 2022 and radiographic images were collected with a minimum of 12 months of follow-up. Medial compartment OA with/without medial meniscus injury patients with unilateral Kellgren /Lawrence grade 3-4 was confirmed by X-ray. All incisions of the lower extremity healed well after the HTO operation without incision infection. Joint space width (JSW) was measured by uploading to ImageJ software. The Knee injury and Osteoarthritis Outcome Score (KOOS) toolkit was applied to assess the pain level. Outerbridge scores were obtained from a second-look arthroscopic examination. RNA was extracted to quantify catabolic targets and pro-inflammatory genes (QiaGen). Student's *t* test for two group comparisons and ANOVA analysis for differences between more than 2 groups were utilized.

To understand the role of mechanical loading-induced cartilage repair, we measured the serial changes of joint space width (JSW) after HTO for assessing the state of the cartilage stabilization. Our data showed that HTO increased the JSW, decreased the VAS score and improved the KOOS score significantly. We further scored cartilage lesion severity using the Outerbridge classification under a second-look arthroscopic examination while removing the HTO plate. It showed the cartilage lesion area decreased significantly, the full thickness of cartilage increased and mechanical

**27-29 SEPTEMBER | PORTO, PORTUGAL**

strength was better compared to the pre-HTO baseline. HTO dampened medial tibiofemoral cartilage degeneration and accelerate cartilage repair from Outerbridge grade 2 to 3 to Outerbridge 0 to 1 compared to untreated varus OA. It suggested that physical loading was involved in HTO-induced cartilage regeneration. Given that HTO surgery increases joint space width and creates a physical loading environment, we hypothesize that HTO could increase cartilage composition and collagen accumulation. Consistent with our observation, a group of cartilage-resident MSCs was identified. Our data further showed decreased expression of RUNX2, COL10 and increased SOX9 in MSCs at the RNA level, indicating that catabolic activities were halted during mechanical off-loading. To understand the role of cartilage-resident MSCs in cartilage repair in a biophysical environment, we investigated the differentiation potential of MSCs under 3-dimensional mechanical loading conditions. The physical loading inhibited catabolic markers (IL-1 and IL-6) and increased anabolic markers (SOX9, COL2).

Knee-preserved HTO intervention alleviates varus malalignment-related knee joint pain, improves daily and recreation function, and repairs degenerated cartilage of medial compartment OA. The off-loading effect of HTO may allow the mechanoregulation of cartilage repair through the differentiation of endogenous cartilage-derived MSCs.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Comparison of Autologous Adipose-Derived Tissue Stromal Vascular Fraction (AD-tSVF) Biomedical Instruments for their Stem Cell Content**

Ibrahim Vargel<sup>1</sup>, M. Furkan Açil<sup>1</sup>, S. Ali Tuncel<sup>2</sup>, Nilsu Baysal<sup>3</sup>, Irem Hartuç<sup>4</sup>, Hamza Okur<sup>5</sup>, Feza Korkusuz<sup>4</sup>

Hacettepe University (1) Faculty of Medicine, Department of Plastic Reconstructive and Aesthetic Surgery, (2) Faculty of Engineering, Department of Chemical Engineering, (3) Faculty of Medicine, (4) Faculty of Medicine, Department of Sports Medicine (5) Faculty of Medicine, Department of Pediatric Hematology, Ankara 06230, Turkey,

Deriving autologous mesenchymal stem cells (MSCs) from adipose tissues without using enzymes requires sophisticated biomedical instruments. Applied pressure on tissues and cells are adjusted manually although centrifugation and filtration systems are frequently used. The number of derived MSCs therefore could differ between instruments. We compared the number of MSCs obtained from four commercially available devices and our newly designed and produced instrument (A2, B3, L3, M2 and T3). Three-hundred mL of adipose tissue was obtained from a female patient undergoing liposuction using the transillumination solution. Obtained tissue was equally distributed to each device and handled according to the producers' guides. After handling, 3 mL stromal vascular fraction (SVF) was obtained from each device (1). Freshly isolated SVF was characterized using multi-color flow cytometry (Navios Flow Cytometer, Beckman Coulter, USA). Cell surface antigens were chosen according to IFATS and ISCT. CD31-FITC, CD34-PC5,5, CD73-PE, CD90-PB and CD45-A750 (Beckman Coulter, USA) fluorochrome-labeled monoclonal antibodies were assessed. Markers were combined with ViaKrome (Beckman Coulter, USA) to determine cell viability. At least  $10^5$  cells were acquired from each sample. A software (Navios EX, Beckman Coulter, USA) was used to create dot plots and to calculate the cell composition percentages. The data was analyzed in the Kaluza 2.1 software package (Beckman Coulter, USA). Graphs were prepared in GraphPad Prism. CD105 PC7/CD31 FITC cell percentages were 23,9%, 13,5%, 24,6%, 11,4% and 28,8% for the A2, B3, L3, M2 and T3 devices, respectively. We conclude that the isolated MSC percentage ranged from 11,4% to 28,8% between devices. The number of MSCs in SVF are key determinants of success in orthobiological treatments. Developing a device should focus on increasing the number of MSCs in the SVF while preserving its metabolic activity.

**Reference: (1)** Vargel I et al, Autologous adipose-derived tissue stromal vascular fraction (AD-tSVF) for knee osteoarthritis. (DOI: 10.3390/ijms232113517) Int J Mol Sci (ISSN: 1422-0067) 2022; 23, 13517.

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

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**Optimal concentration of mesenchymal stem cells for fracture healing in a rat model with long bone fracture**Myung-Seo Kim<sup>1</sup>, Kang-Il Kim,<sup>1</sup>

<sup>1</sup>Department of Orthopaedic Surgery, School of Medicine, Kyung Hee University and Kyung Hee University Hospital at Gangdong, Seoul 05278, South Korea

There is still no consensus on which concentration of mesenchymal stem cells (MSCs) to use for promoting fracture healing in a rat model of long bone fracture.

To assess the optimal concentration of MSCs for promoting fracture healing in a rat model.

Wistar rats were divided into four groups according to MSC concentrations: Normal saline (C),  $2.5 \times 10^6$  (L),  $5.0 \times 10^6$  (M), and  $10.0 \times 10^6$  (H) groups. The MSCs were injected directly into the fracture site. The rats were sacrificed at 2 and 6 weeks post-fracture. New bone formation [bone volume (BV) and percentage BV (PBV)] was evaluated using micro-computed tomography (CT). Histological analysis was performed to evaluate fracture healing score. The protein expression of factors related to MSC migration [stromal cell-derived factor 1 (SDF-1), transforming growth factor-beta 1 (TGF- $\beta$ 1)] and angiogenesis [vascular endothelial growth factor (VEGF)] was evaluated using western blot analysis. The expression of cytokines associated with osteogenesis [bone morphogenetic protein-2 (BMP-2), TGF- $\beta$ 1 and VEGF] was evaluated using real-time polymerase chain reaction.

Micro-CT showed that BV and PBV was significantly increased in groups M and H compared to that in group C at 6 weeks post-fracture ( $P = 0.040$ ,  $P = 0.009$ ;  $P = 0.004$ ,  $P = 0.001$ , respectively). Significantly more cartilaginous tissue and immature bone were formed in groups M and H than in group C at 2 and 6 weeks post-fracture ( $P = 0.018$ ,  $P = 0.010$ ;  $P = 0.032$ ,  $P = 0.050$ , respectively). At 2 weeks post fracture, SDF-1, TGF- $\beta$ 1 and VEGF expression were significantly higher in groups M and H than in group L ( $P = 0.031$ ,  $P = 0.014$ ;  $P < 0.001$ ,  $P < 0.001$ ;  $P = 0.025$ ,  $P < 0.001$ , respectively). BMP-2 and VEGF expression were significantly higher in groups M and H than in group C at 6 weeks postfracture ( $P = 0.037$ ,  $P = 0.038$ ;  $P = 0.021$ ,  $P = 0.010$ ). Compared to group L, TGF- $\beta$ 1 expression was significantly higher in groups H ( $P = 0.016$ ). There were no significant differences in expression levels of chemokines related to MSC migration, angiogenesis and cytokines associated with osteogenesis between M and H groups at 2 and 6 weeks post-fracture.

The administration of at least  $5.0 \times 10^6$  MSCs was optimal to promote fracture healing in a rat model of long bone fractures.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Nr4a1 promotes osteogenic differentiation of mesenchymal stem cells and improves inflammation-inhibited bone regeneration**

Yangshuai Gao<sup>1</sup>, Xiuhua Wu<sup>1</sup>, Zhongmin Zhang<sup>1</sup>, Jiajia Xu<sup>1</sup>

<sup>1</sup>Division of Spine Surgery, Department of Orthopaedics, Nanfang Hospital, Southern Medical University, China

Stem cell therapy is an effective means to address the repair of large segmental bone defects. However, the intense inflammatory response triggered by the implants severely impairs stem cell differentiation and tissue regeneration. High-dose transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), the most locally expressed cytokine in implants, inhibits osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and promotes tissue fibrosis, severely compromising the efficacy of stem cell therapy<sup>1</sup>. Small molecule inhibitors of TGF- $\beta$ 1 can be used to ameliorate the osteogenic disorders caused by high concentrations of TGF- $\beta$ 1, but systemic inhibition of TGF- $\beta$ 1 function will cause strong adverse effects. How to find safe and reliable molecular targets to antagonize TGF- $\beta$ 1 remains to be elucidated. Orphan nuclear receptor Nr4a1, an endogenous inhibitory molecule of TGF- $\beta$ 1, suppresses tissue fibrosis<sup>2</sup>, but its role in BMSC osteogenesis is unclear. We found that TGF- $\beta$ 1 inhibited Nr4a1 expression through HDAC4. Overexpression of Nr4a1 in BMSCs reversed osteogenic differentiation inhibited by high levels of TGF- $\beta$ 1. Mechanistically, RNA sequencing showed that Nr4a1 activated the ECM-receptor interaction and Hippo signaling pathway, which in turn promoted BMSC osteogenesis. In bone defect repair and fracture healing models, transplantation of Nr4a1-overexpressing BMSCs into C57BL/6J mice or treatment with the Nr4a1 agonist Csn-B significantly ameliorated inflammation-induced bone regeneration disorders. In summary, our findings confirm the endogenous inhibitory effect of Nr4a1 on TGF- $\beta$ 1 and uncover the effectiveness of Nr4a1 agonists as a therapeutic tool to improve bone regeneration, which provides a new solution strategy for the treatment of clinical bone defects and inflammatory skeletal diseases.

**References:** 1. Xu J, *et al.* High-dose TGF- $\beta$ 1 impairs mesenchymal stem cell-mediated bone regeneration via Bmp2 inhibition. *J Bone Miner Res* **35**, 167-180 (2020); 2. Katrin Palumbo-Zerr, *et al.* Orphan nuclear receptor NR4A1 regulates transforming growth factor- $\beta$  signaling and fibrosis. *Nat Med* **21**, 150-158 (2015)

**27-29 SEPTEMBER | PORTO, PORTUGAL****Nanovesicular therapeutics and drug delivery systems in orthopaedics**

Mario Gimona

Paracelsus Medical University

Nanovesicle-based therapy is increasingly being pursued as a safe, cell-free strategy to combat various immunological, musculoskeletal and neurodegenerative diseases. Small secreted extracellular vesicles (sEVs) obtained from multipotent mesenchymal stromal cells (MSCs) are of particular interest for therapeutic use since they convey anti-inflammatory, anti-scarring and neuroprotective activities to the recipient cells. Cell-derived vesicles (CDVs) produced by a proprietary extrusion process are surrounded by a lipid bilayer membrane with correct membrane topology, display biological activities similar to MSC-derived EVs and may find specific application for organ-targeted drug delivery systems. Translation of nanovesicle-based therapeutics into clinical application requires quantitative and reproducible analysis of bioactivity and stability, and the potential for GMP-compliant manufacturing. Manufacturing and regulatory considerations as well as preclinical models to support clinical translation will be discussed.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Polarized macrophages-derived extracellular vesicles as potential mediators of tendon repair**

Ana Luísa Graça<sup>1,2</sup>, Márcia T. Rodrigues<sup>1,2</sup>, Rui M. A. Domingues<sup>1,2</sup>, Manuela E. Gomes<sup>1,2</sup>, Manuel Gomez-Florit<sup>3</sup>

<sup>1</sup>3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, Barco, 4805-017 Guimarães, Portugal; <sup>2</sup>ICVS/3B's—PT Government Associate Laboratory, 4805-017 Guimarães, Portugal; <sup>3</sup>Health Research Institute of the Balearic Islands (IdISBa), 07010 Palma, Spain

Macrophages play a critical role in innate immunity by promoting or inhibiting tissue inflammation and repair. Classically, macrophages can differentiate into either pro-inflammatory (M1) or pro-reparative (M2) phenotypes in response to various stimuli. Therefore, this study aimed to address how extracellular vesicles (EVs) derived from polarized macrophages can affect the inflammatory response of tendon cells.

For that purpose, human THP-1 cells were stimulated with lipopolysaccharide (LPS), and interleukins -4 and -13 (IL-4, IL-13), to induce macrophage polarization into M1, M2, and hybrid M1/M2 phenotypes. Subsequently, the EVs were isolated from the culture medium by ultracentrifugation. The impact of these nanovesicles on the inflammation and injury scenarios of human tendon-derived cells (hTDCs), which had previously been stimulated with interleukin-1 beta (IL-1 $\beta$ ) to mimic an inflammatory scenario, was assessed.

We were able to isolate three different nanovesicles populations, showing the typical shape, size and surface markers of EVs. By extensively analyzing the proteomic expression profiles of M1, M2, and M1/M2, distinct proteins that were upregulated in each type of macrophage-derived EVs were identified. Notably, most of the detected pro-inflammatory cytokines and chemokines had higher expression levels in M1-derived EVs and were mostly absent in M2-derived EVs. Hence, by acting as a biological cue, we observed that M2 macrophage-derived EVs increased the expression of the tendon-related marker tenomodulin (TNMD) and tended to reduce the presence of pro-inflammatory markers in hTDCs. Overall, these preliminary results show that EVs derived from polarized macrophages might be a potential tool to modulate the immune system responses becoming a valuable asset in the tendon repair and regeneration fields worthy to be further explored.

**Comparing extracellular vesicles derived from platelets and mesenchymal stromal cells for therapeutic use**

Marian Forteza-Genestra

Cell Therapy and Tissue Engineering Group (TERCIT), Spain

Extracellular Vesicles (EVs) have emerged as potential functional therapeutic effectors in regenerative medicine, for example in the field of osteochondral injuries such as osteoarthritis (OA). However, their cargoes remain yet to be thoroughly evaluated; being non-coding micro-RNAs (miRNA) among the most interesting cargoes in the vesicles, since they are important regulators of gene expression. In this study, our aim was to evaluate the therapeutic effect of platelet lysates (PL)-derived EVs (pEVs) and human umbilical cord mesenchymal stromal cells (hUC-MSC) derived EVs (cEVs) on an osteoarthritis in vitro model and to study their differential miRNA content to contribute elucidating their biological effect. Isolated EVs by Size Exclusion Chromatography (SEC) from PL and hUC-MSC conditioned medium were characterized by Transmission Electron Microscopy (TEM), Western Blot (WB) and Nanoparticle Tracking Analysis (NTA). EVs therapeutic functionality was evaluated by collagen quantification on an OA in vitro model using human cartilage explants. Then, EVs miRNA content was evaluated using the gene chip miRNA 4.0 array and the data was analysed with the Transcriptome Analysis Console (TAC). pEVs and cEVs presented significant differences in their morphological characterization in terms of concentration and size distribution. The biological effect was also different since cartilage explants treated with pEVs showed statistically higher collagen content compared to the explants treated with cEVs. In agreement, a differential profile in miRNA content is observed between both EVs groups. In conclusion, pEVs and cEVs show different miRNA content that might influence their biological activity on the OA-induced cartilage explants. However, a more exhaustive analysis of the EVs molecular content is needed to elucidate the mechanisms underlying their biological effects.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Production of clinical grade extracellular vesicles (EVs) secreted by induced pluripotent stem cell-derived mesenchymal stem cells and mesenchymal stem cells for the treatment of osteoarthritis**

Palamà M.E.F.<sup>1</sup>, Gorgun C.<sup>1</sup>, Shaw G.<sup>2</sup>, Mauphy M.<sup>2</sup>, Gentili C.<sup>1</sup>

<sup>1</sup>Department of Experimental Medicine (DIMES), University of Genova, Genoa, Italy.

<sup>2</sup>Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Ireland

Mesenchymal stem cells (MSCs) have been studied for the treatment of Osteoarthritis (OA), a potential mechanism of MSC therapies has been attributed to paracrine activity, in which extracellular vesicles (EVs) may play a major role. It is suggested that MSCs from younger donor compete with adult MSC in their EV production capabilities. Therefore, MSCs generated from induced pluripotent mesenchymal stem cells (iMSC) appear to provide a promising source. In this study, MSCs and iMSC during long term-expansion using a serum free clinical grade condition, were characterized for surface expression pattern, proliferation and differentiation capacity, and senescence rate. Culture media were collected continuously during cell expansion, and EVs were isolated. Nanoparticle tracking analysis (NTA), transmission electron microscopy, western blots, and flow cytometry were used to identify EVs. We evaluated the biological effects of MSC and iMSC-derived EVs on human chondrocytes treated with IL-1 $\alpha$ , to mimic the OA environment.

In both cell types, from early to late passages, the amount of EVs detected by NTA increased significantly, EVs collected during cells expansion, retained tetraspanins (CD9, CD63 and CD81) expression. The anti-inflammatory activity of MSC-EVs was evaluated in vitro using OA chondrocytes, the expression of IL-6, IL-8 and COX-2 was significantly reduced after the treatment with hMSC-derived EVs isolated at early passage. The miRNA content of EVs was also investigated, we identify miRNA that are involved in specific biological function.

At the same time, we defined the best culture conditions to maintain iMSC and define the best time window in which to isolate EVs with highest biological activity.

In conclusion, a clinical grade serum-free medium was found to be suitable for the isolation and expansion of MSCs and iMSC with increased EVs production for therapeutic applications.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Platelet-derived Extracellular Vesicles Promote Stem Cells Tenogenic Commitment in a Bioengineered Tendon 3D Model**

Ana Luísa Graca<sup>1,2,3</sup>, Rui M. A. Domingues<sup>1,2</sup>, Denitsa Docheva<sup>3</sup>, Manuel Gomez-Florit<sup>4</sup>, Manuela E. Gomes<sup>1,2</sup>

<sup>1</sup>3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, Barco, 4805-017 Guimarães, Portugal

<sup>2</sup>ICVS/3B's—PT Government Associate Laboratory, 4805-017 Guimarães, Portugal

<sup>3</sup>Department of Musculoskeletal Tissue Regeneration, Orthopaedic Hospital König-Ludwig-Haus, University of Würzburg, Friedrich-Bergius-Ring 15, 97076, Würzburg, Germany

<sup>4</sup>Health Research Institute of the Balearic Islands (IdISBa), 07010 Palma, Spain

Worldwide, tendon disorders are one of the main causes of disability that decrease the quality of life of individuals and represent a substantial economic burden on society. Currently, the main therapies used for tendon injuries are not able to restore tendon functionality, and due to tendons' hypovascular and hypocellular nature, they present a reduced healing capacity, which also limits the success of the available therapies. In order to discover new therapies, extracellular vesicles (EVs), key players in cell-cell communication, have been widely explored for tissue engineering and regenerative medicine applications. Thus, the aim of this study is to assess the role of EVs derived from platelets in stem cell tenogenic commitment using a bioengineered tendon in vitro model for potential use as tendon therapeutic agents. Biomimetic platelet-derived EVs were produced by freeze-thaw cycles of platelets and isolation at different centrifugation speed. To recreate the architecture of tendons, a 3D system consisting of electrospun anisotropic nanofiber scaffolds coated with collagen encapsulating human adipose stem cells (hASCs) and different types of platelet-derived EVs, were produced. Then, the influence of the tendon-mimetic constructs and the distinct EVs populations in the hASCs tenogenic differentiation were assessed over culture time. We observed that the hASCs on the nanofibrous tendon scaffolds, show high cytoskeleton anisotropic organization that is characteristic of tenocytes. Moreover, acting as biological cues, platelet-derived EVs boosted hASCs tenogenic commitment, supported by the increased gene expression of tendon-related markers (SCX and TNMD). Additionally, EVs enhanced the deposition of tendon like extracellular matrix (ECM), as evidenced by the increased gene expression of ECM-related markers such as COL1, COL3, DCN, TNC, and MMP-3, which are fundamental for ECM synthesis and degradation balance. Moreover, EVs induced lower collagen matrix contraction on hASCs, which has been related with lower myofibroblast differentiation. Overall, the results revealed that EVs are capable of modulating stem

## **27-29 SEPTEMBER | PORTO, PORTUGAL**

cells' behavior boosting their tenogenic commitment, through the increased expression of healthy tendon cell markers, potentiating ECM deposition and decreasing cell contractility. Therefore, platelet EVs are a promising biochemical tool, worthy to be further explored, as paracrine signaling that might potentiate tendon repair and regeneration.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Stem cells in bone regeneration, the future is NOW**

Cecilie Gjerde

University of Bergen, Norway

The aim of the ongoing projects was to demonstrate the efficacy of autologous bone marrow derived stem cells (MSC) combined with biomaterial to induced new bone formation in a randomized multicenter controlled clinical trial.

Patients with a need for bone reconstruction of residual edentulous ridges in both the mandible and maxilla due to bone defects with a vertical loss of alveolar bone volume and/or knife edge ridges ( $\leq$  than 4,5 mm) unable to provide adequate primary stabilization for dental implants were included in the clinical study. Autologous bone marrow MSC were expanded, loaded on BCP and used to augment the alveolar ridges. After five months bone biopsies were harvested at the implant position site and implants were installed in the regenerated bone. The implants were loaded after 8 - 12 weeks. Safety, efficacy, quality of life and success/survival were assessed. Five clinical centers, 4 different countries participated. Bone grafts harvested from the ramus of the mandibles were used as control in the projects.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Extracellular Vesicles Secreted by Osteogenic-Differentiated Mesenchymal Stem Cells Promote Bone Formation in Rat Calvarial Defect**

Niyaz Al-Sharabi

University of Bergen, Norway

Growing evidence has suggested that paracrine mechanisms of Mesenchymal stem cell (MSC) may be involved in the underlying mechanism of MSC after transplantation, and extracellular vesicles (EVs) are an important component of this paracrine role. The aim of this study was to investigate the in vitro osteogenic effects of EVs derived from undifferentiated mesenchymal stem cells and from chemically induced to differentiate into osteogenic cells for 7 days. Further, the osteoinductive potential of EVs for bone regeneration in rat calvarial defects was assessed.

We could isolate and characterize EVs from naïve and osteogenic-induced MSCs. Proteomic analysis revealed that EVs contained distinct protein profiles, with Osteo-EVs having more differentially expressed proteins with osteogenic properties. EVs were found to enhance the proliferation and migration of cultured MSC. In addition, the study found that Osteo-EVs/MEM combination scaffolds could enhance greater bone formation after 4 weeks as compared to native MEM loaded with serum-free media.

The study suggests that EVs derived from chemically osteogenic-induced MSCs for 7 days can significantly enhance both the osteogenic differentiation activity of cultured hMSCs and the osteoinductivity of MEM scaffolds. The results indicate that Osteo-MSC-secreted nanocarriers-EVs combined with MEM scaffolds can be used for repairing bone defects.

**Source of mesenchymal stem cells is vital for bone regenerative applications**

Samih Mohamed-Ahmed

University of Bergen, Norway

Mesenchymal stem cells (MSC) have been used for bone regenerative applications as an alternative approach to bone grafting. Selecting the appropriate source of MSC is vital for the success of this therapeutic approach. MSC can be obtained from various tissues, but the most used sources of MSC are Bone marrow (BMSC), followed by adipose tissue (ASC). A donor-matched comparison of these two sources of MSC ensures robust and reliable results.

Despite the similarities in morphology and immunophenotype of donor-matched ASC and BMSC, differences existed in their proliferation and in vitro differentiation potential, particularly osteogenic differentiation that was superior for BMSC, compared to ASC. However, these differences were substantially influenced by donor variations. In vivo, although the upregulated expression of osteogenesis-related genes in both ASC and BMSC, more bone was regenerated in the calvarial defects treated with BMSC compared to ASC, especially during the initial period of healing. According to these findings, compared to ASC, BMSC may result in faster regeneration and healing, when used for bone regenerative applications.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Phase separation and particulate leaching integrated 3D printing of porous scaffolds for bone tissue engineering applications**

Mehmet Serhat Aydin

University of Bergen, Norway

Conventional 3D printing by itself is incapable of creating pores on a micro scale within deposited filaments throughout 3D scaffolds. These pores and hence larger surface areas are needed for cells to be adhered, proliferated, and differentiated. The aim of this work was to fabricate 3D polycaprolactone (PCL) scaffolds with internal multiscale porosity by using two different 3D printing techniques (ink/pellet of polymer-salt composite in low/high temperature printing) combined with salt leaching to improve cell adhesion, and cell proliferation besides to change degradation rate of PCL scaffolds:

1. Non-solvent phase separation integrated 3D printing of polymer-salt inks with various salt content (i.e., low temperature ink-based printing, LT).
2. FDM printing of composite polymer-salt pellets which will be obtained by casting and evaporating of prepared ink (i.e., high temperature composite-pellet-based printing, HT).

Further, the two approaches were followed by post salt leaching. Stem cells were able to attach on the surface and grow up to 14 days based on increasing cellular activities.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Triazine-trione based composite materials for potential bone tissue engineering**

Åshild Johansen

University of Bergen, Norway

Several synthetic polymers have been widely investigated for their use in bone tissue engineering applications, but the ideal material is yet to be engineered. Triazine-trione (TATO) based materials and their derivatives are novel in the field of biomedical engineering but have started to draw interest. Different designs of the TATO monomers and introduction of different chemical linkages and end-groups widens the scope of the materials due to a range of mechanical properties.

The aim of our work is to investigate novel TATO based materials, with or without hydroxyapatite filler, for their potential in bone tissue engineering constructs. Initially the biocompatibility of the materials was tested, indirectly and directly, according to ISO standards. Following this the osteoconductive properties were investigated with primary osteoblasts and an osteoblastic cell line. Bone marrow derived mesenchymal stem cells were used to evaluate the osteogenic differentiation and consequently the materials potential in bone tissue engineering applications.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Anatomy and microenvironment of the intervertebral disk**

Holger Jahr

RWTH Aachen University, Germany

Degeneration of the intervertebral disk (IVD), and subsequent low back pain, is an almost inevitable cause of disability. The underlying mechanisms are complex and current therapeutic strategies mainly focus on symptomatic relief rather than on the intrinsic regeneration of the IVD. This talk will provide an overview of special anatomical features and the composition of the IVD as well as its cellular microenvironment. Selected promising conceptional regenerative approaches will be discussed.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Role of Mitochondria in intervertebral disc health and disease**

Makarand V. Risbud

Thomas Jefferson University, Philadelphia, USA

The hypoxic nucleus pulposus cells were thought to contain few, functionally redundant mitochondria. However in contrast to this widely held notion, new evidence shows presence of functional mitochondrial networks in disc cells. The lecture will discuss this evidence and provide insights into how microenvironmental cues govern mitochondrial function. The lecture will also discuss emerging evidence on how mitochondrial dysfunction of nucleus pulposus cells results in metabolic dysregulation and acquisition of a state that promotes inflammation and degeneration.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

**Applications of artificial intelligence in the early diagnosis of chronic low back pain**  
Luca Ambrosio

Università Campus Bio-Medico di Roma, Italy

In the last decades, the use of artificial intelligence (AI) has been increasingly investigated in intervertebral disc degeneration (IDD) and chronic low back pain (LBP) research. To date, several AI-based cutting-edge technologies, such as computer vision, computer-assisted diagnosis, decision support system and natural language processing have been utilized to optimize LBP prevention, diagnosis, and treatment. This talk will provide an outline on contemporary AI applications to IDD and LBP research, with a particular attention towards actual knowledge gaps and promising innovative tools.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Translational potential of mesenchymal stem cell therapy for intervertebral disc regeneration**

Gianluca Vadalá

Università Campus Bio-Medico di Roma, Italy

The use of mesenchymal stem cell (MSCs) for intervertebral disc (IVD) regeneration has been extensively explored in the last two decades. MSCs are potent cell types that can be easily and safely harvested due to their abundance and availability. Moreover, they are characterized by the capacity to differentiate towards IVD cells as well as release growth factors to support resident cell metabolism and recruit local progenitor cells to induce endogenous repair of degenerated IVDs. This talk will outline the characteristics of the main MSC sources and their effect towards IVD regeneration based on available preclinical and clinical evidence. In addition, innovative aspects of MSC-derived cell-free therapies will also be discussed.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Mesendoderm progenitor cells derived from pluripotent stem cells for disc regeneration: a preliminary study in a ovine model**

C. Cicione<sup>1</sup>, V. Tilotta<sup>1</sup>, G. Di Giacomo<sup>1</sup>, L. Ambrosio<sup>2</sup>, F. Russo<sup>2</sup>, R. Papalia<sup>2</sup>, G. Vadalà<sup>2</sup> and V. Denaro<sup>2</sup>

<sup>1</sup>Laboratory of Regenerative Orthopaedic, Research Unit of Orthopaedics and Traumatology, Campus Bio-Medico University, Rome, Italy; <sup>2</sup>UOR of Orthopaedics and Traumatology, Campus Bio-Medico University Hospital Foundation, Rome, Italy

Low back pain (LBP) is a worldwide leading cause of disability. Treatment of intervertebral disc (IVD) with stem cells has been used on degenerate discs (IDD), cause of around 40% of LBP cases. Despite pain reduction, clinical studies' follow-up have not shown a structural IVD improvement. A valid alternative may be the use of notocordal cells (NC) or their precursors. Mesendoderm progenitor cells (MEPC) have the ability to replicate and differentiate toward NC. In this preliminary study we evaluated in a preclinical IDD model the viability and NC differentiation of MEPC derived from induced pluripotent stem cells (iPSC).

MEPC derived from iPSC were developed during the iPSpine project (# 825925), thawed, plated for 24h on laminin and labeled with PKH26.

Two adult sheep were subjected to nucleotomy of five lumbar discs for the induction of IDD. After 5 weeks, 3 degenerated discs were treated with MEPC at 3 different doses (low, medium and high). One sheep was sacrificed after 7 days and one after 30 days. Clinical parameters were collected to evaluate the safety of treatment. Discs were analysed using histological techniques. Survival (PKH26), proliferation (PCNA), notocordal cell differentiation (Brachyury, Cytokeratin 8/18/19, Sox9, Foxa2) and endodermal differentiation (Sox17) were evaluated.

At 7 days from treatment, both sheep lost about 20% of body weight. Only in discs treated with the highest dose PKH26 stained cells were alive up to 30 days. These cells turn out to be: proliferating (PCNA); positive for Brachyury, cytokeratin 8/18/19 and Foxa2; positive for SOX17 in a small percentage.

This preliminary study shows that MEPC, derived from iPSC and injected into ovine discs degenerated by nucleotomy, are able to survive up to 30 days and differentiate within the disc predominantly towards the notocordal phenotype.

**Bone from fat: enhancing osteogenesis from adipose stem cells**

Gun-Il Im

Research institute for Convergence Life Science Dongguk University, Goyang, Korea

Extensive bone defects, caused by severe trauma or resection of large bone tumors, are difficult to treat. Regenerative medicine, including stem cell transplantation, may provide a novel solution for these intractable problems and improve the quality of life in affected patients. Adipose-derived stromal/stem cells (ASCs) have been extensively studied as cell sources for regenerative medicine due to their excellent proliferative capacity and the ability to obtain a large number of cells with minimal donor morbidity. However, the osteogenic potential of ASCs is lower than that of bone marrow-derived stromal/stem cells. To address this disadvantage, our group has employed various methods to enhance osteogenic differentiation of ASCs, including factors such as bone morphogenetic protein or Vitamin D, coculture with bone marrow stem cells, VEGF transfection, and gene transfer of Runx-2 and osterix. Recently, we mined a marker that can predict the osteogenic potential of ASC clones and also investigated the usefulness of the molecule as the enhancer of osteogenic differentiation of ASCs as well as its mechanism of action. Through RNA-seq gene analysis, we discovered that GSTT1 was the most distinguished gene marker between highly osteogenic and poorly osteogenic ASC clones. Knockdown of GSTT1 in high osteogenic ASCs by siGSTT1 treatment reduced mineralized matrix formation while GSTT1 overexpression by GSTT1 transfection or GSTT1 recombinant protein treatment enhanced osteogenic differentiation of low osteogenic ASCs. Metabolomic analysis confirmed significant changes of metabolites related to bone differentiation in ASCs transfected with GSTT1. A high total antioxidant capacity, low levels of cellular reactive oxygen species and increased GSH/GSSG ratios were also detected in GSTT1-transfected ASCs. GSTT1 can be a useful marker to screen the highly osteogenic ASC clones and also a therapeutic factor to enhance the osteogenic differentiation of poorly osteogenic ASC clones.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Sclerostin-Mediated Impaired Osteogenesis by Fibroblast-Like Synoviocytes in the Particle-Induced Osteolysis Model**

Lee Sang-Soo

Institute for Skeletal Aging and Orthopedic Surgery, Hallym University-Chuncheon Sacred Heart Hospital, Chuncheon, Korea

Wear debris from implant interfaces is the major factor leading to periprosthetic osteolysis. Fibroblast-like synoviocytes (FLSs) populate the intimal lining of the synovium and are in direct contact with wear debris. This study aimed to elucidate the effect of Ti particles as wear debris on human FLSs and the mechanism by which they might participate in the bone remodeling process during periprosthetic osteolysis. FLSs were isolated from synovial tissue from patients, and the condition medium (CM) was collected after treating FLSs with sterilized Ti particles. The effect of CM was analyzed for the induction of osteoclastogenesis or any effect on osteogenesis and signaling pathways. The results demonstrated that Ti particles could induce activation of the NF $\kappa$ B signaling pathway and induction of COX-2 and inflammatory cytokines in FLSs. The amount of RANKL in the conditioned medium collected from Ti particle-stimulated FLSs (Ti CM) showed the ability to stimulate osteoclast formation. The Ti CM also suppressed the osteogenic initial and terminal differentiation markers for osteoprogenitors, such as alkaline phosphate activity, matrix mineralization, collagen synthesis, and expression levels of Osterix, Runx2, collagen 1 $\alpha$ , and bone sialoprotein. Inhibition of the WNT and BMP signaling pathways was observed in osteoprogenitors after the treatment with the Ti CM. In the presence of the Ti CM, exogenous stimulation by WNT and BMP signaling pathways failed to stimulate osteogenic activity in osteoprogenitors. Induced expression of sclerostin (SOST: an antagonist of WNT and BMP signaling) in Ti particle-treated FLSs and secretion of SOST in the Ti CM were detected. Neutralization of SOST in the Ti CM partially restored the suppressed WNT and BMP signaling activity as well as the osteogenic activity in osteoprogenitors. Our results reveal that wear debris-stimulated FLSs might affect bone loss by not only stimulating osteoclastogenesis but also suppressing the bone-forming ability of osteoprogenitors. In the clinical setting, targeting FLSs for the secretion of antagonists like SOST might be a novel therapeutic approach for preventing bone loss during inflammatory osteolysis.

**Possible Roles of Antioxidants in Rotator cuff tendinopathy**

Hyung Bin Park

Gyeongsang National University Changwon Hospital, Korea

The rotator cuff tendinopathy is one of the most common shoulder problems leading to full-thickness rotator cuff tendon tear and, eventually, to degenerative arthritis. Recent research on rotator cuff tendon degeneration has focused on its relationship to cell death. The types of cell death known to be associated with rotator cuff tendon degeneration are apoptosis, necrosis, and autophagic cell death. The increased incidence of cell death in degenerative tendon tissue may affect the rates of collagen synthesis and repair, possibly weakening tendon tissue and increasing the risk of tendon rupture. The biomolecular mechanisms of the degenerative changes leading to apoptotic cell death in rotator cuff tenofibroblasts have been identified as oxidative-stress-related cascade mechanisms. Furthermore, apoptosis, necrosis, and autophagic cell death are all known to be mediated by oxidative stress, a condition in which ROS (reactive oxygen species) are overproduced. Lower levels of oxidative stress trigger apoptosis; higher levels mediate necrosis. Although the signaltransduction pathway leading to autophagy has not yet been fully established, ROS are known to be essential to autophagy. A neuronal theory regarding rotator cuff degeneration has been developed from the findings that glutamate, a neural transmitter, is present in increased concentrations in tendon tissues with tendinopathy and that it induces rat supraspinatus tendon cell death. Recent studies have reported that hypoxia involved in rotator cuff tendon degeneration. Because antioxidants are known to scavenge for intracellular ROS, some studies have been conducted to determine whether antioxidants can reduce cell death in rotator cuff tendon-origin fibroblasts. The first study reported that an antioxidant has the ability to reduce apoptosis in oxidative-stressed rotator cuff tenofibroblasts. The second study reported that antioxidants have both antiapoptotic effects and antinecrotic effects on rotator cuff tendon-origin fibroblasts exposed to an oxidative stimulus. The third study reported that an antioxidant has antiautophagic-cell-death effects on rotator cuff tendon-origin fibroblasts exposed to an oxidative stimulus. The fourth study reported that glutamate markedly increases cell death in rotator cuff tendonorigin fibroblasts. The glutamate-induced cytotoxic effects were reduced by an antioxidant, demonstrating its cytoprotective effects against glutamate-induced tenofibroblast cell death. The fifth study reported that hypoxia significantly increases intracellular ROS and apoptosis. The hypoxia-induced cytotoxic effects were markedly attenuated by antioxidants, demonstrating their cytoprotective effects against hypoxia-induced tenofibroblast cell death. In conclusion, antioxidants have cytoprotective effects on tenofibroblasts exposed in vitro to an oxidative stressor, a neurotransmitter, or hypoxia. These cytoprotective effects result from antiapoptotic, antinecrotic, and antiautophagic actions involving the inhibition of ROS formation.

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

These findings suggest that antioxidants may have therapeutic potential for rotator cuff tendinopathy. Further studies must be conducted in order to apply these in vitro findings to clinical situations.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Feasibility of Loop-Mediated Isothermal Amplification for Rapid Detection of Methicillin-Susceptible and Methicillin-Resistant Staphylococcus aureus in Tissue Samples**

Sang-Gyun Kim

Department of Orthopaedic Surgery, National Medical Center, Seoul, Korea

To date, few studies have investigated the feasibility of the loop-mediated isothermal amplification (LAMP) assay for identifying pathogens in tissue samples. This study aimed to investigate the feasibility of LAMP for the rapid detection of methicillin-susceptible or methicillin-resistant *Staphylococcus aureus* (MSSA or MRSA) in tissue samples, using a bead-beating DNA extraction method. Twenty tissue samples infected with either MSSA (n = 10) or MRSA (n = 10) were obtained from patients who underwent orthopedic surgery for suspected musculoskeletal infection between December 2019 and September 2020. DNA was extracted from the infected tissue samples using the bead-beating method. A multiplex LAMP assay was conducted to identify MSSA and MRSA infections. To recognize the *Staphylococcus* genus, *S. aureus*, and methicillin resistance, 3 sets of 6 primers for the 16S ribosomal ribonucleic acid (rRNA) and the *femA* and *mecA* genes were used, respectively. The limit of detection and sensitivity (detection rate) of the LAMP assay for diagnosing MSSA and MRSA infection were analyzed. The results of this study suggest that the LAMP assay performed with tissue DNA samples can be a useful diagnostic method for the rapid detection of musculoskeletal infections caused by MSSA and MRSA.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Role of polaprezinc in fracture healing by differentiations of osteoblast and osteoclast**

Kwang Hwan Park

Department of Orthopaedic Surgery, Yonsei University College of Medicine, Seoul, Korea

Fractures and related complications are a common challenge in the field of skeletal tissue engineering. Vitamin D and calcium are the only broadly available medications for fracture healing, while zinc has been recognized as a nutritional supplement for healthy bones. Here, we aimed to use polaprezinc, an anti-ulcer drug and a chelate form of zinc and L-carnosine, as a supplement for fracture healing. Polaprezinc induced upregulation of osteogenesis-related genes and enhanced the osteogenic potential of human bone marrow-derived mesenchymal stem cells and osteoclast differentiation potential of mouse bone marrow-derived monocytes. In mouse experimental models with bone fractures, oral administration of polaprezinc accelerated fracture healing and maintained a high number of both osteoblasts and osteoclasts in the fracture areas. Collectively, polaprezinc promotes the fracture healing process efficiently by enhancing the activity of both osteoblasts and osteoclasts. Therefore, we suggest that drug repositioning of polaprezinc would be helpful for patients with fractures.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Evaluation of a Calcium Phosphate-collagen Matrix Bone Graft with Needle-shaped Submicron Surface Topography in a Clinically Relevant Sheep Posterolateral Lumbar Spine Fusion Model**

Nathan W. Kucko<sup>1</sup>, James Crowley<sup>2</sup>, Daniel Wills<sup>2</sup>, Tian Wang<sup>2</sup>, Matthew Pelletier<sup>2</sup>, Huipin Yuan<sup>1</sup>, Guido Houtzager<sup>1</sup>, Charlie Champion<sup>1</sup>, William R. Walsh<sup>2</sup>, Joost de Bruijn<sup>1</sup>, Florence Barrère-de Groot<sup>1</sup>

<sup>1</sup>Kuros Biosciences BV, Bilthoven, Netherlands; <sup>2</sup>University of New South Wales, Australia

Biphasic calcium phosphate (BCP) with a characteristic needle-shaped submicron surface topography (MagnetOs) has attracted much attention due to its unique bone-forming ability which is essential for repairing critical-size bone defects such as those found in the posterolateral spine [1,2]. Previous in vitro and ex-vivo data performed by van Dijk LA [3] and Yuan H [4] demonstrated that these specific surface characteristics drive a favorable response from the innate immune system.

This study aimed to evaluate and compare the in vivo performance of three commercially-available synthetic bone grafts, (1) i-FACTOR Putty<sup>®</sup>, (2) OssDsign<sup>®</sup> Catalyst Putty and (3) FIBERGRAFT<sup>®</sup> BG Matrix, with that of a novel synthetic bone graft in a clinically-relevant instrumented sheep posterolateral lumbar spine fusion (PLF) model. The novel synthetic bone graft comprised of BCP granules with a needle-shaped submicron surface topography (MagnetOs) embedded in a highly porous and fibrillar collagen matrix (MagnetOs Flex Matrix).

Four synthetic bone grafts were implanted as standalone in an instrumented sheep PLF model for 12 weeks (n=3 bilateral levels per group; levels L2/3 & L4/5), after which spinal fusion was determined by manual palpation, radiograph and  $\mu$ CT imaging (based on the Lenke scale), range-of-motion mechanical testing, and histological and histomorphological evaluation.

Radiographic fusion assessment determined bilateral robust bone bridging (Lenke scale A) in 3/3 levels for MagnetOs Flex Matrix compared to 1/3 for all other groups. For  $\mu$ CT, bilateral fusion (Lenke scale A) was found in 2/3 levels for MagnetOs Flex Matrix, compared to 0/3 for i-FACTOR Putty<sup>®</sup>, 1/3 for OssDsign<sup>®</sup> Catalyst Putty and 0/3 for FIBERGRAFT<sup>®</sup> BG Matrix. Fusion assessment for MagnetOs Flex Matrix was further substantiated by histology which revealed significant graft resorption complemented by abundant bone tissue and continuous bony bridging between vertebral transverse processes resulting in bilateral spinal fusion in 3/3 implants.

These results show that MagnetOs Flex Matrix achieved better fusion rates compared to three commercially-available synthetic bone grafts when used as a standalone in a clinically-relevant instrumented sheep PLF model.

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Novel Piezoelectric and Osteoconductive Nanofibers for Bone Tissue Engineering****Frederico Barbosa<sup>1,2</sup>, Fábio F. Garrudo<sup>1,2,3</sup>, Paola S. Alberte<sup>1,2</sup>, Marta S. Carvalho<sup>1,2</sup>, Frederico Castelo Ferreira<sup>1,2</sup>, João C. Silva<sup>1,2</sup>**

<sup>1</sup>Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>2</sup>Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>3</sup>Department of Bioengineering and Instituto de Telecomunicações, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

The current procedures being applied in the clinical setting to address osteoporosis-related delayed union and nonunion bone fractures have been found to present mostly suboptimal outcomes. As a result, bone tissue engineering (BTE) solutions involving the development of implantable biomimetic scaffolds to replace damaged bone and support its regeneration are gaining interest. The piezoelectric properties of the bone tissue, which stem primarily from the significant presence of piezoelectric type I collagen fibrils in the tissue's extracellular matrix (ECM), play a key role in preserving the bone's homeostasis and provide integral assistance to the regeneration process. However, despite their significant potential, these properties of bone tend to be overlooked in most BTE-related studies. In order to bridge this gap in the literature, novel hydroxyapatite (HAp)-filled osteoinductive and piezoelectric poly(vinylidene fluoride-co-tetrafluoroethylene) (PVDF-TrFE) electrospun nanofibers were developed to replicate the bone's fibrous ECM composition and electrical features. Different HAp nanoparticle concentrations (1-10%, wt%) were tested to assess their effect on the physicochemical and biological properties of the resulting fibers. The fabricated scaffolds displayed biomimetic collagen fibril-like diameters, while also presenting mechanical features akin to type I collagen. The increase in HAp presence was found to enhance both surface and piezoelectric properties of the fibers, with an improvement in scaffold wettability and increase in  $\beta$ -phase nucleation (translating to increased piezoelectricity) being observed. The HAp-containing scaffolds also exhibited an augmented bioactivity, with a more comprehensive surface mineralization of the fibers being obtained for the scaffolds with the highest HAp concentrations. Improved osteogenic differentiation of seeded human mesenchymal stem/stromal cells was achieved with the addition of HAp, as confirmed by an increased ALP activity, calcium deposition and upregulated expression of key osteogenic markers. Overall, our findings highlight, for the first time, the potential of combining PVDF-TrFE and HAp to develop electroactive and osteoinductive nanofibers for BTE.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Investigation of intervertebral disc herniation through a human Anulus Fibrosus pro-inflammatory model**AL Castro<sup>1,2,3</sup>, MA Barbosa<sup>1,2,3</sup>, RM Gonçalves<sup>1,2,3</sup>

<sup>1</sup>i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; <sup>2</sup>INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal; <sup>3</sup>ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

Intervertebral disc (IVD) herniation involves a chronic inflammatory process and failure of the annulus fibrosus (AF), in a process still under investigated. Inflammation can lead to disorganization of extracellular matrix (ECM) and fibrotic alterations, among others. Most of the ongoing research relies on *in vivo* or *ex vivo* models that do not truly mimic the human microenvironment. Here, we developed a 3D *in vitro* model aimed at recapitulating inflammation progression in human AF (hAF) to improve the understanding of AF failure.

hAF of herniated samples from low back pain patients were collected and digested as previously established [1]. hAF cells were cultured in 2D and 3D, embedded in collagen-type 1 hydrogels. hAF cells in 2D and 3D were then stimulated with IL-1 $\beta$  (10ng/mL), in acute (48h) and chronic (7days) conditions. After 7 days, cell morphology together with extracellular matrix (ECM) (collagen I and fibronectin) were assessed by immunofluorescence. Inflammatory cytokine secretion were analyzed by a protein array.

2D experiments showed alteration of cell morphology, with cell area reduction upon inflammatory stimulation. The profile of inflammatory cytokines secreted showed that: i) IL-1 $\alpha$  increases with time in culture, while TNF- $\alpha$  decreases; and ii) IL-1 $\alpha$  and MIG/CXCL9 are increased in chronic vs acute inflammation. ECM analysis showed no differences. In 3D culture, AF cell morphologic alterations with inflammation were also observed. Concerning ECM production, collagen I production was reduced with chronic inflammation, while fibronectin remained unaltered. Collagen II and aggrecan appear to increase with chronic inflammation. Cytokine production from 3D culture is currently being assessed. ECM alterations are being complemented by characterization at biomechanical (nanoindentation) level.

Our data show that chronic inflammation is crucial to understand AF failure mechanisms and improve the resemblance of human IVD herniation.

**Reference:** [1] Castro, AL et al., 17Jan.2022, doi:10.1186/s13075-021-02690-w

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Quantifying Variability in Daily Accelerations Recorded by Inertial Sensors in Healthy Individuals: Implications for Gait Measurement in Free-Living Environments**

Arash Ghaffari<sup>1</sup>, John Rasmussen<sup>2</sup>, Søren Kold<sup>1</sup>, Ole Rahbek<sup>1</sup>

<sup>1</sup>Interdisciplinary Orthopaedics, Aalborg University Hospital, Aalborg, Denmark;

<sup>2</sup>Department of Materials and Production, Aalborg University, Aalborg East, Denmark

Gait measurements can vary due to various intrinsic and extrinsic factors, and this variability becomes more pronounced using inertial sensors in a free-living environment. Therefore, identifying and quantifying the sources of variability is essential to ensure measurement reliability and maintain data quality.

This study aimed to determine the variability of daily accelerations recorded by an inertial sensor in a group of healthy individuals. Ten participants, four males and six females, with a mean age of 50 years (range: 29–61) and BMI of 26.9 kg/m<sup>2</sup> (range: 21.4–36.8), were included. A single accelerometer continuously recorded lower limb accelerations over two weeks. We extracted and analyzed the accelerations of three consecutive strides within walking bouts if the time difference between the bouts was more than two hours. Multivariate mixed-effects modeling was performed on both the discretized acceleration waveforms at 101 points (0-100) and the harmonics of the signals in the frequency domain to determine the variance components for different subjects, days, bouts, and steps as the random effect variables. Intraclass correlation coefficients (ICCs) were calculated for between-day, between-bout, and between-step comparisons.

The results showed that the ICCs for the between-day, between-bout, and between-step comparisons were 0.73, 0.82, 0.99 for the vertical axis; 0.64, 0.75, 0.99 for the anteroposterior axis; and 0.55, 0.96, 0.97 for the mediolateral axis. For the signal harmonics, the respective ICCs were 0.98, 0.98, 0.99 for the vertical axis; 0.54, 0.93, 0.98 for the anteroposterior axis; and 0.69, 0.78, 0.95 for the mediolateral axis.

Overall, this study demonstrated that accelerations recorded continuously for multiple days in a free-living environment exhibit high variability, mainly between days, and some variability arising from differences between walking bouts during different times within days. However, reliable and repeatable gait measurements can be obtained by identifying and quantifying the sources of variability.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Advanced *in vitro* 3D tissue culture model for the assessment of titanium implant osteointegration**Maglio M<sup>1</sup>, Tschon M<sup>1</sup>, Sartori M<sup>1</sup>, Martini L<sup>1</sup>, Rocchi M<sup>2</sup>, Dallari D<sup>2</sup>, Giavaresi G<sup>1</sup>, Fini M<sup>3</sup><sup>1</sup>IRCCS Istituto Ortopedico Rizzoli, Surgical Sciences and Technologies; <sup>2</sup> IRCCS Istituto Ortopedico Rizzoli, Reconstructive Orthopaedic Surgery and Innovative Techniques - Musculoskeletal Tissue Bank; <sup>3</sup> IRCCS Istituto Ortopedico Rizzoli, Scientific Direction

The use of implant biomaterials for prosthetic reconstructive surgery and osteosynthesis is consolidated in the orthopaedic field, improving the quality of life of patients and allowing for healthy and better ageing. However, there is the lack of advanced innovative methods to investigate the potentialities of smart biomaterials, particularly for the study of local effects of implant and osteointegration. Despite the complex process of osseointegration is difficult to recreate *in vitro*, the growing challenges in developing alternative models require to set-up and validate new approaches. Aim of the present study is to evaluate an advanced *in vitro* tissue culture model of osteointegration of titanium implants in human trabecular bone. Cubic samples (1.5x1.5 cm) of trabecular bone were harvested as waste material from hip arthroplasty surgery (CE AVEC 829/2019/Sper/IOR); cylindrical defects (2 mm Ø, 6 mm length) were created, and tissue specimens assigned to the following groups: 1) empty defects- CTR-; 2) defects implanted with a cytotoxic copper pin (Merck cod. 326429)- CTR+; 3) defects implanted with standard titanium pins of 6 µm-rough (ZARE S.r.l) -Ti6. Tissue specimens were cultured in mini rotating bioreactors in standard conditions, weekly assessing viability. At the 8-week-timepoint, immunoenzymatic, microtomographic, histological and histomorphometric analyses were performed. The model was able to simulate the effects of implantation of the materials, showing a drop in viability in CTR+, differently from Ti6 which appears to have a trophic effect on the bone. MicroCT and histological analysis supported the results, with lower BV/TV and Tb.Th values observed in CTR- compared to CTR+ and Ti6 and signs of matrix and bone deposition at the implant site. The collected data suggest the reliability of the tested model which can recreate the osseointegration process *in vitro* and can therefore be used for preliminary evaluations to reduce and refine *in vivo* preclinical models.

**References:** Maglio M et al. J Cell Physiol 2019. doi:10.1002/jcp.27457**Acknowledgment:** This work was supported by Emilia-Romagna Region for the project “Sviluppo di modelli biologici *in vitro* ed *in silico* per la valutazione e predizione dell’osteointegrazione di dispositivi medici da impianto nel tessuto osseo”

27-29 SEPTEMBER | PORTO, PORTUGAL

## The epigenetic landscape in soft-tissue fibrosis

Akbar M<sup>1</sup>, Crowe LAN<sup>1</sup>, Woolcock K<sup>1</sup>, Cole J<sup>1</sup>, McInnes IB<sup>1</sup>, Millar NL<sup>1</sup>

<sup>1</sup>School of Infection and Immunity, University of Glasgow, Glasgow, UK

Dupuytren's disease (DD) is a fibroproliferative soft tissue disease affecting the palmar fascia of the hand causing permanent and irreversible flexion contracture. Aberrant fibrosis is likely to manifest through a combination of extrinsic, intrinsic, and environmental factors, including genetics and epigenetics. However, the role of epigenetics in soft tissue fibrosis in diseases such as DD is not well established. Therefore, we conducted a comprehensive multi-omic study investigating the epigenetic profiles that influence gene expression in DD pathology. Using control (patients undergoing carpal tunnel release) and diseased fibroblasts (patients undergoing Dupuytren's fasciectomy), we conducted ATAC-seq to assess differential chromatin accessibility between control and diseased fibroblasts. Additionally, ChIP-seq mapped common histone modifications (histone H4; H3K4me3, H3K9me3, H3K27me3, H4K16Ac, H4K20Me3) associated with fibrosis. Furthermore, we extracted RNA from control and DD tissue and performed bulk RNA-seq.

ATAC-seq analysis identified 2470 accessible genomic loci significantly more accessible in diseased fibroblasts compared to control. Comparison between diseased and control cells identified numerous significantly different peaks in histone modifications (H4K20me3, H3K27me3, H3K9me3) associated with gene repression in control cells but not in diseased cells. Pathway analysis demonstrated a substantial overlap in genes being de-repressed across these histone modifications (Figure 1). Both, ATAC-seq and ChIP-seq analysis indicated pathways such as cell adhesion, differentiation, and extracellular matrix organisation were dysregulated as a result of epigenetic changes. Moreover, *de novo* motif enrichment analysis identified transcription factors that possibly contributed to the differential gene expression between control and diseased tissue, including HIC1, NFATC1 and TEAD2. RNA-seq analysis found that these transcription factors were upregulated in DD tissue compared to control tissue.

The current epigenetic study provides insights into the aberrant fibrotic processes associated with soft tissue diseases such as DD and indicates that epigenetic-targeted therapies may be an interesting viable treatment option in future.

**Monitoring *in vitro* and *ex vivo* inflammation using single-walled carbon nanotubes (SWCNTs) sensor technology**

Laura Belcastro<sup>1</sup>, Vitalijs Zubkovs<sup>2</sup>, Miha Markocic<sup>2</sup>, Sayyed Hashem Sajjadi<sup>3</sup>, Christian Peez<sup>1</sup>, Riccardo Tognato<sup>1</sup>, Ardemis Anoush Boghossian<sup>3</sup>, Stefano Cattaneo<sup>2</sup>, Sibylle Grad<sup>1</sup>, Valentina Basoli<sup>1,4</sup>

<sup>1</sup>AO Research Institute (ARI), Davos

<sup>2</sup>Centre Suisse d'Electronique et de Microtechnique (CSEM), Landquart

<sup>3</sup>École Polytechnique Fédérale de Lausanne (EPFL), Lausanne

<sup>4</sup>University of Basel, Department of Biomedical Engineering, Basel

*Osteoarthritis* (OA) is a degenerative joint disease affecting millions worldwide. Early detection of OA and monitoring its progression is essential for effective treatment and for preventing irreversible damage. Although sensors have emerged as a promising tool for monitoring analytes in patients, their application for monitoring the state of pathology is currently restricted to specific fields (such as diabetes). In this study, we present the development of an optical sensor system for real-time monitoring of inflammation based on the measurement of nitric oxide (NO), a molecule highly produced in tissues during inflammation.

Single-walled carbon nanotubes (SWCNT) were functionalized with a single-stranded DNA (ssDNA) wrapping designed using an artificial intelligence approach and tested using S-nitroso-N-acetyl penicillamine (SNAP) as a standard released-NO marker. An optical SWIR reader with LED excitation at 650 nm, 730 nm and detecting emission above 1000 nm was developed to read the fluorescence signal from the SWCNTs. Finally, the SWCNT was embedded in GelMa to prove the feasibility of monitoring the release of NO in bovine chondrocyte and osteochondral inflamed cultures (1-10 ng/ml IL1 $\beta$ ) monitored over 48 hours. The stability of the inflammation model and NO release was indirectly validated using the Griess and DAF-FM methods. A microfabricated sensor tag was developed to explore the possibility of using ssDNA-SWCNT in an *ex vivo* anatomic set-up for surgical feasibility, the limit of detection, and the stability under dynamic flexion.

The SWCNT sensor was sensitive to NO in both *in silico* and *in vitro* conditions during the inflammatory response from chondrocyte and osteochondral plug cultures. The fluorescence signal decreased in the inflamed group compared to control, indicating increased NO concentration. The micro-tag was suitable and stable in joints showing a readable signal at a depth of up to 6 mm under the skin.

The ssDNA-SWCNT technology showed the possibility of monitoring inflammation continuously in an *in vitro* set-up and good stability inside the joint. However, further studies *in vivo* are needed to prove the possibility of monitoring disease progression and treatment efficacy *in vivo*.

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Towards understanding how neutrophil instruct the immune response to biomaterials**Ezgi Irem Bektas<sup>1</sup>, Marinus A. Wesdorp<sup>2</sup>, Andrea Schwab<sup>1</sup>, Martin J. Stoddart<sup>1</sup>, Alvaro Mata<sup>3</sup>, Gerjo J.V.M. Van Osch<sup>2,4,5</sup>, Matteo D'Este<sup>1</sup>

<sup>1</sup>AO Research Institute Davos, Switzerland; <sup>2</sup>Department of Orthopaedics and Sports Medicine, Erasmus MC, University MC Rotterdam, The Netherlands; <sup>3</sup>Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom; <sup>4</sup>Department of Otorhinolaryngology, Erasmus MC, University MC Rotterdam, The Netherlands; <sup>5</sup>Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials

Biomaterials with mechanical or biological competence are ubiquitous in musculoskeletal disorders, and understanding the inflammatory response they trigger is key to guide tissue regeneration. While macrophage role has been widely investigated, immune response is regulated by other immune cells, including neutrophils, the most abundant leukocyte in human blood. As first responders to injury, infection or material implantation, neutrophils recruit other immune cells, and therefore influence the onset and resolution of chronic inflammation, and macrophage polarization [1]. This response depends on the physical and chemical properties of the biomaterials, among other factors [2]. In this study we report an in vitro culture model to describe the most important neutrophil functions in relation to tissue repair.

We identified neutrophil survival and death, neutrophils extracellular trap formation, release of reactive oxygen species and degranulation with cytokines release as key functions and introduced a corresponding array of assays. These tests were suitable to identify clear differences in the response by neutrophils that were cultured on material of different origin, stiffness and chemical composition. Overall, substrates from biopolymers of natural origin resulted in increased survival, less neutrophil extracellular trap formation, and more reactive oxygen species production than synthetic polymers [3]. Within the range of mechanical properties explored (storage modulus below 5 k Pa), storage modulus of covalently crosslinked hyaluronic acid hydrogels did not significantly alter neutrophils response, whereas polyvinyl alcohol gels of matching mechanical properties displayed a response indicating increased activation.

Additionally, we present the effect of material stiffness, charge, coating and culture conditions in the measured neutrophils response. Further studies are needed to correlate the neutrophil response to tissue healing.

By deciphering how neutrophils initiate and modulate the immune response to material implantation, we aim at introducing new principles to design immunomodulatory biomaterials for musculoskeletal disorders.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Acknowledgments

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**The role of pericytes in regulating cartilage degradation and fibrosis**Huan Meng<sup>1</sup>, Sophie Verrier<sup>1</sup>, Sibylle Grad<sup>1</sup>, Zhen Li<sup>1</sup><sup>1</sup>AO Research Institute Davos, Davos, Switzerland

Pericytes are contractile, motile cells that surround the capillary. Recent studies have shown that pericytes promoted joint fibrosis<sup>1,2</sup> and induced subchondral bone angiogenesis<sup>3</sup>, indicating the role of pericytes in osteoarthritis (OA). However, whether pericytes are involved in regulating inflammatory and catabolic response, as well as fibrotic repair of cartilage is still unclear. Here we used *2D* and *3D* models to investigate the communication of pericytes and chondrocytes under inflammatory osteoarthritis conditions.

CD34-CD146+ pericytes were isolated and sorted from human bone marrow. Human OA chondrocytes were isolated from OA joints. In *2D* studies, monolayer cultured chondrocytes were treated +/- pericyte conditioned media, +/- 1ng/ml IL1 $\beta$  for 24h. In *3D* studies, pericytes and chondrocytes were cultured within fibrin gel in 3D polyurethane scaffolds, separately or combined for 7 days, followed by treatment of +/- IL1 $\beta$  for another 7 days (Fig 2A). The inflammatory response, catabolic activity and expression of fibrosis markers of chondrocytes and pericytes were measured by ELISA and/or q-rtPCR.

Pericytes had weak inflammatory, catabolic and fibrotic response to IL1 $\beta$  (data not shown). *The 2D* study showed that pericyte conditioned media promoted inflammation, catabolism and fibrosis markers of chondrocytes, in the absence of IL1 $\beta$  treatment (Figure 1). However, study in *3D* showed that coculture of chondrocytes and pericytes reduced the inflammatory and catabolic response of chondrocytes to IL1 $\beta$  and induced fibrosis markers in chondrocytes (Figure 2).

Pericytes are involved in regulating inflammatory response, catabolic response and fibrosis of chondrocytes. The opposite results from *2D* and *3D* experiments indicate the variety of the regulatory role of pericytes in the interaction with chondrocytes within different culture models. The underlying mechanism is under evaluation with on-going studies.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****iPSpine: where dreams were born and ideas start to become reality in a team effort**  
Marianna Tryfonidou

Utrecht University, The Netherlands

Tryfonidou leads the Horizon 2020 consortium (iPSpine; 2019-2023) bringing a transdisciplinary team of 21 partners together to address the challenges and bottlenecks of iPS-based advanced therapies towards their transition to the clinic. Here, chronic back pain due to intervertebral disc degeneration is employed as a show case. The project develops the iPS-technology and designed smart biomaterials to carry, protect and instruct the iPS cells within the degenerate disc environment. This work will be presented including ongoing activities focus on translating the developed methodology and tools towards clinically relevant animal models.

The consortium optimized the protocol for the differentiated iPS-notochordal-like cells (iPS-NLCs) and shortlisted two biomaterials shortlisted based on their physicochemical, cytotoxicity, biomechanical and biocompatibility testing. Both were shown to be safe and have been tested with the progenitors of iPS-NLCs. An advanced platform (e.g., the dynamic loading bioreactor for disc tissue) was used to evaluate their performance: the biomaterials supported the iPS-NLC progenitors after injection into the degenerate disc and seem to also support their maturation towards NLCs. Furthermore, we confirmed the capacity of these cells to survive inside degenerated discs at 30 days upon injection in sheep, whereafter we continued with their evaluation at 3 months post-injection. We achieved full evaluation of the sheep spines, including biomechanical analysis using the portable spine biomechanics tester prior analysis at the macro- and microscopic, and biochemical level.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Clinical Trial Quality Assessment in Intervertebral Disc Regeneration: Insights from Publication Status and Funding Sources

Luca Ambrosio<sup>1,2</sup>, Gianluca Vadalà<sup>1,2</sup>, Giorgia Petrucci<sup>1</sup>, Fabrizio Russo<sup>1,2</sup>, Rocco Papalia<sup>1,2</sup>, Vincenzo Denaro<sup>2</sup>

<sup>1</sup>Laboratory for Regenerative Orthopaedics, Research Unit of Orthopaedic and Trauma Surgery, Department of Medicine and Surgery, Università Campus Bio-Medico di Roma, Rome, Italy; <sup>2</sup>Operative Research Unit of Orthopaedic and Trauma Surgery, Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy

Low back pain (LBP) is the main cause of disability worldwide and is primarily triggered by intervertebral disc degeneration (IDD). Although several treatment options exist, no therapeutic tool has demonstrated to halt the progressive course of IDD<sup>1</sup>. Therefore, several clinical trials are being conducted to investigate different strategies to regenerate the intervertebral disc, with numerous studies not reaching completion nor being published<sup>2</sup>. The aim of this study was to analyze the publication status of clinical trials on novel regenerative treatments for IDD by funding source and identify critical obstacles preventing their conclusion.

Prospective clinical trials investigating regenerative treatments for IDD and registered on ClinicalTrials.gov were included. Primary outcomes were publication status and investigational treatment funding. Fisher's exact test was utilized to test the association for categorical variables between groups.

25 clinical trials were identified. Among these, only 6 (24%) have been published. The most common source of funding was university (52%), followed by industry (36%) and private companies (12%). Investigational treatments included autologous (56%) or allogeneic (12%) products alone or in combination with a carrier or delivery system (32%). The latter were more likely utilized in industry or privately funded studies (Fig. 1,  $p=0.0112$ ). No significant difference was found in terms of funding regarding the publication status of included trials (Table 1,  $p=0.9104$ ).

Most clinical trials investigating regenerative approaches for the treatment of IDD were never completed nor published. This is likely due to multiple factors, including difficult enrollment, high dropout rate, and publication bias<sup>3</sup>. More accurate design and technical support from stakeholders and clinical research organization (CROs) may likely increase the quality of future clinical trials in the field.

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**Anti-inflammatory effects of preconditioned Bone Marrow MSCs derived secretome on degenerated human nucleus pulposus cells in vitro**

V. Tilotta<sup>1</sup>, G. Di Giacomo<sup>1</sup>, C. Cicione<sup>1</sup>, L. Ambrosio<sup>2</sup>, F. Russo<sup>2</sup>, R. Papalia<sup>2</sup>, G. Vadalà<sup>2</sup> and V. Denaro<sup>2</sup>

<sup>1</sup>Laboratory of Regenerative Orthopaedic, Research Unit of Orthopaedics and Traumatology, Campus Bio-Medico University, Rome, Italy; <sup>2</sup>UOR of Orthopaedics and Traumatology, Campus Bio-Medico University Hospital Fondazione Rome, Italy

Intervertebral disc degeneration (IDD) is a degenerative disease involving a variety of musculoskeletal and spinal disorders such as lower back pain (LBP)<sup>1,2</sup>. Secretome derived from mesenchymal stem cells (MSCs) have exerted beneficial effect on tissue regeneration<sup>3</sup>. In this study, the goal was to investigate the paracrine and the anti-inflammatory effects of secretome from interleukin IL1 $\beta$  preconditioned Bone Marrow MSCs (BMSCs) on human nucleus pulposus cells (hNPCs) in a 3D *in vitro* model.

Secretome was collected from BMSCs (BMSCs-sec) after preconditioning with 10 ng/mL IL1 $\beta$ . hNPCs were isolated from surgical specimens, culture expanded *in vitro*, encapsulated in alginate beads and treated with: growth medium; IL1 $\beta$  10 ng/mL; IL1 $\beta$  10 ng/mL for 24 hours and then BMSCs-sec. We examined: i) cell proliferation and viability (flow cytometry), ii) nitrite production (Griess assay) and ROS quantification (Immunofluorescence) iii) glycosaminoglycan (GAG) amount (DMBB) and iv) gene expression levels of extracellular matrix (ECM) components and inflammatory mediators (qPCR). One-way ANOVA analysis was used to compare the groups under exam and data were expressed as mean  $\pm$  S.D.

*In vitro* tests showed an enhancement of hNPCs proliferation after treatment with BMSCs-sec ( $p \leq 0.05$ ) compared to IL1 $\beta$  group. After 24 hours, the percentage of dead cells was higher in IL1 $\beta$  treated hNPCs compared to control group and decreased significantly in combined IL1 $\beta$  and BMSCs-sec sample group ( $p \leq 0.01$ ). Nitrite and ROS production were significantly mitigated and GAGs content was improved by preconditioned BMSCs-sec ( $p \leq 0.05$ ). Furthermore, gene expression levels were modulated by BMSCs-sec treatment compared to controls.

Our results supported the potential use of BMSCs' secretome as a cell-free strategy for IDD, overcoming the side effects of cell-therapy. Moreover, secretome derived from IL1 $\beta$  preconditioned BMSCs was able to reduce hNPCs death, attenuate ECM degradation and oxidative stress counteracting IDD progression.

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# EORS 2023

31st Annual Meeting of the  
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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****A model to explore intervertebral disc cell activity in adverse biochemical environments**Sofia Tseranidou<sup>1</sup>, Paola Bermudez-Lekerika<sup>2</sup>, Maria Segarra-Queralt<sup>1</sup>, Benjamin Gantenbein<sup>2</sup>, Christine Le Maitre<sup>3</sup>, Janet Piñero<sup>4</sup>, Jérôme Noailly<sup>1</sup><sup>1</sup>BCN MedTech (Universitat Pompeu Fabra), Spain;<sup>2</sup>Department for BioMedical Research (DBMR), Faculty of Medicine, University of Bern, Switzerland;<sup>3</sup> Department of Oncology and Metabolism, University of Sheffield, United Kingdom;<sup>4</sup>IMIM - Hospital del Mar Medical Research Institute, Spain

Intervertebral disc (IVD) degeneration (IDD) involves imbalance between the anabolic and the catabolic processes that regulate the extracellular matrix of its tissues. These processes are complex, and improved integration of knowledge is needed. Accordingly, we present a nucleus pulposus cell (NPC) regulatory network model (RNM) that integrates critical biochemical interactions in IVD regulation and can replicate experimental results. The RNM was built from a curated corpus of 130 specialized journal articles (Fig.1). Proteins were represented as nodes that interact through activation and inhibition edges. Semi-quantitative steady states (SS) of node activations were calculated [1]. Then, a full factorial sensitivity analysis (SA) identified which out of the RNM 15 cytokines, and 4 growth factors affected most the structural proteins and degrading enzymes. The RNM was further evaluated against metabolic events measured in non-healthy human NP explant cultures, after 2 days of 1ng/ml IL-1B catabolic induction. The RNM represented successfully an anabolic basal SS, as expected in normal IVD (Fig. 2, blue bars). IL-1B was able to increase catabolic markers and angiogenic factors and decrease matrix proteins (Fig.2). Such activity was confirmed by the explant culture measurements (Fig.3A-E). The SA identified TGF- $\beta$  and IL1RA as the two most powerful rescue mediators (Fig.4). Accordingly, TGF $\beta$  signaling-based IDD treatments have been proposed [2] and IL-1RA gene therapy diminished the expression of proteases [3]. It resulted challenging to simulate rescue strategies by IL-10, but interestingly, IL-1B could not induce IL-10 expression in the explant cultures (Fig.3F). Our RNM was confronted to independent in vitro measurements and stands for a unique model, to integrate soluble protein signaling and explore IDD.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## ***In vitro* models mimicking the bone remodeling cycle**

Sandra Hofmann

Bioengineering Bone, Department of Biomedical Engineering and Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven, The Netherlands

Metabolic bone diseases, such as osteoporosis and osteopetrosis, result from an imbalanced bone remodeling process. *In vitro* bone models are often used to investigate either bone formation or resorption independently, while *in vivo*, these processes are coupled. Combining these processes in a co-culture is challenging as it requires finding the right medium components to stimulate each cell type involved without interfering with the other cell type's differentiation. Furthermore, differentiation stimulating factors often comprise growth factors in supraphysiological concentrations, which can overshadow the cell-mediated crosstalk and coupling.

To address these challenges, we aimed to recreate the physiological bone remodeling process, which follows a specific sequence of events starting with cell activation and bone resorption by osteoclasts, reversal, followed by bone formation by osteoblasts. We used a mineralized silk fibroin scaffold as a bone-mimetic template, inspired by bone's extracellular matrix composition and organization. Our model supported osteoclastic resorption and osteoblastic mineralization in the specific sequence that represents physiological bone remodeling.

We also demonstrated how culture variables, such as different cell ratios, base media, and the use of osteogenic/osteoclast supplements, and the application of mechanical load, can be adjusted to represent either a high bone turnover system or a self-regulating system. The latter system did not require the addition of osteoclastic and osteogenic differentiation factors for remodeling, therefore avoiding growth factor use.

Our *in vitro* model for bone remodeling has the potential to reduce animal experiments and advance *in vitro* drug development for bone remodeling pathologies like osteoporosis. By recreating the physiological bone remodeling cycle, we can investigate cell-cell and cell-matrix interactions, which are essential for understanding bone physiology and pathology. Furthermore, by tuning the culture variables, we can investigate bone remodeling under various conditions, potentially providing insights into the mechanisms underlying different bone disorders.

**Modelling paracrine chondrocyte communication effects at the tissue level**Andreu Pascuet-Fontanet<sup>1</sup>, Maria Segarra-Queralt<sup>1</sup>, Jérôme Noailly<sup>1</sup><sup>1</sup>BCN-MedTech, Universitat Pompeu Fabra (UPF), Barcelona, Spain

Osteoarthritis (OA) leads to articular cartilage degradation, following complex dysregulation of chondrocyte's metabolism towards a catabolic state. Mechanical and biochemical signals are involved and need to be considered to understand the condition [1]. Regulatory network-based models (RNM) successfully simulated the biological activity of the chondrocyte [2] and the transduction of mechanical signals at the molecular and cell levels [3]. However, the knowledge gap between single-cell regulation and intercellular communication in tissue volumes hinders the interpretability of such models at larger scales. Accordingly, a novel tissue-level biochemical model is proposed. We hypothesise that it is possible to simulate interacting network effects through the transport of diluted species in a finite-element model, to grasp relevant dynamics of cell and tissue regulation in OA. Chondrocyte RNM equations were translated into a reaction term of 18 multi-species diffusion model (e.g., 3 anti-inflammatory and 8 pro-inflammatory interleukins, 3 pro-anabolic and 1 pro-catabolic growth factors, 2 nociceptive factors and 2 pro-inflammatory cytokines). Elements with RNM reaction terms represented the chondrocytes and were distributed randomly through the model, according to known cellular density in the knee cartilage, and could both react to and produce diffusive entities through the pericellular matrix, associated with reduced diffusion coefficients. The model was constructed over a 2D square of 0.47 mm sides considered to be in the middle of the cartilage, so boundary conditions were settled as periodic. Different simulations were initialised with initial concentrations of either healthy or pro-OA mediators. Preliminary results showed that, independently of the initial conditions, the chondrocytes successfully evolved into anabolic states, in absence of sustained pro-catabolic external stimulations, in contrast to single-cell RNM [2]. Our intercellular model suggests that paracrine communication may increase robustness towards cartilage maintenance, and future tests shall reveal new OA dynamics.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## 2D static and 3D dynamic co-cultures of human bone marrow mesenchymal stem cells as novel in vitro bioengineered myogenic models

Pasqualina Scala<sup>1</sup>, Valentina Giudice<sup>1</sup>, Carmine Selleri<sup>1</sup>, Nicola Maffulli<sup>1,2</sup>, Laura Rehak<sup>3</sup>, Giovanna Della Porta<sup>1,4</sup>

<sup>1</sup>Department of Medicine, Surgery and Dentistry, University of Salerno, via S. Allende, 84081 Baronissi (SA), IT; <sup>2</sup>Centre for Sports and Exercise Medicine, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 275 Bancroft Road, London E1 4DG, UK; <sup>3</sup>Athena Biomedical innovations, viale Europa 139, Florence, 50126, IT; <sup>4</sup>Interdepartment Centre BIONAM, University of Salerno, via Giovanni Paolo I, 84084 Fisciano (SA), IT.

Spontaneous muscle regenerative potential is limited, as severe injuries incompletely recover and result in chronic inflammation (1). Current therapies are restricted to conservative management, not providing a complete restitutio ad integrum; therefore, alternative therapeutic strategies are welcome, such as cell-based therapies with stem cells or Peripheral Blood Mononuclear Cells (PBMCs). Here, we described two different in vitro myogenic models: a 2D perfused system and a 3D bioengineered scaffold within a perfusion bioreactor. Both models were assembled with human bone marrow-derived mesenchymal stem cells (hBM-MSCs) and human primary skeletal myoblasts (hSkMs) to study induction and maintenance of myogenic phenotype in presence of PBMCs (2-3). When hBM-MSCs were cultured with human primary skeletal myoblasts (hSkMs) in medium supplemented with 10 ng/mL of bFGF; cells showed increased expression of myogenic-related gene, such as Desmin and Myosin Heavy Chain II (MYH2) after 21 days, and a prevalent expression of anti-inflammatory cytokines (IL10, 15-fold). Next, PBMCs were added in an upper transwell chamber and hBM-MSCs significantly upregulated myogenic genes throughout the culture period, while pro-inflammatory cytokines (e.g., IL12A) were downregulated. In 3D, hBM-MSCs plus hSkMs embedded in fibrin-based scaffolds, cultured in dynamic conditions, showed that all myogenic-related genes tended to be upregulated in the presence of PBMCs, and Desmin and MYH2 were also detected at protein level, while pro-inflammatory cytokine genes were significantly downregulated in the presence of PBMCs. In conclusion, our works suggest that hBM-MSCs have a versatile myogenic potential, enhanced and modulated by PMBCs. Moreover, our 3D biomimetic approach seemed to better resemble the tissue architecture allowing an efficient in vitro cellular cross-talk.

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**Antihypertrophic effect of PGE2 on chondrocytes suggests adjustments of OA analgesia**Schmidt S<sup>1</sup>, Klampfleuthner F<sup>1</sup>, Diederichs S<sup>1</sup><sup>1</sup>Research Centre for Experimental Orthopaedics, Orthopaedic University Hospital Heidelberg

The signaling molecule prostaglandin E2 (PGE2), synthesized by cyclooxygenase-2 (COX-2), is immunoregulatory and reported to be essential for skeletal stem cell function. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in osteoarthritis (OA) analgesia, but cohort studies suggested that long-term use may accelerate pathology. Interestingly, OA chondrocytes secrete high amounts of PGE2. Mesenchymal stromal cell (MSC) chondrogenesis is an in vitro OA model that phenocopies PGE2 secretion along with a hypertrophic OA-like cell morphology. Our aim was to investigate cause and effects of PGE2 secretion in MSC-based cartilage neogenesis and hypertrophy and identify molecular mechanisms responsible for adverse effects in OA analgesia.

Human bone marrow-derived MSCs were cultured in chondrogenic medium with TGFβ (10ng/mL) and treated with PGE2 (1μM), celecoxib (COX-2 inhibitor; 0.5μM), AH23848/AH6809 (PGE2 receptor antagonists; 10μM), or DMSO as a control (n=3-4). Assessment criteria were proteoglycan deposition (histology), chondrocyte/hypertrophy marker expression (qPCR), and ALP activity. PGE2 secretion was measured (ELISA) after TGFβ withdrawal (from day 21, n=2) or WNT inhibition (2μM IWP-2 from day 14; n=3).

Strong decrease in PGE2 secretion upon TGFβ deprivation or WNT inhibition identified both pathways as PGE2 drivers. Homogeneous proteoglycan deposition and *COL2A1* expression analysis showed that MSC chondrogenesis was not compromised by any treatment. Importantly, hypertrophy markers (*COL10A1*, *ALPL*, *SPP1*, *IBSP*) were significantly reduced by PGE2 treatment, but increased by all inhibitors. Additionally, PGE2 significantly decreased ALP activity (2.9-fold), whereas the inhibitors caused a significant increase (1.3-fold, 1.7-fold, 1.8-fold). This identified PGE2 as an important inhibitor of chondrocyte hypertrophy.

Although TGFβ and WNT are known pro-arthritis signaling pathways, they appear to induce a PGE2-mediated antihypertrophic effect that can counteract pathological cell changes in chondrocytes. Hampering this rescue mechanism via COX inhibition using NSAIDs thus risks acceleration of OA progression, indicating the need of OA analgesia adjustment.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Senescence or apoptosis: chondrocyte response to inflammation *in vitro* is dependent on its dimensional environment**

Estelle Strangmark<sup>1</sup>, Jia Hua Wang<sup>1</sup>, Rawiya Al Hosni<sup>1</sup>, Hayat Muhammad<sup>1</sup>, Mohammad Alkhrayef<sup>1</sup>, Eve Robertson-Waters<sup>1</sup>, Alexandra MacMillan<sup>1</sup>, Benjamin Gompels<sup>1</sup>, Antonia Vogt<sup>1</sup>, Wasim Khan<sup>1</sup>, Mark Birch<sup>1</sup>, Andrew McCaskie<sup>1,2</sup>

<sup>1</sup>Division of Trauma and Orthopaedic Surgery, Department of Surgery, University of Cambridge; <sup>2</sup>Wellcome-MRC Stem Cell Institute, University of Cambridge

Cell culture on tissue culture plastic (TCP) is widely used across biomedical research to understand the *in vivo* environment of a targeted biological system. However, growing evidence indicates that the characteristics of cells investigated in this way differ substantially from their characteristics in the human body. The limitations of TCP monolayer cell cultures are especially relevant for chondrocytes, the cell population responsible for producing cartilage matrix, because their zonal organization in hyaline cartilage is not preserved in a flattened monolayer assay. Here, we contrast the response of primary human chondrocytes to inflammatory cytokines, tumor necrosis factor-alpha and interferon-gamma, via transcriptional, translational, and histological profiling, when grown either on TCP or within a 3D cell pellet (scaffold-less). We focus on anti-apoptotic (Bcl2), pro-apoptotic (Bax, Mff, Fis1), and senescent (MMP13, MMP1, PCNA, p16, p21) markers. We find that the 3D environment of the chondrocyte has a profound effect on the behavior and fate of the cell; in TCP monolayer cultures, chondrocytes become anti-apoptotic and undergo senescence in response to inflammatory cytokines, whereas in 3D cell pellet cultures, they exhibit a pro-apoptotic response. Our findings demonstrate that chondrocyte culture environment plays a pivotal role in cell behavior, which has important implications for the clinical applicability of *in vitro* research of cartilage repair. Although there are practical advantages to 2D cell cultures, our data suggest researchers should be cautious when drawing conclusions if they intend to extrapolate findings to *in vivo* phenomena. Our data demonstrates opposing chondrocyte responses in relation to apoptosis and senescence, which appear to be solely reliant on the environment of the culture system. This biological observation highlights that proper experimental design is crucial to increase the clinical utility of cartilage repair experiments and streamline their translation to therapy development.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Biofabrication and 3D Bioprinting Strategies for Musculoskeletal Tissue Regeneration**

Daniel Kelly

Trinity Centre for Biomedical Engineering, School of Engineering, Trinity College Dublin, Ireland

Our musculoskeletal system has a limited capacity for repair. This has led to increased interest in the development of tissue engineering and biofabrication strategies for the regeneration of musculoskeletal tissues such as bone, ligament, tendon, meniscus and articular cartilage. This talk will demonstrate how different musculoskeletal tissues, specifically cartilage, bone and osteochondral defects, can be repaired using emerging biofabrication and 3D bioprinting strategies. This will include examples from our lab where cells and/or growth factors are bioprinted into constructs that can be implanted directly into the body, to approaches where biomimetic tissues are first engineered *in vitro* before *in vivo* implantation. The efficacy of these different biofabrication strategies in different preclinical studies will be reviewed, and lessons from the relative successes and failures of these approaches to tissue regeneration will be discussed.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Fabrication of a high-throughput 3D printed osteogenic coral-containing scaffold**

Stephanie Doyle<sup>1</sup>, Deirdre Winrow<sup>1</sup>, Temi Aregbesola<sup>2</sup>, James Martin<sup>3</sup>, Elin Pernevik<sup>4</sup>, Volodymyr Kuzmenko<sup>4</sup>, Linda Howard<sup>1</sup>, Kerry Thompson<sup>2</sup>, Martin Johnson<sup>3</sup>, Cynthia Coleman<sup>1</sup>

<sup>1</sup>College of Medicine, Nursing and Health Science, School of Medicine, Regenerative Medicine Institute (REMEDI), University of Galway, Galway, Ireland; <sup>2</sup>College of Medicine, Nursing and Health Science, School of Medicine, Anatomy Imaging and Microscopy (AIM), School of Medicine, University of Galway, Galway, Ireland; <sup>3</sup>Zoan Nuáil Teoranta T/A Zoan BioMed, The Hatchery Building, Cloonacarton, Recess, Galway; <sup>4</sup>CELLINK, Gothenburg, Sweden

In 2021 the bone grafting market was worth €2.72 billion globally [1]. As allograft bone has a limited supply and risk of disease transmission, the demand for synthetic grafting substitutes (BGS) continues to grow while allograft bone grafts steadily decrease [1]. Synthetic BGS are low in mechanical strength and bioactivity, inspiring the development of novel grafting materials, a traditionally laborious and expensive process. Here a novel BGS derived from sustainably grown coral was evaluated. Coral-derived scaffolds are a natural calcium carbonate bio-ceramic, which induces osteogenesis in bone marrow mesenchymal stem cells (MSCs), the cells responsible for maintaining bone homeostasis and orchestrating fracture repair [2]. By 3D printing MSCs in coral-laden bioinks (Fig. 1) we utilise high throughput (HT) fabrication and evaluation of osteogenesis, overcoming the limitations of traditional screening methods.

MSC and coral-laden GelXA (CELLINK) bioinks were 3D printed in square bottom 96 well plates using a CELLINK BIO X printer with pneumatic adapter. Samples were non-destructively monitored during the culture period, evaluating both the sample and the culture media for metabolism (PrestoBlue), cytotoxicity (lactose dehydrogenase (LDH)) and osteogenic differentiation (alkaline phosphatase (ALP)). Endpoint, destructive assays used included qRT-PCR and SEM imaging.

The inclusion of coral in the printed bioink was biocompatible with the MSCs, as reflected by maintained metabolism and low LDH release. The inclusion of coral induced osteogenic differentiation in the MSCs as seen by ALP secretion and increased RUNX2, collagen I and osteocalcin transcription.

Sustainably grown coral was successfully incorporated into bioinks, reproducibly 3D printed, non-destructively monitored throughout culture and induced osteogenic differentiation in MSCs. This HT fabrication and monitoring workflow offers a faster, less labour-intensive system for the translation of bone substitute materials to clinic.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Development of 4D Printing Strategy for Skeletal Muscle Tissue Engineering

Emre Ergene<sup>1,2</sup>, Gorkem Liman<sup>2</sup>, Gokhan Demirel<sup>2</sup>, Pinar Yilgor<sup>1</sup>

<sup>1</sup>Department of Biomedical Engineering, Ankara University, 06830 Ankara, Turkiye.

<sup>2</sup>Bio-inspired Materials Research Laboratory (BIMREL), Department of Chemistry, Gazi University, 06500 Ankara, Turkiye.

Skeletal muscle tissue engineering has made progress towards production of functional tissues in line with the development in materials science and fabrication techniques. In particular, combining the specificity of 3D printing with smart materials has introduced a new concept called the 4D printing. Inspired by the unique properties of smart/responsive materials, we designed a bioink made of gelatin, a polymer with well-known cell compatibility, to be 3D printed on a magnetically responsive substrate. Gelatin was made photocrosslinkable by the methacrylate reaction (GELMA), and its viscosity was finetuned by blending with alginate which was later removed by alginate lyase treatment, so that the printability of the bioink as well as the cell viability can be finetuned. C2C12 mouse myoblasts-laden bioink was then 3D printed on a magnetic substrate for 4D shape-shifting. The magnetic substrate was produced using silicon rubber (EcoFlex) and carbonyl iron powders. After 3D printing, the bioink was crosslinked on the substrate, and the substrate was rolled with the help of a permanent magnet. Unrolled (Open) samples were used as the control group. The stiffness of the bioink matrix was found to be in the range of 13-45 kPa (1), which is the appropriate value for the adhesion of C2C12 cells. In the cell viability analysis, it was observed that the cells survived and could proliferate within the 7-day duration of the experiment. As a result of the immunofluorescence test, compared to the Open Group, more cell nuclei were observed overlapping MyoD1 expression in the Rolled Group; this indicated that the cells in these samples had more cell-cell interactions and therefore tended to form more myotubes.

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**3D Bioprinting Tissue Engineered Meniscal Constructs**

Grace McDermott<sup>1</sup>, Marco Domingos<sup>2</sup>, Bilal Barkatali<sup>3</sup>, Stephen Richardson<sup>1</sup>

<sup>1</sup>Faculty of Biology, Medicine and Health, The University of Manchester, UK

<sup>2</sup>Faculty of Science and Engineering, The University of Manchester, UK

<sup>3</sup>Salford Royal University Teaching NHS Trust, UK

Meniscal injuries affect over 1.5 million people across Europe and the USA annually. Injury greatly reduces knee joint mobility and quality of life and frequently leads to the development of osteoarthritis. Tissue engineered strategies have emerged in response to a lack of viable treatments for meniscal pathologies. However, to date, constructs mimicking the structural and functional organisation of native tissue, whilst promoting deposition of new extracellular matrix, remains a bottleneck in meniscal repair. 3D bioprinting allows for deposition and patterning of biological materials with high spatial resolution. This project aims to develop a biomimetic 3D bioprinted meniscal substitute.

Meniscal tissue was characterised to effectively inform the design of biomaterials for bioprinting constructs with appropriate structural and functional properties. Histology, gene expression and mass spectrometry were performed on native tissue to investigate tissue architecture, matrix components, cell populations and protein expression regionally across the meniscus. 3D laser scanning and magnetic resonance imaging were employed to acquire the external geometrical information prior to fabrication of a 3D printed meniscus. Bioink suitability was investigated through regional meniscal cell encapsulation in blended hydrogels, with the incorporation of growth factors and assessed for their suitability through rheology, scanning electron microscopy, histology and gene expression analysis.

Meniscal tissue characterisation revealed regional variations in matrix compositions, cellular populations and protein expression. The process of imaging through to 3D printing highlighted the capability of producing a construct that accurately replicated meniscal geometries. Regional meniscal cell encapsulation into hydrogels revealed a recovery in cell phenotype, with the incorporation of growth factors into the bioink's stimulating cellular re-differentiation and improved zonal functionality.

Meniscus biofabrication highlights the potential to print patient specific, customisable meniscal implants. Achieving zonally distinct variations in cell and matrix deposition highlights the ability to fabricate a highly complex tissue engineered construct.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## The impact of metabolism dysregulation in scaffold-guided large volume bone regeneration

Daniela B. Dias<sup>1</sup>, Raphaela Fritsche-Guenther<sup>2</sup>, WingLee Chan<sup>1</sup>, Agnes Ellinghaus<sup>1,3</sup>, Georg N. Duda<sup>1,3</sup>, Jennifer Kirwan<sup>2,4</sup> and Patrina S.P. Poh<sup>1</sup>

<sup>1</sup>Julius Wolff Institute, Berlin Institute of Health (BIH) at Charité—Universitätsmedizin Berlin, 13353 Berlin, Germany; <sup>2</sup>Berlin Institute of Health at Charité—BIH Metabolomics Platform, 10178 Berlin, Germany; <sup>3</sup>Berlin Institute of Health Center for Regenerative Therapies, Berlin Institute of Health at Charité—Universitätsmedizin Berlin, 13353 Berlin, Germany; <sup>4</sup>Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Robert-Rössle-Str 10, 13125 Berlin, Germany.

The ability of the body to constantly maintain metabolism homeostasis while fulfilling the heightened energy and macromolecule demand is crucial to ensure successful tissue healing outcomes. Studies investigating the local metabolic environment during healing are scarce to date. Here, using Type 2 Diabetes (T2D) as a study model, we investigate the impact of metabolism dysregulation on scaffold-guided large-volume bone regeneration. Our study treated wild-type or T2D rats with 5 mm critical-sized femoral defects with 3D-printed polycaprolactone (PCL) scaffolds with 70% porosity. Metabolomics was leveraged for a holistic view of metabolism alteration as healing progress and correlated to regenerated bone tissue volume and quality assessed using micro-computed tomography ( $\mu$ -CT), histology, and immunohistology. Semi-targeted metabolomics analysis indicated dysregulation in the glycolysis and TCA cycle – the main energy production pathways, in T2D compared to healthy animals. The abundance of metabolites substrates, i.e., amino acids – for protein/ extracellular matrix synthesis was also affected in T2D. Tissue-level metabolites observations aligned with morphological observation with less newly formed bone observed in T2D than wild-type rats. This study enlightens the metabolism landscape during scaffold-guided large-volume bone regeneration in wild-type vs. T2D to further guide the personalization of the scaffold to drive successful regeneration.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Exploring the ECM from young donors to enhance the impaired osteogenic properties of aged cells**Marta S. Carvalho<sup>1,2</sup>, Joaquim M.S. Cabral<sup>1,2</sup>, Cláudia L. da Silva<sup>1,2</sup>

<sup>1</sup>Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>2</sup>Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

Mesenchymal stromal cells (MSC) have been proposed as an emerging cell therapy for bone tissue engineering applications. However, the healing capacity of the bone tissue is often compromised by patient's age and comorbidities, such as osteoporosis. In this context, it is important to understand the impact of donor age on the therapeutic potential of MSC. Importantly, the impact on donor age is not restricted to cells themselves but also to their microenvironment that is known to affect cell function. The extracellular matrix (ECM) has an important role in stem cell microenvironment, being able to modulate cell proliferation, self-renewal and differentiation. Decellularized cell-derived ECM (dECM) has been explored for regenerative medicine applications due to its bioactivity and its resemblance to the *in vivo* microenvironment. Thus, dECM offers the opportunity not only to develop microenvironments with customizable properties for improvement of cellular functions but also as a platform to study cellular niches in health and disease. In this study, we investigated the capacity of the microenvironment to rescue the impaired proliferative and osteogenic potential of aged MSC. The goal of this work was to understand if the osteogenic capacity of MSC could be modulated by exposure to a dECM derived from cells obtained from young donors. When aged MSC were cultured on dECM derived from young MSC, their *in vitro* proliferative and osteogenic capacities were enhanced. Our results suggest that the microenvironment, specifically the ECM, plays a crucial role in the osteogenic differentiation capacity of MSC. dECM might be a valuable clinical strategy to overcome the age-related decline in the osteogenic potential of MSC by recapitulating a younger microenvironment, attenuating the effects of aging on the stem cell niche. Overall, this study opens new possibilities for developing clinical strategies for elderly patients with limited bone formation capacity who currently lack effective treatments.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Deciphering cell–cell interactions of human bone marrow mononuclear cells in 3D culture to enhance the regenerative potential of bone marrow autografts**

Sebastian Häusner<sup>1,2</sup>, Konstantin Horas<sup>2</sup>, Torsten Blunk<sup>3</sup>, Marietta Herrmann<sup>1,2</sup>

<sup>1</sup>University Hospital Würzburg (UKW), IZKF Research Group Tissue regeneration in musculoskeletal diseases, Röntgenring 11, 97070 Würzburg; <sup>2</sup>University of Würzburg, Orthopedic Department, Bernhard-Heine-Center for Locomotion Research, Brettreichstraße 11, 97074 Würzburg; <sup>3</sup>University Hospital Würzburg (UKW), Department of Trauma, Hand, Plastic and Reconstructive Surgery, Oberdürrbacher Str. 6, 97080, Würzburg, Germany

Autografts containing bone marrow (BM) are current gold standard in the treatment of critical size bone defects, delayed union and bone nonunion defects. Although reaching unprecedented healing rates in bone reconstruction(1), the mode of action and cell-cell interactions of bone marrow mononuclear cell (BM-MNC) populations have not yet been described. BM-MNCs consist of a heterogeneous mixture of hematopoietic and non-hematopoietic lineage fractions. Cell culture in a 3D environment is necessary to reflect on the complex mix of these adherent and non-adherent cells in a physiologically relevant context. Therefore, the main aim of this approach was to establish conditions for a stable 3D BM-MNC culture to assess cellular responses on fracture healing strategies.

BM samples were obtained from residual material after surgery with positive ethical vote and informed consent of the patients. BM-MNCs were isolated by density gradient centrifugation, and cellular composition was determined by flow cytometry to obtain unbiased data sets on contained cell populations. Collagen from rat tail and human fibrin was used to facilitate a 3D culture environment for the BM-MNCs over a period of three days. Effects on cellular composition that could improve the regenerative potential of BM-MNCs within the BM autograft were assessed using flow cytometry. Cell-cell-interactions were visualized using confocal microscopy over a period of 24 hours. Cell localization and interaction partners were characterized using immunofluorescence labeled paraffin sectioning.

Main BM-MNC populations like Monocytes, Macrophages, T cells and endothelial progenitor cells were determined and could be conserved in 3D culture over a period of three days. The 3D cultures will be further treated with already clinically available reagents that lead to effects even within a short-term exposure to stimulate angiogenic, osteogenic or immunomodulatory properties. These measures will help to ease the translation from “bench to bedside” into an intraoperative protocol in the end.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Effect of PPARG inhibition on human BMSC cell fate**Maria Rosa Iaquinta<sup>1</sup>, Carmen Lanzillotti<sup>1</sup>, Mauro Tognon<sup>1</sup>, Fernanda Martini<sup>1</sup>, Martin J Stoddart<sup>2</sup>, Elena Della Bella<sup>2</sup><sup>1</sup>Dept. Medical Sciences, University of Ferrara, Ferrara, Italy; <sup>2</sup>AO Research Institute Davos, Davos Platz, Switzerland

The effects of dexamethasone (dex), during *in vitro* human osteogenesis, are contrasting [1]. Indeed, dex downregulates *SOX9* during osteogenic differentiation of human bone marrow mesenchymal stromal cells (HBMSCs). However, dex also promotes *PPARG* expression, resulting in the formation of adipocyte-like cells within the osteogenic monolayers. The regulation of both *SOX9* and *PPARG* seems to be downstream the transactivation activity of the glucocorticoid receptor (GR), thus the effect of dex on *SOX9* downregulation is indirect. This study aims at determining whether PPAR- $\gamma$  regulates *SOX9* expression levels, as suggested by several studies.

HBMSCs were isolated from bone marrow of patients with written informed consent. HBMSCs were cultured in different osteogenic induction media containing 10 or 100 nM dex. Undifferentiated cells were used as controls. Cells were treated either with a pharmacological PPAR- $\gamma$  inhibitor T0070907 (donors n=4) or with a *PPARG*-targeting siRNA (donors n=2). Differentiation markers or PPAR- $\gamma$  target genes were analysed by RT-qPCR. Mineral deposition was assessed by ARS staining. Two-way ANOVA followed by a Tukey's multiple comparison test compared the effects of treatments.

At day 7, T0070907 downregulated *ADIPOQ* and upregulated *CXCL8*, respectively targets of PPAR- $\gamma$ -mediated transactivation and transrepression. *RUNX2* and *SOX9* were also significantly downregulated in absence of dex. *PPARG* was successfully downregulated by siRNA. *ADIPOQ* expression was also inhibited, while *CXCL8* did not show any significant difference between siRNA treatment groups. *RUNX2* was downregulated by the *PPARG*-siRNA treatment in presence of 100 nM dexamethasone, while *SOX9* levels were not affected. ARS showed no change in the mineralization levels when *PPARG* expression or activity was inhibited.

Understanding how dex regulates HBMSC differentiation is of pivotal importance to refine current *in vitro* models. These results suggest that *PPARG* does not mediate *SOX9* downregulation. Unexpectedly, *RUNX2* expression was also unaltered or even downregulated after PPAR- $\gamma$  inhibition.

**Reference:** [1] Della Bella et al. Int J Mol Sci. 2021; 22: 4785.**Acknowledgements:** AO Foundation, AO Research Institute (CH) and PRIN 2017 MUR (IT) for financial support.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Mapping the spatial and temporal heterogeneity of mesenchymal stromal cells when used as a cell therapy in osteochondral repair**

M Seah<sup>1</sup>, M Birch<sup>1</sup>, I Moutsopoulos<sup>1</sup>, I Mohorianu<sup>1</sup>, A McCaskie<sup>1</sup>

<sup>1</sup>Division of Trauma and Orthopaedic Surgery, University of Cambridge, UK

Despite osteoarthritis (OA) representing a large burden for healthcare systems, there remains no effective intervention capable of regenerating the damaged cartilage in OA. Mesenchymal stromal cells (MSCs) are adult-derived, multipotent cells which are a candidate for musculoskeletal cell therapy. However, their precise mechanism of action remains poorly understood.

The effects of an intra-articular injection of human bone-marrow derived MSCs into a knee osteochondral injury model were investigated in C57Bl/6 mice. The cell therapy was retrieved at different time points and single cell RNA sequencing was performed to elucidate the transcriptomic changes relevant to driving tissue repair. Mass cytometry was also used to study changes in the mouse immune cell populations during repair.

Histological assessment reveals that MSC treatment is associated with improved tissue repair in C57Bl/6 mice. Single cell analysis of retrieved human MSCs showed spatial and temporal transcriptional heterogeneity between the repair tissue (in the epiphysis) and synovial tissue. A transcriptomic map has emerged of some of the distinct genes and pathways enriched in human MSCs isolated from different tissues following osteochondral injury. Several MSC subpopulations have been identified, including proliferative and reparative subpopulations at both 7 days and 28 days after injury. Supported by the mass cytometry results, the immunomodulatory role of MSCs was further emphasised, as MSC therapy was associated with the induction of increased numbers of regulatory T cells correlating with enhanced repair in the mouse knee.

The transcriptomes of a retrieved MSC therapy were studied for the first time. An important barrier to the translation of MSC therapies is a lack of understanding of their heterogeneity, and the consequent lack of precision in its use. MSC subpopulations with different functional roles may be implicated in the different phases of tissue repair and this work offers further insights into repair process.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

**There is no such thing as luck. There are only choices.**

José Micard Teixeira, Author, Mentor & Coach, Portugal

José Micard Teixeira was born in Aveiro - Portugal, in 1961. He graduated in International Relations from the University of Minho - Braga and was a trainee at the Economic and Social Committee of the European Communities. He then joined SONAE (one of the biggest Portuguese multinationals) where he held general management positions in several companies. At the age of 40, he decides to change his life and direction and chooses to help people find their way in life. He did various training courses in personal development and ended up being internationally certified as a Life Coach by the ICC-UK, International Coaching Community. Currently, he gives Life Coaching consultations and lectures on a wide variety of topics of the human condition. He has written seven books of motivational texts and is preparing the launch of his new book for later this year. He is an advocate of freedom and truth as a means for each person to live their own life.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Can we use smartphones to monitor orthopaedic patients' physical activities during the perioperative period? A prospective observational study.**

Arash Ghaffari<sup>1</sup>, Rikke Emilie Kildahl Lauritsen<sup>1</sup>, Michael Christensen<sup>2</sup>, Trine Rolighed Thomsen<sup>3,4</sup>, Harshit Mahapatra<sup>2</sup>, Robert Heck<sup>4</sup>, Søren Kold<sup>1</sup>, Ole Rahbek<sup>1</sup>

<sup>1</sup>Interdisciplinary Orthopaedics, Aalborg University Hospital, Aalborg, Denmark; <sup>2</sup>Alexandra Institute, Aarhus N, Denmark; <sup>3</sup> Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark; <sup>4</sup>The Danish Technological Institute, Aarhus C, Denmark

Smartphones are often equipped with inertial sensors capable of measuring individuals' physical activities. Their role in monitoring the patients' physical activities in telemedicine, however, needs to be explored. The main objective of this study was to explore the correlation between a participant's daily step counts and the daily step counts reported by their smartphone. This prospective observational study was conducted on patients undergoing lower limb orthopedic surgery and a group of non-patients. The data collection period was from 2 weeks before until four weeks after the surgery for the patients and two weeks for the non-patients. The participants' daily steps were recorded by physical activity trackers employed 24/7, and an application recorded the number of daily steps registered by the participants' smartphones. We compared the cross-correlation between the daily steps time-series taken from the smartphones and physical activity trackers in different groups of participants. We also employed mixed modeling to estimate the total number of steps. Overall, 1067 days of data were collected from 21 patients (11 females) and 10 non-patients (6 females). The cross-correlation coefficient between the smartphone and physical activity tracker was 0.70 [0.53–0.83]. The correlation in the non-patients was slightly higher than in the patients (0.74 [0.60–0.90] and 0.69 [0.52–0.81], respectively). Considering the ubiquity, convenience, and practicality of smartphones, the high correlation between the smartphones and the total daily step time-series highlights the potential usefulness of smartphones in detecting the change in the step counts in remote monitoring of the patient's physical activity.

**Mining of biomechanical and geometry data of IVD FE simulations**Estefano Muñoz-Moya, Carlos Ruiz, Gemma Piella, Jérôme Noailly

Department of Information and Communications Technologies, Pompeu Fabra University, Spain

This study investigates the relationships between Intervertebral Disc (IVD) morphology and biomechanics [2] using patient-specific (PS) finite element (FE) models and poromechanical simulations [1].

169 3D lumbar IVD shapes from the European project MySpine (FP7-269909), spanning healthy to Pfirrmann grade 4 degeneration, were obtained from MRIs [3]. A Bayesian Coherent Point Drift algorithm aligned meshes [4] to a previously validated structural FE mesh of the IVD [1]. After mesh quality analyses and Hausdorff distance measurements, mechanical simulations were performed: 8 and 16 hours of sleep and daytime, respectively, applying 0.11 and 0.54 MPa of pressure on the upper cartilage endplate (CEP). Simulation results were extracted from the anterior (ATZ) and posterior regions (PTZ) and the center of the nucleus pulposus (CNP) (Figure 1). Data mining was performed using Linear Regression, Support Vector Machine, and eXtreme Gradient Boosting techniques. Mechanical variables of interest in DD, such as pore fluid velocity (FLVEL), water content, and swelling pressure, were examined. The morphological variables of the simulated discs were used as input features (Figure). Local morphological variables significantly impacted the local mechanical response. The local disc heights, respectively in the mid (mh), anterior (ah), and posterior (ph) regions, were key factors in general. Additionally, fluid transport, reflected by FLVEL, was greatly influenced ( $r^2$  0.69) by the shape of the upper and lower cartilage endplates (CEPs).

This study suggests that disc morphology affects Mechanical variables of interest in DD. Attention should be paid to the antero-posterior distribution and local effects of disc heights. Surprisingly, the CEP morphology remotely affected the fluid transport in NP volumes around mid-height, and mechanobiological implications shall be explored. In conclusion, patient-specific IVD modeling has strong potential to unravel important correlations between IVD phenotypes and local tissue regulation.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Chronic stress results in increased pain perception in osteoarthritis *in vivo*

G. Rösch<sup>1</sup>, A. E. Rapp<sup>1</sup>, P. Tsai<sup>2</sup>, H. Kohler<sup>1</sup>, S. Taheri<sup>3</sup>, A. F. Schilling<sup>3</sup>, F. Zaucke<sup>1</sup>, D. Slattery<sup>2</sup>, Z. Jenei-Lanzl<sup>1</sup>

<sup>1</sup>Dr Rolf M. Schwiete Research Unit for Osteoarthritis, Department of Orthopedics (Friedrichsheim), University Hospital Frankfurt, Goethe University, 60528 Frankfurt/Main, Germany; <sup>2</sup>Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, University Hospital Frankfurt, Goethe University, 60528 Frankfurt/Main, Germany; <sup>3</sup>Department of Trauma Surgery, Orthopedic Surgery and Plastic Surgery, University Medical Center Göttingen, 37075 Göttingen, Germany

Osteoarthritis (OA) affects the whole joint and leads to chronic pain<sup>1</sup>. The sympathetic nervous system (SNS) seems to be involved in OA pathogenesis, as indicated by *in vitro* studies<sup>2,3</sup> as well as by our latest work demonstrating that sympathectomy in mice results in increased subchondral bone volume in the OA knee joint<sup>4</sup>. We assume that chronic stress may lead to opposite effects, such as an increased bone loss in OA due to an elevated sympathetic tone. Therefore, we analyzed experimental OA progression in mice exposed to chronic stress. OA was induced in male C57BL/6J mice by surgical destabilization of the medial meniscus (DMM)<sup>5</sup> and Sham as well as non-operated mice served as controls. Half of these groups were exposed to chronic unpredictable mild stress (CUMS)<sup>6</sup>. After 12 weeks, chronic stress efficiency was assessed using behavioral tests. In addition to measuring body weight and length, changes in subchondral bone were analyzed by  $\mu$ CT. Dynamic Weight Bearing system was used to monitor OA-related pain. Histological scoring will be conducted to investigate the severity cartilage degeneration and synovial inflammation. CUMS resulted in increased anxiety and significant decrease in body weight gain in all CUMS groups compared to non-CUMS groups. CUMS also increased serum corticosterone in healthy mice, with even higher levels in CUMS mice after DMM surgery. CUMS had no significant effect on subchondral bone, but subarticular bone mineral density and trabecular thickness were increased. Moreover, CUMS resulted in significant potentiation of DMM-associated pain. Our results suggest that the autonomic imbalance with increased sympathetic nervous activity induced by chronic stress exacerbates the severity of OA pain perception. We expect significantly increased cartilage degeneration as well as more severe synovial inflammation in CUMS DMM mice compared to DMM mice.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Real World Validation of an Machine Learning Algorithm Predicting Treatment Strategy for Hip Osteoarthritis

Walter van der Weegen<sup>1</sup>, Tristan Warren<sup>2</sup>, Rintje Agricola<sup>1,2</sup>, Dirk Das<sup>1</sup> en Michiel Siebelt<sup>1</sup>

<sup>1</sup>Researcher Health Department, Stichting Imec/Holst centre, Eindhoven, Netherlands;<sup>2</sup>Sports and Orthopaedics Research Center, Anna hospital, Geldrop, Netherlands

Artificial Intelligence (AI) is becoming more powerful but is barely used to counter the growth in health care burden. AI applications to increase efficiency in orthopedics are rare. We questioned if (1) we could train machine learning (ML) algorithms, based on answers from digitalized history taking questionnaires, to predict treatment of hip osteoarthritis (either conservative or surgical); (2) such an algorithm could streamline clinical consultation.

Multiple ML models were trained on 600 annotated (80% training, 20% test) digital history taking questionnaires, acquired before consultation. Best performing models, based on balanced accuracy and optimized automated hyperparameter tuning, were build into our daily clinical orthopedic practice. Fifty patients with hip complaints (>45 years) were prospectively predicted and planned (partly blinded, partly unblinded) for consultation with the physician assistant (conservative) or orthopedic surgeon (operative). Tailored patient information based on the prediction was automatically sent to a smartphone app. Level of evidence: IV.

Random Forest and BernoulliNB were the most accurate ML models (0.75 balanced accuracy). Treatment prediction was correct in 45 out of 50 consultations (90%),  $p < 0.0001$  (sign and binomial test). Specialized consultations where conservatively predicted patients were seen by the physician assistant and surgical patients by the orthopedic surgeon were highly appreciated and effective.

Treatment strategy of hip osteoarthritis based on answers from digital history taking questionnaires was accurately predicted before patients entered the hospital. This can make outpatient consultation scheduling more efficient and tailor pre-consultation patient education.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Gait Analysis in your hand: feasibility study evaluating an AI approach to gait analysis using monocular video from mobile phones**Robert Wendlandt<sup>1,2</sup>, Tabea Volpert<sup>3</sup>, Jörg Schroeter<sup>1,2</sup>, Arndt Peter Schulz<sup>2,4,5</sup>, Andreas Paech<sup>1,2</sup>

<sup>1</sup>University Medical Center Schleswig-Holstein, Campus Lübeck, Clinic for Orthopedics and Trauma Surgery, Lübeck, Germany; <sup>2</sup>University of Lübeck, Lübeck, Germany; <sup>3</sup>Technische Hochschule Lübeck, Lübeck, Germany; <sup>4</sup>BG Trauma Hospital Hamburg, Hamburg, Germany; <sup>5</sup>Fraunhofer Research Institution for Individualized and Cell-Based Medical Engineering, Lübeck, Germany

Gait analysis is an indispensable tool for scientific assessment and treatment of individuals whose ability to walk is impaired. The high cost of installation and operation are a major limitation for wide-spread use in clinical routine.

Advances in Artificial Intelligence (AI) could significantly reduce the required instrumentation. A mobile phone could be all equipment necessary for 3D gait analysis. MediaPipe Pose provided by Google Research is such a Machine Learning approach for human body tracking from monocular RGB video frames (1,2) that is detecting 3D-landmarks of the human body.

Aim of this study was to analyze the accuracy of gait phase detection based on the joint landmarks identified by the AI system.

Motion data from 10 healthy volunteers walking on a treadmill with a fixed speed of 4.5km/h (Callis, Sprintex, Germany) was sampled with a mobile phone (iPhone SE 2nd Generation, Apple). The video was processed with Mediapipe Pose (Version 0.9.1.0) using custom python software. Gait phases (Initial Contact - IC and Toe Off - TO) were detected from the angular velocities of the lower legs (3). For the determination of ground truth, the movement was simultaneously recorded with the AS-200 System (LaiTronic GmbH, Innsbruck, Austria).

The number of detected strides, the error in IC detection and stance phase duration was calculated.

In total, 1692 strides were detected from the reference system during the trials from which the AI-system identified 679 strides. The absolute mean error (AME) in IC detection was  $39.3 \pm 36.6$  ms while the AME for stance duration was  $187.6 \pm 140$  ms. Landmark detection is a challenging task for the AI-system as can clearly be seen by the rate of only 40% detected strides. As mentioned by Fadillioglu et al. (3), error in TO-detection is higher than in IC-detection.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

Ringhof, S., Krafft, F. C., Sell, S., & Stein, T. (2020). Automated gait event detection for a variety of locomotion tasks using a novel gyroscope-based algorithm. *Gait & Posture*, 81, 102–108.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Mid-Term Survivorship and Outcomes for Handheld Robotics Assisted Unicompartmental Knee Arthroplasty and Its Learning Curve – A Single Surgeon Study of 100 Knees**Soon Yaw Walter Wong<sup>1</sup>, Stephen Grant<sup>2</sup>

<sup>1</sup>Department of Orthopaedic Surgery, Sengkang General Hospital, 110 Sengkang East Way, Singapore 544886; <sup>2</sup>Department of Orthopaedic Surgery, University Hospital Hairmyres, 218 Eaglesham Rd, East Kilbride, Glasgow G75 8RG, United Kingdom

Robotic assistance in knee arthroplasty has become increasingly popular due to improved accuracy of prosthetic implantation. However, literature on the mid-term outcomes is limited especially that of hand-held robotic-assisted devices. We present one of the longest follow-up series to date using this novel technology and discuss the learning curve for introducing robotic technology into our practice.

The purpose of this single-surgeon study is to evaluate the survival, patient-reported outcomes and learning curve for handheld boundary-controlled robotic-assisted unicompartmental knee arthroplasties (HBRUKAs) at our hospital.

This retrospective study evaluates 100 cases (94 Medial, 6 Lateral) performed by a single surgeon between October 2012 and July 2018. 52% were males, mean age was 64.5y (range 47.3y-85.2y) and mean BMI was 31.3 (range 21.8-43). Both inlay (40%) and onlay (60%) designs were implanted. Patients were followed up routinely at 1 and 5 years with Oxford Knee Scores (OKS) recorded. The learning curve was determined by tourniquet times.

At a mean follow-up of 4.3 years (range 1.6y-7.3y), survivorship was 97%. There were three revisions: One case of aseptic loosening (1.5y), one case of deep-infection (3.8y) and one case of contralateral compartment osteoarthritis progression (5y). Mean 5-year OKS was 39.8. A 14.3% reduction in mean tourniquet times between the first 25 cases (105.5minutes) and subsequent cases (90.4minutes) was seen.

This single-surgeon study showed good survivorship and patient-reported outcomes for HBRUKAs at our hospital. A learning curve of approximately 25 cases was shown, with significant decreases in tourniquet times with respect to increased surgeon experience.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Human monocytes and mesenchymal stem/stromal cells co-cultured in fibrin have a pro-repair phenotype that influences chondrocyte activity**

Mohammad Alkhrayef<sup>1,3</sup>, Hayat Muhammad<sup>1</sup>, Rawiya Al Hosni<sup>1</sup>, Andrew McCaskie<sup>1,2</sup> and Mark Birch<sup>1</sup>

<sup>1</sup>Division of Trauma and Orthopaedic Surgery, Department of Surgery, University of Cambridge, Cambridge, UK; <sup>2</sup>Wellcome-MRC Stem Cell Institute, University of Cambridge, Cambridge, UK; <sup>3</sup>Healthy Aging Research Institute, King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia

Tissue repair is believed to rely on tissue-resident progenitor cell populations proliferating, migrating, and undergoing differentiation at the site of injury. During these processes, the crosstalk between mesenchymal stromal/stem cells (MSCs) and macrophages has been shown to play a pivotal role. However, the influence of extracellular matrix (ECM) remodelling in this crosstalk, remains elusive.

Human MSCs cultured on tissue culture plastic (TCP) and encased within fibrin *in vitro* were treated with/without TNF $\alpha$  and IFN $\gamma$ . Human monocytes were cocultured with untreated/pretreated MSCs on TCP or within fibrin. After seven days, the conditioned media (CM) were collected. Human chondrocytes were exposed to CM in a migration assay. The impact of TGF $\beta$  was assessed by adding an inhibitor (TGF $\beta$ Ri). Cell activity was assessed using RT-qPCR and XL-protein-profiler-array.

Previously, we demonstrated that culturing human MSCs within 3D-environments significantly enhances their immunoregulatory activity in response to pro-inflammatory stimuli. In this study, monocytes were co-cultured with MSCs within fibrin, acquiring a distinct M2-like repair macrophage phenotype in contrast to TCP co-cultures. MSC/macrophage CM characterization using a protein array demonstrated differences in release of several factors, including chemokines, growth factors and ECM components. Chondrocyte migration was significantly reduced in CM from untreated MSC/monocytes co-cultures in fibrin compared to CM of untreated MSCs/monocytes on TCP. This impact on migration was not seen with chondrocytes cultured in CM of monocytes co-cultured with pretreated MSCs in fibrin. The CM of monocytes co-cultured with pretreated MSCs in fibrin up-regulates COL2A1 and SOX9 compared to TCP. Chondrogenesis and migration were TGF $\beta$  dependent.

MSC/macrophage crosstalk and responsiveness to cytokines are influenced by the ECM environment, which subsequently impacts tissue-resident cell migration and chondrogenesis. The direct effects of ECM on MSC/macrophage secretory phenotype is complemented by the dynamic ECM binding and release of growth factors such as TGF $\beta$ .

**27-29 SEPTEMBER | PORTO, PORTUGAL****Determining the Function of Matrix Bound and Secreted Vesicles in Mineralisation****Anghileri, G.<sup>1</sup>, DeVoogt, W.<sup>2</sup>, Seinen CS.<sup>2</sup>, Peacock, B.<sup>3</sup>, Vader P.<sup>2</sup>, Martin-Fabiani, I.<sup>1</sup>, Davies, O.G.<sup>1</sup>**

<sup>1</sup>School of Sport, Exercise and Health Sciences, Loughborough University; <sup>2</sup>Central Diagnostic Laboratory, UMC Utrecht; <sup>3</sup>NanoFCM Co., Ltd, Nottingham, United Kingdom

Matrix-bound vesicles (MBVs) are embedded within osteoid and function as the site of initial mineral formation. However, they remain insufficiently characterised in terms of biogenesis, composition and function while their relationship with secreted culture medium EVs (sEVs) such as exosomes remains debated. We aimed to define the biogenesis and pro-mineralisation capacity of MBVs and sEVs to understand their potential in regenerative orthopaedics.

sEVs and MBVs isolated from conditioned medium (differential ultracentrifugation) and ECM (collagenase digestion and differential ultracentrifugation) of mineralising MC3T3 pre-osteoblast and human bone marrow MSC cultures were characterised by nanoparticle tracking analysis, western blotting, nano-flow cytometry, super resolution microscopy (ONI) and TEM. Immunoprecipitated populations positive for alkaline phosphatase (ALP), a putative marker of mineralisation capacity, were also characterised. Collagen binding efficiency was evaluated using MemGlow staining.

Results reported were comparative across both cell lines. Western blots indicated MBV fractions were positive for markers of endosomal biogenesis (CD9, CD81, ALIX, TSG101) and pro-mineralising proteins (ALP, Pit1, Annexin II, Annexin V), with Annexin V and CD9 present in immunoprecipitated ALP-positive fractions. MBVs were significantly larger than sEVs ( $p < 0.05$ ) and contained a higher amount of ALP ( $p < 0.05$ ) with a significant increase from day 7 to day 14 of cellular mineralisation ( $p < 0.05$ ). This mirrored the pattern of electron-dense vesicles seen via TEM. Super resolution single vesicle analysis revealed for the first-time co-expression of ALP with markers of endosomal biogenesis (CD9, CD63, CD81, ALIX) and Annexin II in both vesicle types, with higher co-expression percentage in MBVs than sEVs. MBVs also exhibited preferential collagen binding.

Advanced imaging methods demonstrated that contrary to opinions in the field, MBVs appear to possess exosomal markers and may arise via endosomal biogenesis. However, it was evident that a higher proportion of MBVs possessed machinery to induce mineralisation and were enriched in mineral-dense material.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Donor Age Matters: Intervertebral Disc Decellularization for Tissue Regeneration

Fiordalisi Morena Francesca<sup>1,2,3</sup>, Ferreira Joana Rita<sup>1,2,3</sup>, Pinto Marta Laranjeiro<sup>4</sup>, Ribeiro-Machado Cláudia<sup>1,2</sup>, Pinto Marta Teixeira<sup>1,5</sup>, Oliveira Maria José<sup>1,2,3,6</sup>, Barbosa Mário Adolfo<sup>1,2,3</sup>, Gonçalves Raquel Madeira<sup>1,2,3</sup> #, Caldeira Joana<sup>1,2</sup> #

<sup>1</sup>IS – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; <sup>2</sup>INEB – Instituto de Engenharia Biomédica, Porto, Portugal; <sup>3</sup>ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; <sup>4</sup>CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; <sup>5</sup>IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; <sup>6</sup>Department of Pathology and Oncology, Faculty of Medicine, University of Porto, Porto, Portugal. #equal contribution.

Intervertebral disc (IVD) degeneration occurs with aging, leading to low back pain (LBP), which is one of the leading conditions of disability worldwide. With the lack of effective treatment, decellularized extracellular matrix (dECM) – based biomaterials have been proposed for IVD regeneration. However, the impact of donor ages on tissue repair had never been explored before in the disc field. Therefore, we aimed to address this question.

For that, a decellularization protocol for bovine nucleus pulposus (NP) of different aged donors (fetus, young and old) was optimized by testing several detergents (SDS and Triton). The process efficiency was evaluated in terms of DNA and cell removal, as well as ECM preservation. Afterwards, dECMs were repopulated with bovine NP cells and cultured *ex vivo*. At day 7, cell behavior, ECM *de novo* synthesis and remodeling were evaluated [1]. Moreover, dECMs' inflammatory response was assessed after *in vivo* CAM assay. Finally, inflammatory and angiogenic cytokines were analyzed in the conditioned media-derived from dECMs by using a cytokine array.

As results, an optimal decellularization protocol (SDS 0.1%, 1h), efficient at removing cells and DNA from bovine NPs, while preserving ECM cues of native tissues, was developed. After repopulation, aggrecan increased in younger NPs, while collagen 2 decreased which may be indicative of matrix remodeling [1]. After *in vivo* CAM assay, fetal dECMs showed the highest inflammatory response. Finally, no statistically significant changes of cytokines were detected in the matrices, despite for a trend of higher IFN- $\alpha$ , IFN- $\gamma$  and LIF in fetal dECMs, IL-1 $\beta$  in young dECMs and Decorin in old dECMs.

Overall, this work uncovered the importance of tissue donor ages for tissue regenerative purpose, opening new avenues for the development of appropriate therapeutic strategies for IVD degeneration.

**Reference:** [1] Fiordalisi M.F., et al., *Biomaterials Advances*, 143, 213192. 2022.

# EORS 2023

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Fully automatic system to detect and segment the proximal femur in pelvic radiographic images for Legg-Calve-Perthes Disease**

S. Ditmer<sup>1</sup>, N. Dwenger<sup>1</sup>, L.N. Jensen<sup>1</sup>, A. Ghaffari<sup>2</sup>, O. Rahbek<sup>3</sup>

<sup>1</sup>Cognitive Science, School of Communication and Cognition, University of Aarhus, Jens Chr. Skous Vej 2, 8000 Aarhus, Denmark; <sup>2</sup>Orthopaedic Research Unit, Department of Orthopedics, Aalborg University Hospital, Hobrovej 18-22, 9000 Aalborg, Denmark; <sup>3</sup>Orthopaedic Research Unit, Department of Clinical Medicine, Aalborg University Hospital, Hobrovej 18-22, 9000 Aalborg, Denmark

The most important outcome predictor of Legg-Calvé-Perthes disease (LCPD) is the shape of the healed femoral head. However, the deformity of the femoral head is currently evaluated by non-reproducible, categorical, and qualitative classifications. In this regard, recent advances in computer vision might provide the opportunity to automatically detect and delineate the outlines of bone in radiographic images for calculating a continuous measure of femoral head deformity. This study aimed to construct a pipeline for accurately detecting and delineating the proximal femur in radiographs of LCPD patients employing existing algorithms. To detect the proximal femur, the pretrained state-of-the-art object detection model, YOLOv5, was trained on 1580 manually annotated radiographs, validated on 338 radiographs, and tested on 338 radiographs. Additionally, 200 radiographs of shoulders and chests were added to the dataset to make the model more robust to false positives and increase generalizability. The convolutional neural network architecture, U-Net, was then employed to segment the detected proximal femur. The network was trained on 80 manually annotated radiographs using real-time data augmentation to increase the number of training images and enhance the generalizability of the segmentation model. The network was validated on 60 radiographs and tested on 60 radiographs. The object detection model achieved a mean Average Precision (mAP) of 0.998 using an Intersection over Union (IoU) threshold of 0.5, and a mAP of 0.712 over IoU thresholds of 0.5 to 0.95 on the test set. The segmentation model achieved an accuracy score of 0.912, a Dice Coefficient of 0.937, and a binary IoU score of 0.854 on the test set. The proposed fully automatic proximal femur detection and segmentation system provides a promising method for accurately detecting and delineating the proximal femoral bone contour in radiographic images, which is necessary for further image analysis.

**The effect of geometries and gradient of strain-dependent solute diffusivity on the metabolic transport in patient-specific intervertebral discs**Zerihun G. Workineh, Estefano Muñoz-Moya, Carlos Ruiz Wills, Jérôme Noailly

Barcelona Centre for New Medical Technologies (BCN MedTech), Department of Information and Communication Technologies, Universitat Pompeu Fabra, Barcelona, Spain

Intervertebral discs (IVD) provide flexibility to the back and ensure functional distributions of the spinal loads. They are avascular, and internal diffusion-dependent metabolic transport is vital to supply nutrients to disc cells<sup>1</sup>, but interactions with personalized IVD shapes and mechanics remain poorly explored. Poromechanical finite element models of seven personalized lumbar IVD geometries, with mean heights ranging from 8 to 16 mm were coupled with a reactive oxygen, glucose and lactate transport model linked with tissue deformations and osmosis<sup>2</sup>. In previous studies<sup>2,3</sup>, reduced formulations of the divergence of the solute flux ( $\nabla \cdot \mathbf{J} = \nabla \cdot (D \nabla C) = \nabla D \cdot \nabla C + D \nabla^2 C$ ) ignored the dependence of the diffusion on the deformation gradients,  $\nabla D \cdot \nabla C$ . We simulated this phenomenon to explore its significance in mechano-metabolic -transport couplings, in the different geometries, over 24h of simulated rest (8h) and physical activity (16h).  $\nabla D \cdot \nabla C$  affected the daily variations of glucose concentrations in IVD thinner than 12 mm (Fig. 1a) but with neglectable variation ranges, while not considering  $\nabla D \cdot \nabla C$  in taller discs only slightly overestimated the glucose concentration (Fig. 1b). Most importantly, tall IVD had nearly 60% less glucose than thin IVD, with local drops below the concentration of 0.5 mM, considered to be critical for disc cells<sup>3</sup>, in the anterior nucleus pulposus (Fig. 1c,d). On the one hand, previous reduced formulations for mechanometabolic -transport models of the IVD seem acceptable, even for patient-specific modelling. On the other hand, tall IVD might suffer from unfortunate combinations of deformation-dependent solute diffusion and large diffusion distances, which may favor early catabolic events in the anterior nucleus pulposus.

**References:** 1. Urban, JPG, et al. Spine 29 (2004): 2700-2709; 2. Wills, C. Ruiz, et al. J Mech Phys Sol 90 (2016): 108-123; 3. Malandrino, A. et al. Front. Bioeng. Biotechnol. 3, (2015)

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Subject Specific Knee Joint Modeling between Young and Elderly

Sang Kuy Han<sup>1</sup>, YeonWoo Yoo<sup>2</sup>, Heawon Choi<sup>1</sup>, Ki Kwang Lee<sup>2</sup>, Rami K. Korhonen<sup>3</sup>, Amir Esrafilian<sup>3</sup>

<sup>1</sup>Korea Institute of Industrial Technology, Ansan, Korea; <sup>2</sup>Kookmin University, Seoul, Korea; <sup>3</sup>University of Eastern Finland, Kuopio, Finland

It is known that the gait dynamics of elderly substantially differs from that of young people. However, it has not been well studied how this age-related gait dynamics affects the knee biomechanics, e.g., cartilage mechanical response. In this study, we investigated how aging affects knee biomechanics in a female population using subject-specific computational models.

Two female subjects (ages of 23 and 69) with no musculoskeletal disorders were recruited. Korea National Institute for Bioethics Policy Review Board approved the study. Participants walked at a self-selected speed (SWS), 110% of SWS, and 120% of SWS on 10 m flat ground. Three-dimensional marker trajectories and ground reaction forces (Motion Analysis, USA), and lower limbs' muscle activities were measured (EMG, Noraxon USA). Knee cartilage and menisci geometries were obtained from subjects' magnetic resonance images (3T, GE Health Care). An EMG-assisted musculoskeletal finite element modeling workflow was used to estimate knee cartilage tissue mechanics in walking trials [1]. Knee cartilage and menisci were modeled using a transversely isotropic poroviscoelastic material model [2].

Walking speed in SWS, 110%, and 120% of SWS were 1.38 m/s, 1.51 m/s, and 1.65 m/s for the young, and 1.21 m/s, 1.34 m/s and 1.46 m/s for the elderly, respectively. The maximum tensile stress in the elderly tibial cartilage was ~25%, ~33%, and ~32% lower than the young at SWS, 110%, and 120% of SWS, respectively. These preliminary results suggest that the cartilage in the elderly may not have enough stimulation even at 20% increases in walking speed, which may be one reason for tissue degeneration. To enhance these findings, further study with more subjects and different genders will investigate how age-related gait dynamics affects knee biomechanics.

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**Application of External Torque Enhances the Detection of Subtle Syndesmotic Ankle Instability in a Weightbearing CT**

M. Huyghe<sup>1</sup>, M. Peiffer<sup>1</sup>, F. Cuigniez, T. Tampere<sup>1</sup>, S. Ashkani-Esfahani<sup>2</sup>, P. D'Hooghe<sup>3</sup>, E. Audenaert<sup>1</sup>, A. Burssens<sup>1</sup>

<sup>1</sup>UZ Gent; <sup>2</sup>Massachusetts General Hospital; <sup>3</sup>Aspetar Orthopaedic and Sportsmedical Hospital

One-fourth of all ankle trauma involve injury to the syndesmotic ankle complex, which may lead to syndesmotic instability and/or posttraumatic ankle osteoarthritis in the long term if left untreated. The diagnosis of these injuries still poses a deceitful challenge, as MRI scans lack physiologic weightbearing and plain weightbearing radiographs are subject to beam rotation and lack 3D information. Weightbearing cone-beam CT (WBCT) overcomes these challenges by imaging both ankles during bipedal stance, but ongoing debate remains whether these should be taken under weightbearing conditions and/or during application of external rotation stress. The aim of this study is study therefore to compare both conditions in the assessment of syndesmotic ankle injuries using WBCT imaging combined with 3D measurement techniques.

In this retrospective study, 21 patients with an acute ankle injury were analyzed using a WBCT. Patients with confirmed syndesmotic ligament injury on MRI were included, while fracture associated syndesmotic injuries were excluded. WBCT imaging was performed in weightbearing and combined weightbearing-external rotation. In the latter, the patient was asked to internally rotate the shin until pain (VAS>8/10) or a maximal range of motion was encountered. 3D models were developed from the CT slices, whereafter. The following 3D measurements were calculated using a custom-made Matlab® script; Anterior tibiofibular distance (AFTD), Alpha angle, posterior Tibiofibular distance (PFTD) and Talar rotation (TR) in comparison to the contralateral non-injured ankle.

The difference in neutral-stressed Alpha angle and AFTD were significant between patients with a syndesmotic ankle lesion and contralateral control (P=0.046 and P=0.039, respectively). There was no significant difference in neutral-stressed PFTD and TR angle.

Combined weightbearing-external rotation during CT scanning revealed an increased AFTD in patients with syndesmotic ligament injuries. Based on this study, application of external rotation during WBCT scans could enhance the diagnostic accuracy of subtle syndesmotic instability.

27-29 SEPTEMBER | PORTO, PORTUGAL

## 3D Quantitative characterization of the human meniscal vascular network using X-ray micro-Computed Tomography

Federica Orellana<sup>1,2</sup>, Alberto Grassi<sup>3</sup>, Peter Wahl<sup>4,5</sup>, Katja Nuss<sup>6</sup>, Antonia Neels<sup>1,2</sup>, Stefano Zaffagnini<sup>3</sup>, Annapaola Parrilli<sup>1</sup>

<sup>1</sup>Empa, Center for X-ray Analytics, 8600 Dübendorf, Switzerland; <sup>2</sup>University of Fribourg, 1700 Fribourg, Switzerland; <sup>3</sup>Rizzoli Orthopaedic Institute, 40136 Bologna, Italy; <sup>4</sup>Kantonsspital Winterthur, 8400 Winterthur, Switzerland; <sup>5</sup>Faculty of Medicine, University of Berne, Berne, Switzerland; <sup>6</sup>Vetsuisse Faculty, University of Zurich, 8057 Zürich, Switzerland

A comprehensive understanding of the self-repair abilities of menisci and their overall function in the knee joint requires three-dimensional information. However, previous investigations of the meniscal blood supply have been limited to two-dimensional imaging methods<sup>1</sup>, which fail to accurately capture tissue complexity. In this study, micro-CT was used to analyse the 3D microvascular structure of the meniscus, providing a detailed visualization and precise quantification of the vascular network. A contrast agent ( $\mu$ Angiofil<sup>®</sup>) was injected directly into the femoral artery of cadaver legs to provide the proper contrast enhancement. First, the entire knee joint was analysed with micro-CT, then to increase the applicable resolution the lateral and medial menisci were excised and investigated with a maximum resolution of up to 4  $\mu$ m. The resulting micro-CT datasets were analysed both qualitatively and quantitatively. Key parameters of the vascular network, such as vascular volume fraction, vessel radius, vessel length density, and tortuosity, were separately determined for the lateral and medial meniscus, and their four circumferential zones defined by Cooper<sup>2</sup>.

In accordance with previous literature, the quantitative micro-CT data confirm a decrease in vascular volume fraction along the meniscal zones. The highest concentration of blood vessels was measured in the meniscocapsular region 0, which is characterized by vascular segments with a significantly larger average radius. Furthermore, the highest vessel length density observed in zone 0 suggests a more rapid delivery of oxygen and nutrients compared to other regions. Vascular tortuosity was detected in all circumferential regions, indicating the occurrence of vascular remodelling in all tissue areas.

In conclusion, micro-CT is a non-invasive imaging technique that allows for the visualization of the internal structure of an object in three dimensions. These advanced 3D vascular analyses have the potential to establish new surgical approaches that rely on the healing potential of specific areas of the meniscus.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Alternation of the Three-Dimensional Subtalar Joint Alignment after Inframalleolar Osteotomy in Progressive Collapsing Flatfoot Deformity

Loïc Raes<sup>1</sup>, Matthias Peiffer<sup>1,2</sup>, Peter Kvarda<sup>3</sup>, Tim Leenders<sup>4</sup>, Emanuel A. Audenaert<sup>1</sup>, Arne Burssens<sup>1</sup>

<sup>1</sup>Ghent University Hospital; <sup>2</sup>Harvard Medical School; <sup>3</sup>Kantonsspital Baselland; <sup>4</sup>AZ Monica Hospital

A medializing calcaneal osteotomy (MCO) is one of the key inframalleolar osteotomies to correct progressive collapsing foot deformity (PCFD). While many studies were able to determine the hind- and midfoot alignment after PCFD correction, the subtalar joint remained obscured by superposition on plain radiography. Therefore, we aimed to perform a 3D measurement assessment of the hind- and subtalar joint alignment pre-compared to post-operatively using weightbearing CT (WBCT) imaging.

Fifteen patients with a mean age of 44,3 years (range 17-65yrs) were retrospectively analyzed in a pre-post study design. Inclusion criteria consisted of PCFD deformity correct by MCO and imaged by WBCT. Exclusion criteria were patients who had concomitant midfoot fusions or hindfoot coalitions. Image data were used to generate 3D models and compute the hindfoot - and talocalcaneal angle as well as distance maps.

Pre-operative radiographic parameters of the hindfoot and subtalar joint alignment improved significantly relative to the post-operative position (HA, MA<sub>Sa</sub>, and MA<sub>Co</sub>). The post-operative talus showed significant inversion, abduction, and dorsiflexion of the talus (2.79° ±1.72, 1.32° ±1.98, 2.11°±1.47) compared to the pre-operative position. The talus shifted significantly different from 0 in the posterior and superior direction (0.62mm ±0.52 and 0.35mm ±0.32). The distance between the talus and calcaneum at the sinus tarsi increased significantly (0.64mm ±0.44).

This study found pre-dominantly changes in the sagittal, axial and coronal plane alignment of the subtalar joint, which corresponded to a decompression of the sinus tarsi. These findings demonstrate the amount of alternation in the subtalar joint alignment that can be expected after MCO. However, further studies are needed to determine at what stage a calcaneal lengthening osteotomy or corrective arthrodesis is indicated to obtain a higher degree of subtalar joint alignment correction.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Evaluation of the antibacterial efficacy of using a bone allograft developed according to the Marburg system of bone bank on a model of chronic osteomyelitis**Amina Koshanova<sup>1</sup>, Berik Tuleubayev<sup>1</sup>, Dina Saginova<sup>2</sup>, Saule Akhmetova, Elyarbek Tashmetov<sup>1</sup>

<sup>1</sup>Department of Surgical Diseases, Karaganda Medical University, Karaganda, Kazakhstan; <sup>2</sup>Center for Applied Scientific Research, National Scientific Center of Traumatology and Orthopaedics named after academician N.D.Batpenov, Nur-Sultan, Kazakhstan; <sup>3</sup>Department of Microbiology, Karaganda Medical University, Karaganda, Kazakhstan

Bone infections due to fractures or implants are a big medical problem [1]. In experimental medicine, many experimental models have been created on different animal species to simulate the disease condition and to do experience treatments [2]. The aim of this paper was to present an antibacterial efficacy of using a bone allograft developed according to the Marburg system of bone bank on a model of chronic osteomyelitis induced in rabbits.

In research was used 54 rabbits. Osteomyelitis was induced in rabbits by a human strain of *St. aureus* ATCC 43300, in the rabbit femur. There have been created 3 groups of animals. In 1<sup>st</sup> group used antibiotic impregnated biodegradable material "PerOssal". In 2<sup>nd</sup> group used antibiotic impregnated whole bone allograft. In 3<sup>rd</sup> group used antibiotic impregnated perforated bone allograft. Evaluation of installation and evolution of the disease was done by microbiological. A separate study of microbiological data is presented here.

This study showed, in the 1<sup>st</sup> and 3<sup>rd</sup> groups there is a persistent decrease in CFU by 14 knocks to 120.4 in the 1<sup>st</sup> group and to 3.5 in the 3<sup>rd</sup> group, and in the 2<sup>nd</sup> group, on the contrary, there is an increase in CFU to 237.33. This shows the lack of effectiveness of using a whole bone allograft.

The results showed, after 7 days there was no statistically significant difference between the groups. After 14 days the perforated bone allograft impregnated with antibiotic was better than the biodegradable material "PerOssal".

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27-29 SEPTEMBER | PORTO, PORTUGAL

## 3D Printed Polyether-ether-ketone (PEEK) Mechanical-adaptive Implants for Immunomodulatory Osseointegration

Hongyun Ma<sup>1,2</sup>, Bo Lei<sup>1,2</sup>, Yingang Zhang<sup>1</sup>

<sup>1</sup>Department of Orthopedics, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, P. R. 710061, China; <sup>2</sup>Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an, P. R. 710000, China

3D Printed polyether-ether-ketone (PEEK) has gained widespread use in clinical practice due to its excellent biocompatibility, biomechanical compatibility, and personalization. However, pre-printed PEEK implants are not without their flaws, including bioinert, optimization distortion of 3D printing digital model and prosthetic mismatching. Recent advancements in mechanical processing technology have made it possible to print bone implants with PEEK fused deposition, allowing for the construction of mechanically adaptable implants [1]. In this study, we aimed to synthesize silanized polycitrate (PCS) via thermal polymerization and in situ graft it to PEEK surface to construct an elastomer coating for 3D printed PEEK implants (PEEK-PCS). This incorporation of PCS allows the implant to exhibit adaptive space filling ability and stress dispersal. In vivo and in vitro results, PEEK-PCS exhibited exceptional osseointegration and osteogenesis properties along with macrophage M2 phenotypic polarization, inflammatory factors reducing, promotion of osteogenic differentiation in bone marrow mesenchymal stem cells (BMSCs). Additionally, PEEK-PCS displays good autofluorescence properties [2] in vitro and in vivo, with stable fluorescence for 14 days, suggesting potential bioimaging applications. The study confirms that PEEK in situ grafting with thermo-polymerized PCS elastomers is a viable approach for creating multifunctional (bone defect adaptation, bioimaging, immune regulation, and osseointegration) implants for bone tissue engineering.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Gabapentinoids in the Symptomatic Management of Canal Stenosis: A Comparative Study on Pain Relief, Ambulation, and Safety Profile**Telmo Martínez<sup>1</sup>, Gonzalo Mariscal<sup>1</sup>, Eduardo Hevia<sup>2</sup>, Carlos Barrios<sup>1</sup><sup>1</sup>Institute for Research on Musculoskeletal Disorders, Valencia Catholic University;  
<sup>2</sup>Spine Unit, University Clinic of Navarra

The multimodal management of canal stenosis increasing, and inhibitors of central sensitization are playing a crucial role in central sensitization processes. Pregabalin and gabapentin are antiepileptic drugs that reduce presynaptic excitability. The objective of this study was to investigate whether the use of pregabalin and gabapentin is effective in the symptomatic management of canal stenosis.

A literature search was conducted in four databases. The inclusion criteria were studies that compared pregabalin or gabapentin with a control group in lumbar canal stenosis. Randomized clinical trials and a comparative retrospective cohort study were included. The main clinical endpoints were VAS/NRS, ODI, and RDQ (Roland Morris Disability Questionnaire) at 2, 4, 8 weeks, and 3 months, adverse events, and walking distance were also collected. Data were combined using Review Manager 5.4 software.

Six studies and 392 patients were included. The mean age was 60.25. No significant differences were observed in VAS at 2, 4, and 8 weeks: (MD: 0.23; 95% CI: -0.63-1.09), (MD: -0.04; 95% CI: -0.64 to -0.57), and (MD: -0.6; 95% CI: -1.22 to 0.02). Significant differences were observed in favor of pregabalin with respect to VAS at three months: (MD: -2.97; 95% CI: -3.43 to -2.51). No significant differences were observed in ODI (MD: -3.47; 95% CI: -7.15 to -0.21). Adverse events were significantly higher in the pregabalin/gabapentin group (OR 5.88, 95%CI 1.28-27.05). Walking distance and RDQ could not be compared, although the results were controversial.

Gabapentinoids have not been shown to be superior to other drugs used in the treatment of LSS or to placebo. However, they have shown a higher incidence of adverse effects, improved results in VAS at 3 months, and a slight improvement in ambulation at 4 months in combination with NSAIDs compared to NSAIDs in monotherapy.

27-29 SEPTEMBER | PORTO, PORTUGAL

## 3D dynamic culture of Tendon stem cells from Tendinopathic explant: a new tool for advanced *in vitro* studies

M. C. Ciardulli<sup>1</sup>, V. Giudice<sup>2</sup>, F. Oliva<sup>3</sup>, C. Selleri<sup>2</sup>, N. Maffulli<sup>4</sup>, G. Della Porta<sup>5</sup>

<sup>1</sup>Department of Medicine, Surgery and Dentistry, University of Salerno, Via S. Allende, 84081 Baronissi (SA), IT; <sup>2</sup>Hematology and Transplant Center, University Hospital "San Giovanni di Dio e Ruggi D'Aragona", 84131 Salerno (SA), Italy; <sup>3</sup>Department of Trauma and Orthopaedic Surgery; University Hospital "San Giovanni di Dio e Ruggi D'Aragona", 84131 Salerno (SA), Italy; <sup>4</sup>Interdepartmental Centre BIONAM, Università di Salerno, via Giovanni Paolo I, 84084 Fisciano (SA), IT.

Poor tendon repair is an unsolved issue in clinical practice, due to complex tendon structure (1). Tendon stem/progenitor cells (TSPCs) play key roles in homeostasis, regeneration, and inflammation regulation in acute tendon injuries, and rely on TGF- $\beta$  signaling for recruitment into degenerative tendons. In this study, we aimed to develop an *in vitro* model for tenogenesis adopting a dynamic culture of a fibrin 3D scaffold, bioengineered with human TSPCs collected from both healthy and tendinopathic surgery explants (Review Board prot./SCCE n.151, 29 October 2020) (2,3,4). 3D culture was maintained for 21 days under perfusion provided by a custom-made bioreactor, in a medium supplemented with hTGF- $\beta$ 1 at 20 ng/mL (5). The data collected suggested that the 3D *in vitro* model well supported survival of both pathological and healthy cells, and that hTGF- $\beta$  signaling, coupled to a dynamic environment, promoted differentiation events. However, pathological hTSPCs showed a different expression pattern of tendon-related genes throughout the culture and an impaired balance of pro-inflammatory and anti-inflammatory cytokines, compared to healthy hTSPCs, as indicated by qRT-PCT and immunofluorescence analyses. Additionally, the expression of both tenogenic and cytokine genes in hTSPCs was influenced by hTGF- $\beta$ 1, indicating that the environment assembled was suitable for studying tendon stem cells differentiation. The study offers insights into the use of 3D cultures of hTSPCs as an *in vitro* model for investigating their behavior during tenogenic events and opens perspectives for following the potential impact on resident stem cells during regeneration and healing events.

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**Novel stem cell strategies for tendon regenerative medicine**

Arlette Alina Haidar Montes<sup>1</sup>, Annunziata Mauro<sup>1</sup>, Adrián Cerveró-Varona<sup>1</sup>, Giuseppe Prencipe<sup>1</sup>, Mohammad El Khatib<sup>1</sup>, Umberto Tosi<sup>1</sup>, Guy Wouters<sup>2</sup>, Johannes Stöckl<sup>3</sup>, Valentina Russo<sup>1</sup>, Barbara Barboni<sup>1</sup>

<sup>1</sup>Unit of Basic and Applied Sciences, Department of Biosciences and Agro-Food and Environmental Technologies, University of Teramo, Teramo, Italy; <sup>2</sup>FAT STEM Company, Erembodegem, Belgium; <sup>3</sup>Centre for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Medical University of Vienna, Vienna, Austria.

Adipose-derived stem cells (ADSCs) are an effective alternative for Teno-regeneration [1]. Despite their applications in tendon engineering, the mechanisms promoting tendon healing still need to be understood. Since there is scattered information on ovine ADSCs, this research aims to investigate *in vitro* their teno-differentiation for potential use in preclinical tendon regeneration models.

Ovine ADSCs were isolated from the tail region according to FAT-STEM laboratories [2], expanded until passage six (P6), and characterized in terms of stemness, adhesion and MHC markers by Flow Cytometry (FCM) and immunocytochemistry (ICC). Cell proliferation and senescence were evaluated with MTT and Beta-galactosidase assays, respectively. P1 ADSCs' teno-differentiation was assessed by culturing them with teno-inductive Conditioned Media (CM) or engineering them on tendon-mimetic PLGA scaffolds [3,4]. ADSCs teno-differentiation was evaluated by morphological, molecular (qRT-PCR), and biochemical (WesternBlot) approaches.

ADSCs exhibited mesenchymal phenotype, positive for stemness (SOX2, NANOG, OCT4), adhesion (CD29, CD44, CD90, CD166) and MHC-I markers, while negative for hematopoietic (CD31, CD45) and MHC-II markers, showing no difference between passages. ICC staining confirmed these results, where ADSCs showed nuclear positivity for SOX2 ( $\cong$  56%) and NANOG ( $\cong$  67%), with high proliferation capacity without senescence until P6. Interestingly, ADSCs cultured with the teno-inductive CM did not express tenomodulin (TNMD) protein or gene. Conversely, ADSCs seeded on scaffolds teno-differentiated, acquiring a spindle shape supported by TNMD protein expression at 48h ( $p < 0.05$  vs. ADSCs 48h) with a significant increase at 14 days of culture ( $p < 0.05$  vs. ADSCs + fleece 48h).

Ovine ADSCs respond differently upon distinct teno-inductive strategies. While the molecules on the CM could not trigger a teno-differentiation in the cells, the scaffold's topological stimulus did, resulting in the best strategy to apply. More insights are requested to better understand ovine ADSCs' tenogenic commitment before using them *in vivo* for tendon regeneration.

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# EORS 2023

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**A role for perfusion delivered shear force in the promotion of TPSC EV production****M. Clerici<sup>1,2</sup>, M.C. Ciardulli<sup>1</sup>, N.R. Forsyth<sup>2</sup>, N. Maffulli<sup>1,3</sup>, G. Della Porta<sup>1,4</sup>**

<sup>1</sup>Department of Medicine, Surgery and Dentistry, University of Salerno, Via S. Allende, 84081 Baronissi (SA), IT; <sup>2</sup>School of Pharmacy and Bioengineering, Keele University, Stoke-on-Trent, Staffordshire ST4 7QB, UK; <sup>3</sup>Department of Trauma and Orthopaedic Surgery; University Hospital "San Giovanni di Dio e Ruggi D'Aragona", 84131 Salerno (SA), Italy; <sup>4</sup>Interdepartmental Centre BIONAM, Università di Salerno, via Giovanni Paolo I, 84084 Fisciano (SA), IT.

Tendon injuries are a common problem that can significantly impact an individual's quality of life. While traditional surgical methods<sup>1</sup> have been used to address this issue, Extracellular Vesicles (EVs) have emerged as a promising approach to promote tendon repair and regeneration mechanisms<sup>2</sup>, as they deliver specific biological signals to neighbouring cells<sup>3</sup>. In this study, we extracted human Tendon Progenitor Stem cells (hTPSCs) from surgery explants and isolated their EVs from perfused and static media.

hTPSCs were isolated from tendon surgery biopsy<sup>4</sup> (Review Board prot./SCCE n.151, 29/10/2020) and cultured in both static and dynamic conditions, using a perfusion bioreactor (1ml/min). When cells reached 80% confluence, they were switched into a serum-free medium for 24 hours for EVs-production. Conditioned media was ultracentrifuged for 90min (100000g). The recovered pellet was then characterized by size and concentration (Nanosight NS300), Zeta potential (Mastersizer S), morphology (SEM and TEM) and protein quantification.

hTPSCs stemness and multipotency were confirmed through CD73, CD90, and CD105 expression and confirmation of quad-lineage (adipo-osteo-chondro-teno) differentiation. After 7 days, hTPSCs were ready for EVs-production.

Ultracentrifugation revealed the presence of particles with a concentration of  $7 \times 10^7$  particles/mL consistent across both cultures. Further characterization indicated that EVs collected from perfused conditions displayed an elevated vesicle mean size (mean  $143 \pm 6.5$  nm) in comparison to static conditions (mean  $112 \pm 7.4$  nm). Consistent with, but not in proportion with, the above protein content was measured at 20 ng/ml (dynamic) and 7 ng/ml (static) indicating a nearly 3-fold increase in concentration associated with a ~22% increase in particle size.

Proposed data showed that sub-200 diameter vesicles were successfully collected from multipotent hTPSCs starvation, and the vesicle size and protein concentration were compatible with established EV literature; furthermore, dynamic culture conditions seemed more suitable for EVs-production. Further characterization will be required to better understand, EVs-compositions and their role in tendon regenerative events.

# EORS 2023

31st Annual Meeting of the  
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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Mechanically-instructive Scaffolds to Steer Tissue Regeneration: Merging Mechanobiology with Biofabrication**

Lorenzo Moroni

Complex Tissue Regeneration department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Universiteitssingel 40, 6229ER, Maastricht, the Netherlands

Regenerative medicine (RM) promises to restore both the mechanical functionality and the biological composition of tissues after damage. Three-dimensional scaffolds are used in RM to host cells and let them produce proteins that are the building blocks of the native tissues. While regenerating tissues evolve over time through dynamic biomechanical and biochemical changes, current scaffolds' generation are passive causing mechanical mismatch, suboptimal growth, and pain. Furthermore, current scaffolds ignore the complexity of the reciprocal bio-mechanics regulation, hindering the design of the next-gen scaffolds. To regenerate tissues and organs, biofabrication strategies that impart spatiotemporal control over cell-cell and cell-extracellular matrix communication, often through control over cell and material deposition and placement, are being developed. To achieve these targets, the spatiotemporal control over biological signals at the interface between cells and materials is often aimed for. Alternatively, biological activity can be triggered through the control of mechanical cues, harnessing more fundamental know-how in mechanobiology that could be combined with biofabrication strategies. Here, I present some of our most recent advancements in merging mechanobiology with biofabrication that enabled the control of cell activity, moving towards enhanced tissue regeneration as well as the possibility to create more complex 3D *in vitro* models to study biological processes.

27-29 SEPTEMBER | PORTO, PORTUGAL

### 3D Writing of Multicellular Tendon-on-CNC-Chip models

Rosa F. Monteiro<sup>1,2</sup>, Syeda M. Bakht<sup>1,2</sup>, Manuel Gomez-Florit<sup>1,2</sup>, Rui L. Reis<sup>1,2</sup>, Manuela E. Gomes<sup>1,2</sup>, Rui M. A. Domingues<sup>1,2</sup>

<sup>1</sup>3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark - Parque de Ciência e Tecnologia, Zona Industrial da Gandra, Barco, Guimarães, 4805-017 Barco, Portugal; <sup>2</sup>ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

Relevant *in vitro* models emulating tendinopathies are highly needed to study these diseases and develop better treatments. We have recently proposed a new strategy that allows the automated 3D writing of microphysiological systems (MPS) embedded into its own biomimetic fibrillar support platform based on the self-assembling of cellulose nanocrystals (CNCs)<sup>1</sup>. Here, we explored this CNC platform for writing humanized *in vitro* tendon models using tendon decellularized extracellular matrix (dECM)-based bioinks to closely recapitulate the biophysical and biochemical cues of tendon cell niche and self-induce the tenogenic differentiation of stem cells<sup>2</sup>. The proposed concept was further explored to study the crosstalk between the tendon core and vascular compartment.

Porcine flexor tendons were decellularized to produce the dECM bioink hydrogel. hASCs were used as cell source and the bioink was directly printed within the CNC fluid gel. Tendon constructs were co-printed with compartmentalized microvascular structures to evaluate the cellular crosstalk with endothelial cells. The tendon-on-chip models showed high cell viability and proliferation during culture up to 21 days, and the synergy between dECM cues and printed patterns induced anisotropic cell organization similar to tendon tissues. Gene and protein analysis showed upregulation of the most important tendon related markers on tendon constructs, demonstrating that the biophysical and biochemical cues of dECM induced hASCs commitment toward tenogenic phenotype. In co-culture system, chemotaxis induced endothelial cells migration toward the tendon compartment, but without significant infiltration. Gene and protein expression results suggest that the cellular crosstalk established in this MPS with endothelial cells boosted hASCs tenogenesis, emulating tendon development stages.

Overall, the proposed system might be promising for the automated fabrication of organotypic tendon-on-chip models that will be a valuable new tool to study tendon physiology, pathology, or the effect of drugs for the treatment of tendinopathy.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **3D printing of PCL to fabricate porous scaffolds for bone tissue engineering applications**

Mehmet Serhat Aydin<sup>1</sup>, Theo Luciani<sup>1</sup>, Samih Mohamed-Ahmed<sup>1</sup>, Mohammed A. Yassin<sup>1</sup>, Kamal Mustafa<sup>1</sup>, Ahmad Rashad<sup>1</sup>

<sup>1</sup>Center of Translational Oral Research (TOR) – Tissue Engineering Group, Department of Clinical Dentistry, University of Bergen, Bergen, Norway

The aim of this study is to print 3D polycaprolactone (PCL) scaffolds at high and low temperature (HT/LT) combined with salt leaching to induced porosity/larger pore size and improve material degradation without compromising cellular activity of printed scaffolds. PCL solutions with sodium chloride (NaCl) particles either directly printed in LT or were casted, dried, and printed in HT followed by washing in deionized water (DI) to leach out the salt. Micro-Computed tomography (Micro-CT) and scanning electron microscope (SEM) were performed for morphological analysis. The effect of the porosity on the mechanical properties and degradation was evaluated by a tensile test and etching with NaOH, respectively. To evaluate cellular responses, human bone marrow-derived mesenchymal stem/stromal cells (hBMSCs) were cultured on the scaffolds and their viability, attachment, morphology, proliferation, and osteogenic differentiation were assessed. Micro-CT and SEM analysis showed that porosity induced by the salt leaching increased with increasing the salt content in HT, however no change was observed in LT. Structure thickness reduced with elevating NaCl content. Mass loss of scaffolds dramatically increased with elevated porosity in HT. Dog bone-shaped specimens with induced porosity exhibited higher ductility and toughness but less strength and stiffness under the tension in HT whereas they showed decrease in all mechanical properties in LT. All scaffolds showed excellent cytocompatibility. Cells were able to attach on the surface of the scaffolds and grow up to 14 days. Microscopy images of the seeded scaffolds showed substantial increase in the formation of extracellular matrix (ECM) network and elongation of the cells. The study demonstrated the ability of combining 3D printing and particulate leaching together to fabricate porous PCL scaffolds. The scaffolds were successfully printed with various salt content without negatively affecting cell responses. Printing porous thermoplastic polymer could be of great importance for temporary biocompatible implants in bone tissue engineering applications.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Multilayer dual-porosity 3D-printed scaffolds to recreate the anisotropic microenvironment of the cartilage**Sandra Ramos-Díez<sup>1</sup>, Sandra Camarero-Espinosa<sup>1,2</sup>

<sup>1</sup>BioSmarTE lab, RPT Group, POLYMAT, University of the Basque Country UPV/EHU, Donostia/San Sebastián 20018, Guipúzcoa, Spain; <sup>2</sup>IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

Articular cartilage is a multi-zonal tissue that coats the epiphysis of long bones and avoids its wear during motion. An unusual friction could micro-fracture this connective membrane and progress into an osteochondral defect (OD), where the affected cartilage suffers inflammation, fibrillation, and forfeiture of its anisotropic structure. Clinical treatment for ODs has been focused on micro-fracture techniques, where the defect area is removed and small incisions are performed in the subchondral bone, which allows the exudation of mesenchymal stem cells (hMSCs) to the abraded zone. However, hMSCs represent less than 0.01% of the total cell population and are not able to self-organise coherently, so the treatments fail in the long term(1). To select, support and steer hMSCs from the bone marrow into a specific differentiation stage, and recreate the cartilage anisotropic microenvironment, multilayer dual-porosity 3D-printed scaffolds were developed.

Dual-porosity scaffolds were printed using prepared inks, containing specific ratios of poly-(d,l)lactide-co-caprolactone copolymer and gelatine microspheres of different diameters, which acted as sacrificial micro-pore templates and were leached after printing. The cell adhesion capability was investigated showing an increased cell number in dual-porosity scaffolds as compared to non-porous ones. To mimic the stiffness of the three cartilage zones, several patterns were designed, printed, and checked by dynamic-mechanical analysis under compression at 37 °C. Three patterns with specific formulations were chosen as candidates to recreate the mechanical properties of the cartilage layers. Differentiation studies in the selected scaffolds showed the formation of mature cartilage by gene expression, protein deposition and biomolecular analysis. Given the obtained results, designed scaffolds were able to guide hMSC behaviour.

In conclusion, biocompatible, multilayer and dual-porosity scaffolds with cell entrapment capability were manufactured. These anisotropic scaffolds were able to recreate the physical microenvironment of the natural cartilage, which in turn stimulated cell differentiation and the formation of mature cartilage.

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27-29 SEPTEMBER | PORTO, PORTUGAL

### 3D printed hybrid scaffolds for cartilage regeneration

Silvia A. Ferreira<sup>1</sup>, Francesca Tallia<sup>2</sup>, Agathe Heyraud<sup>2</sup>, Simone A. Walker<sup>1</sup>, Christoph Salzlechner<sup>3</sup>, Julian R. Jones<sup>2</sup>, Sara M. Rankin<sup>1</sup>

<sup>1</sup>National Heart & Lung Institute, Imperial College London, London, UK; <sup>2</sup>Department of Materials, Imperial College London, London, UK; <sup>3</sup>Centre for Craniofacial and Regenerative Biology, King's College London, London, UK

For chondral damage in younger patients, surgical best practice is microfracture, which involves drilling into the bone to liberate the bone marrow. This leads to a mechanically inferior fibrocartilage formed over the defect as opposed to the desired hyaline cartilage that properly withstands joint loading. While some devices have been developed to aid microfracture and enable its use in larger defects, fibrocartilage is still produced and there is no clear clinical improvement over microfracture alone in the long term. Our goal is to develop 3D printed devices, which surgeons can implant with a minimally invasive technique. The scaffolds should match the functional properties of cartilage and expose endogenous marrow cells to suitable mechanobiological stimuli *in-situ*, in order to promote healing of articular cartilage lesions before they progress to osteoarthritis, and rapidly restore joint health and mobility. Importantly, scaffolds should direct a physiological host reaction, instead of a foreign body reaction, associated with chronic inflammation and fibrous capsule formation, negatively influencing the regenerative outcome.

Our novel silica/polytetrahydrofuran/polycaprolactone hybrids were prepared by sol-gel synthesis and scaffolds were 3D printed by direct ink writing. 3D printed hybrid scaffolds with pore channels of ~250 µm mimic the compressive behaviour of cartilage<sup>1</sup>. Our results show that these scaffolds support human bone marrow stem/stromal cell (hMSC) differentiation towards chondrogenesis *in vitro* under hypoxic conditions to produce markers integral to articular cartilage-like matrix evaluated by immunostaining and gene expression analysis. Macroscopic and microscopic evaluation of subcutaneously implanted scaffolds in mice showed that scaffolds caused a minimal resolving inflammatory response. Our findings show that 3D printed hybrid scaffolds have the potential to support cartilage regeneration.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****A precision health approach for osteoarthritis: prediction of rapid knee osteoarthritis progression using automated machine learning.**Simone Castagno<sup>1</sup>, Mark Birch<sup>1</sup>, Mihaela van der Schaar<sup>1</sup>, Andrew McCaskie<sup>1</sup><sup>1</sup>University of Cambridge

Precision health aims to develop personalised and proactive strategies for predicting, preventing, and treating complex diseases such as osteoarthritis (OA). Due to OA heterogeneity, which makes developing effective treatments challenging, identifying patients at risk for accelerated disease progression is essential for efficient clinical trial design and new treatment target discovery and development.

To create a reliable and interpretable precision health tool that predicts rapid knee OA progression over a 2-year period from baseline patient characteristics using an advanced automated machine learning (autoML) framework, "*Autoprognosis 2.0*".

All available 2-year follow-up periods of 600 patients from the FNIH OA Biomarker Consortium were analysed using "*Autoprognosis 2.0*" in two separate approaches, with distinct definitions of clinical outcomes: multi-class predictions (categorising disease progression into pain and/or radiographic progression) and binary predictions. Models were developed using a training set of 1352 instances and all available variables (including clinical, X-ray, MRI, and biochemical features), and validated through both stratified 10-fold cross-validation and hold-out validation on a testing set of 339 instances. Model performance was assessed using multiple evaluation metrics. Interpretability analyses were carried out to identify important predictors of progression.

Our final models yielded higher accuracy scores for multi-class predictions (AUC-ROC: 0.858, 95% CI: 0.856-0.860) compared to binary predictions (AUC-ROC: 0.717, 95% CI: 0.712-0.722). Important predictors of rapid disease progression included WOMAC scores and MRI features. Additionally, accurate ML models were developed for predicting OA progression in a subgroup of patients aged 65 or younger.

This study presents a reliable and interpretable precision health tool for predicting rapid knee OA progression. Our models provide accurate predictions and, importantly, allow specific predictors of rapid disease progression to be identified. Furthermore, the transparency and explainability of our methods may facilitate their acceptance by clinicians and patients, enabling effective translation to clinical practice.

27-29 SEPTEMBER | PORTO, PORTUGAL

**Distinctive neuronal and immune system signatures, predisposed by genetic background, associate with discogenic intervertebral disc herniation.**

Emanuel J. Novais<sup>1,3</sup>, Eric Brown<sup>4</sup>, Olivia K. Ottone<sup>1,2</sup>, Victoria A. Tran<sup>1</sup>, Angelo C. Lepore<sup>4</sup>, Makarand V. Risbud<sup>1,2</sup>

<sup>1</sup>Department of Orthopaedic Surgery, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, USA; <sup>2</sup>Graduate Program in Cell Biology and Regenerative Medicine, Jefferson College of Life Sciences, Thomas Jefferson University, Philadelphia, USA; <sup>3</sup>Unidade Local de Saúde do Litoral Alentejano, Santiago do Cacém, Portugal; <sup>4</sup>Department of Neuroscience, Vickie and Jack Farber Institute for Neuroscience, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, USA

Despite the clinical relevance of back pain and intervertebral disc herniation, the lack of reliable models has strained their molecular understanding. We characterized the lumbar spinal phenotype of C57BL/6 and SM/J mice during aging. Interestingly, old SM/J lumbar discs evidenced accelerated degeneration, associated with high rates of disc herniation. SM/J AF's and degenerative human's AF transcriptomic profiles showed altered immune cell, inflammation, and p53 pathways. Old SM/J mice presented increased neuronal markers in herniated discs, thicker subchondral bone, and higher sensitization to pain. Dorsal root ganglia transcriptomic studies and spinal cord analysis exhibited increased pain and neuroinflammatory markers associated with altered extracellular matrix regulation. Immune system single-cell and tissue level analysis showed distinctive T-cell and B-cell modulation and negative correlation between mechanical allodynia and INF- $\alpha$ , IL-1 $\beta$ , IL2, and IL4, respectively. This study underscores the multisystemic network behind back pain and highlights the role of genetic background and the immune system in disc herniation disease.

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**Galectins -4 and -8 in Human Intervertebral Disc Degeneration****Strauss C<sup>1,2</sup>, Djojic D<sup>2,3</sup>, Grohs J<sup>3</sup>, Schmidt S<sup>4</sup>, Windhager R<sup>3</sup>, Stadlmann J<sup>5</sup>, Toegel S<sup>1,2</sup>**

<sup>1</sup>Karl Chiari Lab for Orthopaedic Biology, Department of Orthopedics and Trauma Surgery, Medical University of Vienna, Austria; <sup>2</sup>Ludwig Boltzmann Institute for Arthritis and Rehabilitation, Vienna, Austria; <sup>3</sup>Division of Orthopedics, Department of Orthopedics and Trauma Surgery, Medical University of Vienna, Austria; <sup>4</sup>Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Munich, Germany; <sup>5</sup>University of Natural Resources and Life Sciences, Vienna, Austria

Intervertebral disc (IVD) degeneration is responsible for severe clinical symptoms including chronic back pain. Galectins are a family of carbohydrate-binding proteins, some of which can induce functional disease markers in IVD cells [1] and other musculoskeletal diseases. Galectins -4 and -8 were shown to trigger disease-promoting activity in chondrocytes [2, 3] but their effects on IVD cells have not been investigated yet. This study elucidates the role of galectin-4 and -8 in IVD degeneration.

Immunohistochemical evidence for the presence of galectin-4 and -8 in the IVD was comparatively provided in specimens of 36 patients with spondylochondrosis, spondylolisthesis, or spinal deformity. Confocal microscopy revealed co-localization of galectin-4 and -8 in chondrocyte clusters of degenerated cartilage. The immunohistochemical presence of galectin-4 correlated with histopathological and clinical degeneration scores of patients, whereas galectin-8 did not show significant correlations. The specimens were separated into annulus fibrosus (AF), nucleus pulposus (NP) and endplate, which was confirmed histologically. Separate cell cultures of AF and NP (n=20) were established and characterized using cell type-specific markers. Potential binding sites for galectins including sialylated N-glycans and LacdiNAc structures were determined in AF and NP cells using LC/ESI-MS-MS. To assess galectin functions, cell cultures were treated with recombinant galectin-4 or -8, in comparison to IL-1 $\beta$ , and analyzed using RT-qPCR and In-cell Western blot. In vitro, both galectins triggered the induction of functional disease markers (CXCL8 and MMP3) on mRNA level and activated the nuclear factor-kB pathway. NP cells were significantly more responsive to galectin-8 and IL-1 $\beta$  than AF cells. Phosphorylation of p-65 was time-dependently induced by both galectins in both cell types to a comparable extent.

Taken together, this study provides evidence for a functional role of glycobiological processes in IVD degeneration and highlights galectin-4 and -8 as regulators of pro-inflammatory and degenerative processes in AF and NP cells.

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## 27-29 SEPTEMBER | PORTO, PORTUGAL

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## **Tendon bioengineering - challenges for clinical application**

Sebastian Muller

Kantonsspital Baselland, University of Basel, Switzerland

Tendons mainly consist of collagen in order to withstand high tensile forces. Compared to other, high turnover tissues, cellularity and vascularity in tendons are low. Thus, the natural healing process of tendons takes long and can be problematic. In case of injury to the enthesis, the special transition from tendon over cartilage to bone is replaced by a fibrous scar tissue, which remains an unsolved problem in rotator cuff repair.

To improve tendon healing, many different approaches have been described using scaffolds, stem cells, cytokines, blood products, gene therapy and others. Despite promising in vitro and in vivo results, translation to patient care is challenging. In clinics however, tendon auto- or allografts remain still first choice to augment tendon healing if needed.

Therefore, it is important to understand natural tendon properties and natural tendon healing first. Like in other tissues, senescence of tenocytes seems to play an important role for tendon degeneration which is interestingly not age depended. Our in vivo healing studies have shown improved and accelerated healing by adding collagen type I, which is now used in clinics, for example for augmentation of rotator cuff repair. Certain cytokines, cells and scaffolds may further improve tendon healing but are not yet used routinely, mainly due to missing clinical data, regulatory issues and costs.

In conclusion, the correct diagnosis and correct first line treatment of tendon injuries are important to avoid the necessity to biologically augment tendon healing. However, strategies to improve and accelerate tendon healing are still desirable. New treatment opportunities may arise with further advances in tendon engineering in the future.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Revision of Shoulder Replacements using Modular Components: A systematic review**

Dragonas C<sup>1</sup>, Waseem S<sup>1</sup>, Simpson A<sup>2</sup>, Leivadiotou D<sup>1</sup>

<sup>1</sup>Princess Alexandra Hospital, Hamstel Road, Harlow; <sup>2</sup>Guys and St Thomas' NHS Foundation Trust, Great Maze Pond, London

The advent of modular implants aims to minimise morbidity associated with revision of hemiarthroplasty or total shoulder arthroplasty (TSA) to reverse shoulder arthroplasty (RSR) by allowing retention of the humeral stem. This systematic review aimed to summarise outcomes following its use and reasons why modular humeral stems may be revised.

A systematic review of Pubmed, Medline and EMBASE was performed according to PRISMA guidelines of all patients undergoing revision of a modular hemiarthroplasty or TSA to RSR. Primary implants, glenoid revisions, surgical technique and opinion based reports were excluded. Collected data included demographics, outcomes and incidence of complications.

277 patients were included, with a mean age of 69.8 years (44-91) and 119 being female. Revisions were performed an average of 30 months (6-147) after the index procedure, with the most common reason for revision being cuff failure in 57 patients. 165 patients underwent modular conversion and 112 underwent stem revision. Of those that underwent humeral stem revision, 18 had the stem too proximal, in 15 the stem was loose, 10 was due to infection and 1 stem had significant retroversion. After a mean follow up of 37.6 months (12-91), the Constant score improved from a mean of 21.8 to 48.7. Stem revision was associated with a higher complication rate (OR 3.13, 95% CI 1.82-5.39).

The increased use of modular stems has reduced stem revision, however 40% of these implants still require revision due to intra-operative findings. Further large volume comparative studies between revised and maintained humeral stems post revision of modular implants can adequately inform implant innovation to further improve the stem revision rate.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Early Mobilisation versus Immobilisation after Reverse Shoulder Arthroplasty and Total Shoulder Arthroplasty – A Systematic Review**Lily Nolan, John Mahon, Rayyan Mirdad, Rafee Alnajjar, Adam Galbraith, Ken Kaar

University Hospital Galway, Newcastle Road, Galway

Total shoulder arthroplasty (TSA) and Reverse Total shoulder arthroplasty (RSA) are two of the most performed shoulder operations today. Traditionally postoperative rehabilitation included a period of immobilisation, protecting the joint and allowing time for soft tissue healing. This immobilisation period may significantly impact a patient's quality of life (QoL) and ability to perform activities of daily living (ADL's). This period of immobilisation could be safely avoided, accelerating return to function and improving postoperative QoL.

This systematic review examines the safety of early mobilisation compared to immobilisation after shoulder arthroplasty focusing on outcomes at one year.

Methods: A systematic review was performed as per the PRISMA guidelines. Results on functional outcome and shoulder range of motion were retrieved.

Six studies were eligible for inclusion, resulting in 719 patients, with arthroplasty performed on 762 shoulders, with information on mobilisation protocols on 736 shoulders (96.6%) and 717 patients (99.7%). The patient cohort comprised 250 males (34.9%) and 467 females (65.1%). Of the patients that successfully completed follow-up, 81.5% underwent RSA (n = 600), and 18.4% underwent TSA (n = 136). Overall, 262 (35.6%) patients underwent early postoperative mobilisation, and 474 shoulders were (64.4%) immobilised for a length of time. Immobilised patients were divided into three subgroups based on the period of immobilisation: three, four, or six weeks. There were 201 shoulders (27.3%) immobilised for three weeks, 77 (10.5%) for four weeks and 196 (26.6%) for six weeks. Five of the six manuscripts found no difference between clinical outcomes at one year when comparing early active motion versus immobilisation after RSA or TSA.

Early mobilisation is a safe postoperative rehabilitation pathway following both TSA and RSA. This may lead to an accelerated return to function and improved quality of life in the postoperative period.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Influence of Knot Number on Holding Capacity of two High Strength Sutures Tapes – A Biomechanical Analysis**

Tatjana Pastor<sup>1,2</sup>, Ivan Zderic<sup>1</sup>, Peter Varga<sup>1</sup>, Boyko Gueorguiev<sup>1</sup>, Torsten Pastor<sup>3</sup>

<sup>1</sup>AO Research Institute Davos, Davos, Switzerland; <sup>2</sup>Department of Hand surgery, Bern University Hospital, University of Bern, Bern, Switzerland; <sup>3</sup>Department of Orthopaedic and Trauma Surgery, Lucerne Cantonal Hospital, Lucerne, Switzerland

The number of seven needed knots to provide secure hold of high strength sutures was previously reported. New technologies like tape sutures and sutures with a salt infused silicon core have been developed, potentially reducing the number of needed knots. Study aims: To investigate the influence of (1) throw number and (2) different ambient conditions on knot security in two different high-strength sutures, and (3) to compare their biomechanical competence.

Two sutures (SutureTape (FT); n=56 and DynaTape (DT); n=56) were assigned for knot tying. Specimens were exposed to different media during tying, namely air, saline solution, and fat. A monotonic tensile ramp was applied. For each suture and ambient condition, seven specimens with 3 to 7 throws each were tested (n=7), evaluating their slippage and ultimate force to failure. The minimum number of throws preventing suture unraveling was determined in each suture and condition.

For each suture type and condition failure occurred via rupturing in all specimens for the following minimum number of throws: FT: dry-6, wet-6, fatty-wet-6; DT: dry-6; wet-4; fatty-wet-5. No significant differences were found comparing ultimate load to rupture of the two groups with minimum number of needed throws in each media. (FT dry-6 vs. DT dry-6; p<0.07); (FT wet-6 vs. DT wet-4; p<0.20); (FT fat-6 vs. DT fat-5; p<0.58). Knot slippage of DT was significantly higher in wet and fatty conditions compared to ST p<0.001 and p<0.004.

In fatty-wet conditions DT requires 5 throws to achieve a secure knot. In wet conditions this number can be reduced to 4 throws. FT needs 6 throws to provide a stable knot in all conditions. The biomechanical competence of both sutures in terms of knot slippage and peak force are comparable.

**Mechanical stiffness or resolved inflammation – what is more important for tendon rehabilitation?**

Kirsten Legerlotz

Movement Biomechanics, Department of Sport Sciences, Humboldt-Universität zu Berlin, Germany

As high incidences of tendinopathies are observed particularly in those who intensively use their tendons, we assume that pathological changes are caused, at least partially, by mechanical overload. This has led to the so-called overload hypothesis, explaining the development of tendinopathies by structural failure resulting from excessive load. At the same time, tendon loading is an important part in tendon rehabilitation. Currently, exercise treatment approaches such as eccentric training or heavy load resistance training are widely applied in tendinopathy rehabilitation, with good clinical results such as an improvement in function and a reduction in pain. Particularly those rehabilitative approaches which impose high strains on the tendon may induce an adaptation of the tendon's mechanical properties such as increased tendon stiffness (Radovanović et al 2022). An increased tendon stiffness is often interpreted as desirable, as it may protect the tendon from overloading and thus prevent future strain injuries. However, the tendinopathic tendon is not necessarily less stiff than the tendon in the contralateral leg and an improvement in tendon stiffness is not necessarily accompanied by an improvement in tendon pain or function (Radovanović et al 2023). In addition, metabolic factors, resulting e.g. in low-level systemic inflammation, may contribute to pathological tendon tissue changes and are not necessarily affected by an exercise program, while nutritional interventions or dietary supplements may potentially affect tendon cell metabolism. Indeed, dietary supplements have been introduced as an additional therapeutic approach in the treatment of tendinopathies in recent years, and their positive curative effects have been reported for both the general population and athletes (Qiu et al. 2022). In the management of tendinopathies, it may thus be advisable if therapeutic approaches aim to address both tendon mechanics and tendon metabolism for better treatment effectiveness and a sustainable improvement in pain and function.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

Physiotherapeutic Treatment Outcome in Tendinopathy? A Systematic Review and Meta-Analysis. *Journal of Clinical Medicine*. 17;11(6):1666.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Animal Component-Free Workflows to Derive Tenocyte-Like Cells from Human Mesenchymal Stromal Cells and Pluripotent Stem Cells**

Alessandro Dei<sup>1,2</sup>, Mark Hills<sup>3</sup>, Wing Chang<sup>3</sup>, Ravenska Wagey<sup>3</sup>, Allen Eaves<sup>3,4</sup>, Sharon Louis<sup>3</sup>, Dimitrios I. Zeugolis<sup>1,5</sup>, Arthur V. Sampaio<sup>3</sup>

<sup>1</sup>Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Biomedical Sciences Building, National University of Ireland Galway (NUIG), Galway, Ireland; <sup>2</sup>STEMCELL Technologies Ltd., Cambridge, United Kingdom; <sup>3</sup>STEMCELL Technologies Inc., Vancouver, Canada; <sup>4</sup>Terry Fox Laboratory, BC Cancer, Vancouver, Canada; <sup>5</sup>Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), Biomedical Sciences Building (NUIG), Galway, Ireland

Cell-based therapies offer a promising strategy to treat tendon injuries and diseases. Both mesenchymal stromal cells (MSCs) and pluripotent stem cells (PSCs) are good candidates for such applications due to their self-renewing and differentiation capacity. However, the translation of cell-based therapies from bench to bedside can be hindered by the use of animal-derived components in ancillary materials and by the lack of standardised media and protocols for in vitro tenogenic differentiation. To address this, we have optimized animal component-free (ACF) workflows for differentiating human MSCs and PSCs to tenocyte-like cells (TLCs) respectively. MSCs isolated from bone marrow (n = 3) or adipose tissue (n = 3) were expanded using MesenCult™-ACF Plus Culture Kit for at least 2 passages, and differentiated to TLCs in 21 days using a step-wise approach. Briefly, confluent cultures were treated with an ACF tenogenic induction medium for 3 days, followed by treatment with an ACF maturation medium for 18 days. Monolayer cultures were maintained at high density without passaging for the entire duration of the protocol, and the medium was changed every 2 - 3 days. In a similar fashion, embryonic (n = 3) or induced PSCs (n = 3) were first differentiated to acquire a mesenchymal progenitor cell (MPC) phenotype in 21 days using STEMdiff™ Mesenchymal Progenitor Kit, followed by the aforementioned tenogenic protocol for an additional 21 days. In all cases, the optimized workflows using ACF formulations consistently activated a tenogenic transcriptional program, leading to the generation of elongated, spindle-shaped tenomodulin-positive (TNMD+) cells and deposition of an extracellular matrix predominantly composed of collagen type I. In summary, here we describe novel workflows that can robustly generate TLCs from MSCs and hPSC-derived MPCs for potential translational applications.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Remnant-preserved Acute Anterior Cruciate Ligament Reconstruction with Concomitant Medial and Lateral Meniscal Tear Repair: Radiographic, Functional and Patient Reported Outcomes after 12 Month Follow-up**

Sheng Dai<sup>1</sup>, Jianlong Ni<sup>1</sup>, Genwen Mao<sup>1</sup>, Cuiping Mao<sup>2</sup>, Ruiyu Liu<sup>1</sup>, Zhibin Shi<sup>1</sup>, Meng Feng<sup>1</sup>

<sup>1</sup>Department of Orthopedics, the Second Affiliated Hospital of Xi'an Jiaotong University; <sup>2</sup>Department of Radiology, the Second Affiliated Hospital of Xi'an Jiaotong University

Although remnant-preserved ACL reconstruction (ACLR) restores knee joint stability and dampens the problem of acute ACL rupture-induced knee pain, an increasing number of patients still develop post-traumatic osteoarthritis (PTOA) after 10 to 15 years of ACLR. We previously found that remnant-preserved ACLR with concomitant medial and lateral meniscus repair may not prevent cartilage degeneration and weaken muscle strength, while the clinical features of PTOA are not clear. We hypothesized that remnant-preserved ACLR with concomitant medial and lateral meniscus tears is related to early cartilage damage, worse function recovery, patient-reported outcomes (PROs) and delayed duration to return to sports. The aim is to evaluate the remnant-preserved ACLR with complicated meniscal injuries in predicting which patients are at higher risk of osteoarthritic changes, worse function and limited activities after ACLR for 12 months.

Human ethical issue was approved by a committee from Xi'an Jiaotong University. 26 young and active patients (24 male, 2 female) with ACL injuries (Sherman type I and II) with concomitant medial and lateral meniscus within 2 months were included from January 2014 to March 2022. The average age of the ACLR+ meniscus repair was 26.77±1.52 (8 right, 5 left) and isolated ACLR control was 31.92±2.61 years old (7 left, 6 right). Remnant-preserved ACLR with a 5- to 6-strand hamstring tendon graft was operated on by the same sports medicine specialists. MRI CUBE-T<sub>2</sub> scanning with 48 channels was conducted by a professional radiologist. The volume of the ACL graft was created through 3 dimensional MRI model (Mimics 19, Ann Arbor). Anterior Cruciate Ligament OsteoArthritis Score (ACLOAS) was applied to score visible cartilage damage. IKDC 2000 score and VAS were assessed by two blinded researchers. Results were presented as mean± SEM of each group.

The cross-sectional area and 3D volume of the ACL graft were greater in the remnant-preserved ACLR+meniscus group compared with isolated ACLR ( $p=0.01$ ). It showed that ACLR+ meniscus group had early signs of joint damage and delayed meniscus healing regarding ACLOAS compared to control group ( $p=0.045$ ). MRI CUBE-T<sub>2</sub> prediction of radiographic cartilage degeneration was not obvious in both groups post remnant-preserved ACLR over 12 months ( $p>0.05$ ). However, higher VAS scores, lower IKDC scores, and long-last joint swelling were reported in the ACLR+ meniscus repair

**27-29 SEPTEMBER | PORTO, PORTUGAL**

group at the end of 12 months follow-up. Although remnant-preserved ACLR+ meniscus was able to maintain the restore the knee function, it showed delayed timing (>12 months) to return to play at the pre-injury stage, while no difference between the timing of returning to the normal daily routine of their ACLR knee compared to control ( $p=0.30$ ). The cost of ACLR+ meniscus (average 10,520.76\$) was higher than the control group (6,452.92\$,  $p=0.018$ ).

Remnants-preserved ACLR with concomitant injured medial and lateral meniscus repair shows a higher risk of cartilage damage, greater cost, worse functional performance, and longer time for young male patients to return to sports after 12-month follow-up compared to isolated ACLR. Further evidence and long-term follow-up are needed to better understand the association between these results and the risk of development of PTOA in this patient cohort.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Increased CD4<sup>+</sup> to CD8<sup>+</sup> T-cell ratio as a driver of impaired Achilles tendon healing in human patients**

Franka Klatte-Schulz<sup>1,2</sup>, Tobias Gehlen<sup>3</sup>, Nicole Bormann<sup>1,2</sup>, Serafim Tsitsilonis<sup>1,3,4</sup>, Sebastian Manegold<sup>5</sup>, Aysha Schmock<sup>1,2</sup>, Josephine A. Melzer<sup>1</sup>, Katharina Schmidt-Bleek<sup>1,2</sup>, Sven Geißler<sup>1,2</sup>, Georg N. Duda<sup>1,2</sup>, Birgit Sawitzki<sup>6,7</sup> und Britt Wildemann<sup>1,8</sup>

<sup>1</sup>Julius Wolff Institut, Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Germany; <sup>2</sup>BIH-Center for Regenerative Therapies, Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Germany; <sup>3</sup>Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin, Germany; <sup>4</sup>Sporthopaedicum Berlin, Germany; <sup>5</sup>BG Unfallklinik Frankfurt am Main gGmbH, Germany; <sup>6</sup>Center of Immunomics, Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Germany; <sup>7</sup>Institute of Medical Immunology, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt University of Berlin, German; <sup>8</sup>Experimental Trauma Surgery, Department of Trauma-, Hand- and Reconstructive Surgery, Jena University Hospital, Friedrich Schiller University Jena, Germany

Early identification of patients at risk for impaired tendon healing and corresponding novel therapeutic approaches are urgent medical needs. This study aimed to clarify the role of CD3<sup>+</sup> T-cells during acute Achilles tendon (AT) healing. Blood and hematoma aspirate were taken from 26 patients during AT reconstruction, and additional blood samples were obtained during clinical follow-up at 6, 26 and 52 weeks after surgery. T-cell subsets were analyzed by flow cytometry using CD3, CD4, CD8, CD11a, CD57 and CD28 antibodies. Clinical follow-up included functional tests, MRI assessments, and subjective questionnaires. In vitro, the functional behavior of patient-derived tenocytes was investigated in co-cultures with autologous unpolarized CD4<sup>+</sup> or CD8<sup>+</sup> T-cells, or IFN $\gamma$ -polarized CD8<sup>+</sup> or IL17-polarized CD4<sup>+</sup> T-cells (n=5-6). This included alterations in gene expression (qPCR), MMP secretion (ELISA), migration rate (scratch wound healing assay) or contractility (collagen gels). Analysis revealed that elevated CD4<sup>+</sup> T-cell levels and reduced CD8<sup>+</sup> T-cell levels (increased CD4/CD8 ratio) in hematoma aspirate and pre-operative blood were associated with inferior clinical outcomes regarding pain and function at 26 and 52 weeks. Increased levels of CD8<sup>+</sup> -memory T-cell subpopulations in blood 6 weeks after surgery were associated with less tendon elongation. In vitro, tenocytes showed increased MMP1/2/3 levels and collagen III/I ratio in co-culture with unpolarized and/or IL17-polarized CD4<sup>+</sup> T-cells compared to unpolarized CD8<sup>+</sup> T-cells. This coincided with increased IL17 receptor expression in tenocytes co-cultured with CD4<sup>+</sup> T-cells. Exposure of tenocytes to IL17-polarized CD4<sup>+</sup> T-cells decreased their migration rate and increased their matrix contractility, especially compared to IFN $\gamma$ -polarized CD8<sup>+</sup> T-cells. The CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratio could serve as prognostic marker for early identification of patients with impaired AT healing potential. Local reduction of CD4<sup>+</sup> T-cell levels or their IL17

**27-29 SEPTEMBER | PORTO, PORTUGAL**

secretion represent a potential therapeutic approach to improve AT healing and to prevent weakening of the tendon ECM.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Glutamate Expression in Subacromial Bursa is Associated with Rotator Cuff Tear and with Shoulder Pain

Hyung Bin Park<sup>1,2</sup> and Ra Jeong Kim<sup>1</sup>

<sup>1</sup>Gyeongsang Institute of Health Sciences, Gyeongsang National University, Jinju, Republic of Korea; <sup>2</sup>Department of Orthopaedic Surgery, Gyeongsang National University Changwon Hospital, Changwon, Republic of Korea

Glutamate regulates the expression of apoptosis-related genes and triggers the apoptosis of fibroblasts in rotator cuff tendons. Subacromial bursitis is always accompanied by symptomatic rotator cuff tear (RCT). However, no study has been reported on the presence of glutamate in subacromial bursa and on its involvement of shoulder pain in patients who had RCT. The purposes of this study were to determine whether the glutamate expression in subacromial bursa is associated with the presence of RCT and with the severity of shoulder pain accompanying RCT.

Subacromial bursal tissues were harvested from patients who underwent arthroscopic rotator cuff tendon repair or glenoid labral repair with intact rotator cuff tendon. Glutamate tissue concentrations were measured, using a glutamate assay kit. Expressions of glutamate and its receptors in subacromial bursae were histologically determined. The sizes of RCT were determined by arthroscopic findings, using the DeOrio and Cofield classification. The severity of shoulder pain was determined, using visual analog scale (VAS). Any associations between glutamate concentrations and the size of RCT were evaluated, using logistic regression analysis. The correlation between glutamate concentrations and the severity of pain was determined, using the Pearson correlation coefficient. Differences with a probability <0.05 were considered statistically significant.

Glutamate concentrations showed significant differences between the torn tendon group and the intact tendon group ( $P = 0.009$ ). Concentrations of glutamate significantly increased according to increases in tear size ( $P < 0.001$ ). In histological studies, the expressions of glutamate and of its ionotropic and metabotropic receptors have been confirmed in subacromial bursa. Glutamate concentrations were significantly correlated with pain on VAS ( $Rho=0.56$  and  $P = 0.01$ ).

The expression of glutamate in subacromial bursa is significantly associated with the presence of RCT and significantly correlated with its accompanying shoulder pain.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **AO Fracture Monitor: Continuous sensor monitoring for personalised fracture care**

Manuela Ernst<sup>1</sup>, Markus Windolf<sup>1</sup>, Viktor Varjas<sup>1</sup>, Dominic Gehweiler<sup>1</sup>,  
B Gueorguiev-Rüegg<sup>1</sup> R. Geoff Richards<sup>1</sup>

<sup>1</sup>AO Research Institute Davos, Switzerland

In absence of available quantitative measures, the assessment of fracture healing based on clinical examination and X-rays remains a subjective matter. Lacking reliable information on the state of healing, rehabilitation is hardly individualized and mostly follows non evidence-based protocols building on common guidelines and personal experience. Measurement of fracture stiffness has been demonstrated as a valid outcome measure for the maturity of the repair tissue but so far has not found its way to clinical application outside the research space. However, with the recent technological advancements and trends towards digital health care, this seems about to change with new generations of instrumented implants – often unfortunately termed "smart implants" – being developed as medical devices.

The AO Fracture Monitor is a novel, active, implantable sensor system designed to provide an objective measure for the assessment of fracture healing progression (1). It consists of an implantable sensor that is attached to conventional locking plates and continuously measures implant load during physiological weight bearing. Data is recorded and processed in real-time on the implant, from where it is wirelessly transmitted to a cloud application via the patient's smartphone. Thus, the system allows for timely, remote and X-ray free provision of feedback upon the mechanical competence of the repair tissue to support therapeutic decision making and individualized aftercare.

The device has been developed according to medical device standards and underwent extensive verification and validation, including an in-vivo study in an ovine tibial osteotomy model, that confirmed the device's capability to depict the course of fracture healing as well as its long-term technical performance. Currently a multi-center clinical investigation is underway to demonstrate clinical safety of the novel implant system. Rendering the progression of bone fracture healing assessable, the AO Fracture Monitor carries potential to enhance today's postoperative care of fracture patients.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Commercially Pure (Cp-Ti) Titanium Medical Implant Production using Laser Powder Bed Fusion (L-PBF) Technology****Fatma Nur Depboylu<sup>1</sup>, Evren Yasa<sup>2</sup>, Özgür Poyraz<sup>3</sup>, Feza Korkusuz<sup>4</sup>**

<sup>1</sup>Department of Bioengineering, Hacettepe University Institute of Science and Technology, Beytepe, 06800, Ankara, Turkey, fatmanur.depboylu@gmail.com;

<sup>2</sup>Department of Mechanical Engineering, Eskisehir Osmangazi University, 26480, Eskisehir, Turkey, ebalyasa@gmail.com / eyasa@ogu.edu.tr;

<sup>3</sup>Department of Mechanical Engineering, Eskisehir Technical University, 26555, Eskisehir, Turkey, poyrazozgur@gmail.com;

<sup>4</sup>Faculty of Medicine, Department of Sports Medicine, Hacettepe University, Sıhhiye, 06100, Ankara, Turkey, feza.korkusuz@gmail.com

Decreasing the bulk weight without losing the biomechanical properties of commercial pure titanium (Cp-Ti) medical implants is now possible by using Laser Powder Bed Fusion (L-PBF) technology [1]. Gyroid lattice structures that have precious mechanical and biological advantages because of similarity to trabecular bone. The aim of the study was to design and develop L-PBF process parameter optimization for manufacturing gyroid lattice Cp-Ti structures. The cleaning process was then optimized to remove the non-melted powder from the gyroid surface without mechanical loss.

Gyroid cubic designs were created with various relative densities by nTopology. L-PBF process parameter optimization was progressed using with Cp-Ti (EOS TiCP Grade2) powder in the EOS M290 machine to achieve parts that have almost full dense and dimensional accuracy. The metallography method was made for density. Dimensional accuracy at gyroid wall thicknesses was investigated between designed and manufactured via stereomicroscope, also mechanical tests were applied with real time experiment and numerical analysis (ANSYS). Mass loss and strut thickness loss were investigated for chemical etching cleaning process.

Gyroid parts had 99,5% density. High dimensional accuracy was achieved during L-PBF process parameters optimization. Final L-PBF parameters gave the highest 19% elongation and 427 MPa yield strength values at tensile test. Mechanical properties of gyroid were controlled with changing relative density. A minute chemical etching provided to remove non-melted powders.

Compression test results of gyroids at numerical and real-time analysis gave unrelated while deformation behaviors were compatible with each other. Gyroid Cp-Ti osteosynthesis mini plates will be produced with final L-PBF process parameters. MTT cytotoxicity test will be characterized for cell viability.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**Influence of fluid uptake on the mechanical and thermal properties of PMMA-based bone cement**

Crystal Emonde<sup>1</sup>, Magnus Reulbach<sup>1</sup>, Patrick Evers<sup>2</sup>, Hannah Behnsen<sup>3</sup>, Florian Nürnberger<sup>2</sup>, Eike Jakobowitz<sup>1</sup>, Henning Windhagen<sup>1</sup>

<sup>1</sup>Laboratory for Biomechanics and Biomaterials (LBB), Department of Orthopaedic Surgery, Hannover Medical School, Anna-von-Borries-Strasse 1-7, 30625 Hannover, Germany; <sup>2</sup>Institute of Materials Science (Werkstoffkunde), Leibniz University Hannover, An der Universitaet 2, 30823 Garbsen, Germany; <sup>3</sup>Institute of Plastics and Circular Economy, Leibniz University Hannover, An der Universitaet 2, 30823 Garbsen, Germany

According to the latest report from the German Arthroplasty Registry, aseptic loosening is the primary cause of implant failure following primary hip arthroplasty [1]. Osteolysis of the proximal femur due to the stress-shielding of the bone by the implant causes loss of fixation of the proximal femoral stem, while the distal stem remains fixed [2], [3].

Removing a fixed stem is a challenging process. Current removal methods rely on manual tools such as chisels, burrs, osteotomes, drills and mills, which pose the risk of bone fracture and cortical perforation [4], [5]. Others such as ultrasound and laser, generate temperatures that could cause thermal injury to the surrounding tissues and bone [6]. It is crucial to develop techniques that preserve the host bone, as its quality after implant removal affects the outcome of a revision surgery.

A gentler removal method based on the transcutaneous heating of the implant by induction is proposed [7]. By reaching the glass transition temperature ( $T_G$ ) of the periprosthetic cement, the cement is expected to soften, enabling the implant to be gently pulled out. The in-vivo environment comprises body fluids and elevated temperatures, which deteriorate the inherent mechanical properties of bone cement, including its  $T_G$  [8]. We aimed to investigate the effect of fluid absorption on the  $T_G$  (ASTM E2716-09) and Vicat softening temperature (VST) (ISO 306) of Palacos R cement (Heraeus Medical GmbH) when dry and after storage in Ringer's solution for up to 8 weeks.

Samples stored in Ringer's solution exhibited lower  $T_G$  and VST than those stored in air. After 8 weeks, the  $T_G$  decreased from 95.2°C to 81.5°C in the Ringer's group, while the VST decreased from 104.4°C to 91.9°C. These findings will be useful in the ultimate goal of this project which is to design an induction-based system for implant removal.

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27-29 SEPTEMBER | PORTO, PORTUGAL

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Development of a dynamic coil-shaped scaffold for articular cartilage tissue engineering****Pedro J. Díaz-Payno<sup>1</sup>, Javier Llorca<sup>2,3</sup>, Andrés Díaz Lantada<sup>4</sup>, Jennifer Patterson<sup>1</sup>**

<sup>1</sup>Biomaterials and Regenerative Medicine group, IMDEA Materials Institute, Tecogetafe, Madrid, Spain; <sup>2</sup>Bio/Chemo/Mechanics of Materials group, IMDEA Materials Institute, Tecogetafe, Madrid, Spain; <sup>3</sup>Department of Materials Science, Polytechnic University of Madrid, Madrid, Spain; <sup>4</sup>ETSI Industriales, Polytechnic University of Madrid, Madrid, Spain

Even minor lesions in articular cartilage (AC) can cause underlying bone damage creating an osteochondral (OC) defect. OC defects can cause pain, impaired mobility and can develop to osteoarthritis (OA). OA is a disease that affects nearly 10% of the population worldwide<sup>[1]</sup>, and represents a significant economic burden to patients and society<sup>[2]</sup>. While significant progress has been made in this field, realising an efficacious therapeutic option for unresolved OA remains elusive and is considered one of the greatest challenges in the field of orthopaedic regenerative medicine<sup>[3]</sup>. Therefore, there is a societal need to develop new strategies for AC regeneration. In recent years there has been increased interest in the use of tissue-specific aligned porous freeze-dried extracellular matrix (ECM) scaffolds as an off-the-shelf approach for AC repair, as they allow for cell infiltration, provide biological cues to direct target-tissue repair and permit aligned tissue deposition, desired in AC repair<sup>[4]</sup>. However, most ECM-scaffolds lack the appropriate mechanical properties to withstand the loads passing through the joint<sup>[5]</sup>. One solution to this problem is to reinforce the ECM with a stiffer framework made of synthetic materials, such as polylactic acid (PLA)<sup>[6]</sup>. Such framework can be 3D printed to produce anatomically accurate implants<sup>[7]</sup>, attractive in personalized medicine. However, typical 3D prints are static, their design is not optimized for soft-hard interfaces (OC interface), and they may not adapt to the cyclic loading passing through our joints, thus risking implant failure. To tackle this limitation, more compliant or dynamic designs can be printed, such as coil-shaped structures<sup>[8]</sup>. Thus, in this study we use finite element modelling to create different designs that mimic the mechanical properties of AC and prototype them in PLA, using polyvinyl alcohol as support. The optimal design will be combined with an ECM scaffold containing a tailored microarchitecture mimicking aspects of native AC.

**References:** [1] Felson 2000, [2] Hunter 2014, [3] Hunter and Bierma-Zeinstra 2019, [4] Cunniffe and Díaz-Payno 2019, [5] Putra 2022, [6] Rodrigues 2012, [7] Daly 2016, [8] Kolken 2021.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Stability of biofunctionalized high-performance oxide ceramics against radiation sterilization

Philipp Schröder<sup>1</sup>, Alejandro Gómez Montoya<sup>1</sup>, Norina Labude-Weber<sup>2</sup>, Jörg Eschweiler<sup>3</sup>, Sabine Neuss<sup>2,4</sup>, Horst Fischer<sup>1</sup>

<sup>1</sup>Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany; <sup>2</sup>Helmholtz Institute for Biomedical Engineering, Biointerface Group, RWTH Aachen University Hospital, Aachen, Germany; <sup>3</sup>Department for Orthopaedics, Trauma and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany; <sup>4</sup>Institute of Pathology, RWTH Aachen University Hospital, Aachen, Germany

While high-performance ceramics like alumina and zirconia exhibit excellent wear resistance, they provide poor osseointegration capacity. As osseointegration is crucial for non-cemented joint prostheses, new techniques have been successfully developed for biofunctionalizing high-performance ceramic surfaces. Stable cell adhesion can be achieved by covalently bound specific peptides. In this study we investigate the effect of sterilization processes on organo-chemically functionalized surfaces.

To enhance the performance of alumina-toughened zirconia ceramics (ATZ), a 3-aminopropyl-diisopropylethoxysilane (APDS) monolayer was applied and coupled with cyclo-RGD peptides (cRGD) by using bifunctional crosslinker bis(sulfosuccinimidyl)suberat (BS<sup>3</sup>). The samples were sterilized using e-beam or gamma-sterilization at 25 kGy, either before or after biofunctionalization with cRGD. Functionalization stability was investigated by contact angle measurements. The functionality of cRGD after sterilization was demonstrated using proliferation tests and cytotoxicity assays. Immunofluorescence staining (pFAK, Actin, DAPI) was conducted to evaluate the adhesion potential between the samples and human mesenchymal stem cells (hMSCs).

Functionalized samples before and after sterilization showed no significant difference regarding their contact angles. A proliferation test demonstrated that the cells on functionalized samples proliferate significantly more than on untreated samples before and after sterilization. hMSCs showed a significant higher proliferation on gamma sterilized samples compared to all other groups after 14 days. It was confirmed that the samples did not exhibit cytotoxic behavior before or after sterilization. Fluorescence microscopy demonstrated that both, cells on sterilized and on non-sterilized samples, expressed high levels of pFAK-Y397.

The investigated functionalization enables improved adhesion and proliferation of hMSCs and is stable against the investigated sterilization processes. This is of importance as the option of having a sterile product enables the start of the translation of this biofunctional coating towards preclinical and subsequently first-in-man applications.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Microenvironmental dependence of proper chondrocytic maturation**

Holger Jahr

Dept. of Anatomy and Cell Biology and Institute of Structural Mechanics and Lightweight Design, RWTH Aachen University, Germany

Articular cartilage is a relatively hypoxic tissue with a unique extracellular matrix that is enriched with cations, resulting in an elevated interstitial fluid osmolarity. Several biomechanical and physicochemical stimuli are reported to influence chondrocyte metabolism. For regenerative *in vitro* applications, increasing the extracellular osmolarity above plasma level to more physiological values induces chondrogenic marker expression and the differentiation of chondroprogenitor cells. Calcineurin inhibitor FK506 modulates the differentiation of primary chondrocytes under such conditions and its effect on cell proliferation, extracellular matrix quality, and BMP- and TGF- $\beta$  signaling will be described. Supraphysiological osmolarity compromises chondrocyte proliferation, while physosmolarity or FK506 did not. Rather, the combination of the latter increased proteoglycan and collagen expression in chondrocytes *in vitro* and *in situ*, affecting expression of TGF- $\beta$ -inducible protein TGFBI and chondrogenic (SOX9, Col2) as well as terminal differentiation markers (e.g., Col10). Surprisingly, expression of particularly minor collagens (e.g., Col9, Col11) was improved. Physiological osmolarity seems to promote terminal chondrogenic differentiation of progenitor cells through sensitization of TGF- $\beta$  superfamily signaling at the type I receptor. While hyperosmolarity alone facilitates TGF- $\beta$  superfamily signaling, FK506 seems to enhance signaling by releasing the FKBP12 break from the type I receptor to improve collagenous marker expression. Our data help explaining seemingly contradictory earlier findings and potentially benefit future cell-based cartilage repair strategies.

**The role of histone modifiers and oxidative stress in osteoarthritis**

Wei-Shiung Lian

Core Laboratory for Phenomics and Diagnostics and Center for Mitochondrial Research and Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

Development of osteoarthritis (OA) correlates with epigenetic alteration in chondrocytes. H3K27me3 demethylase UTX is known to regulate tissue homeostasis, but its role in the homeostasis of articulating joint tissue is poorly understood. Forced UTX expression upregulated H3K27me3 enrichment at the Sox9 promoter region to inhibit key extracellular matrix (ECM) molecules, like e.g. type II collagen, aggrecan, and glycosaminoglycans in articular chondrocytes. Utx loss in vitro altered the H3K27me3-binding epigenomic landscape, which contributes to mitochondrial activity, cellular senescence, and cartilage development. Functional target genes of Utx comprise insulin-like growth factor 2 (Igf2) and polycomb repressive complex 2 (PRC2) core components Eed and Suz12. Specifically, Utx deletion promoted Tfam transcription, mitochondrial respiration, ATP production and Igf2 transcription, but inhibited Eed and Suz12 expression. Igf2 inhibition or forced Eed or Suz12 expression increased H3K27 trimethylation and H3K27me3 enrichment at the Sox9 promoter, compromising Utx loss-induced ECM overproduction. Overexpression of Utx in murine knee joints aggravated OA development, including articular cartilage damage, synovitis, osteophyte formation, and subchondral bone loss. Transgenic mice with a chondrocytespecific Utx knockout develop thicker articular cartilage as compared to wild-type controls and show fewer gonarthrotic symptoms during destabilized medial meniscus- and collagenase-induced joint injury. In summary, UTX represses chondrocytic activity and accelerates cartilage degradation during OA, while Utx loss promotes cartilage integrity through epigenetic stimulation of mitochondrial biogenesis and Igf2 transcription. This highlights a novel noncanonical role of Utx that regulates articular chondrocyte anabolism and OA development.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Myokine Fndc5 compromises mitochondrial dysfunction in osteoarthritis**

Yu-Shan Chen

Center for Mitochondrial Research and Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

Chondrocytic activity is downregulated by compromised autophagy and mitochondrial dysfunction to accelerate the development of osteoarthritis (OA). Irisin is a cleaved form of fibronectin type III domain containing 5 (FNDC5) and known to regulate bone turnover and muscle homeostasis. However, little is known about the role of irisin in chondrocytes and the development of OA. This talk will shed light on FNDC5 expression by human articular chondrocytes and compare normal and osteoarthritic cells with respect to autophagosome marker LC3-II and oxidative DNA damage marker 8-hydroxydeoxyguanosine (8-OHdG). In chondrocytes *in vitro*, irisin improves IL-1 $\beta$ -mediated growth inhibition, loss of specific cartilage markers and glycosaminoglycan production. Irisin further suppressed Sirt3 and UCP-1 to improve mitochondrial membrane potential, ATP production, and catalase. This attenuated IL-1 $\beta$ -mediated production of reactive oxygen species, mitochondrial fusion, mitophagy, and autophagosome formation. In a surgical murine model of destabilization of the medial meniscus (DMM) intra-articular administration of irisin alleviates symptoms like cartilage erosion and synovitis. Furthermore, gait profiles of the treated limbs improved. In chondrocytes, irisin treatment upregulates autophagy, 8-OHdG and apoptosis in cartilage of DMM limbs. Loss of FNDC5 in chondrocytes correlates with human knee OA and irisin repressed inflammation-mediated oxidative stress and deficient extracellular matrix synthesis through retaining mitochondrial biogenesis and autophagy. The talk sheds new light on the chondroprotective actions of this myokine and highlights the remedial effects of irisin during progression of OA.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Novel insights from the gut-joint axis: microbial contribution to skeletal homeostasis**

Feng-Sheng Wang

Dept. of Medical Research, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

Osteoporosis (OP) and osteoarthritis (OA) are leading causes of musculoskeletal dysfunction in elderly, with chondrocyte senescence, inflammation, oxidative stress, subcellular organelle dysfunction, and genomic instability as prominent features. Age-related intestinal disorders and gut dysbiosis contribute to host tissue inflammation and oxidative stress by affecting host immune responses and cell metabolism. Not surprisingly, the development of OP and OA correlate with dysregulations of the gut microflora in rodents and humans. Intestinal microorganisms produce metabolites, including short-chain fatty acids, bile acids, trimethylamine N-oxide, and liposaccharides, affecting mitochondrial function, metabolism, biogenesis, autophagy, and redox reactions in chondrocytes to regulate joint homeostasis. Modulating the abundance of specific gut bacteria, like *Lactobacillus* and *Bifidobacterium*, by probiotics or fecal microbiota transplantation appears to suppress age-induced chronic inflammation and oxidative damage in musculoskeletal tissue and holds potential to slow down OP development. The talk will highlight treatment options with probiotics or metabolites for modulating the progression of OA and OP.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **EVs-based therapies for intervertebral disc regeneration: where do we stand?**

Marianna Tryfonidou

Utrecht University, The Netherlands

Within the field of disc degeneration-related low back pain, the spine community has been increasingly acknowledging the regenerative potential of extracellular vesicles (EVs). EVs are small lipid bilayer-delimited particles naturally released by cells, involved in intercellular signaling. They do so by interacting with recipient cells and releasing their biological cargo (e.g., mRNA, miRNA, DNA, protein, lipid)

EVs derived from mesenchymal stromal cells and, more recently, also EVs from notochordal cells, the cells residing within the core of the juvenile human disc, are being actively studied. In general, they have been proposed to mitigate inflammation/catabolic processes, reduce apoptosis, stimulate proliferation and even improve the matrix producing capacity of the treated cells. Within this context, appropriate characterization of EVs is essential to increase the level of evidence that the reported effects are indeed EV-associated. To analyze the purity and biochemical composition of EV preparations the International Society for Extracellular Vesicles (ISEV) has prepared guidelines recommending the analysis of multiple (EV) markers, as well as proteins co-isolated/recovered with EVs. Alongside, to prove that the effects are EV-associated and not due to co-isolated factors from the tissue or cells used to derive the EVs, appropriate technical controls need to be taken along (during cell/tissue culture). As such the question arises: “what is the evidence so far?”

While from a fundamental perspective EVs are very appealing, the use of natural EVs in clinical applications is challenging. It comes with drawbacks, including biologic variability, yield, cumbersome isolation, and challenging upscaling and storage to achieve industrial levels. To date there is no FDA-approved EV-based therapy for disc-related lower back pain.

Nonetheless, EV-based therapeutic approaches have unique advantages over the use of (pluripotent) stem cell-based therapies, such as a high biologic, but low immunogenic and tumorigenic potential.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Advancing messenger RNA therapeutics for ortho-regeneration**Elizabeth Rosado Balmayor<sup>1,2</sup>

<sup>1</sup>Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Germany;

<sup>2</sup>Rehabilitation Medicine Research Center, Mayo Clinic, Rochester, MN, USA.

Messenger RNA (mRNA) is a new class of drug that can be used to express a therapeutic protein and, in contrast to DNA, is safer and inexpensive. Among its advantages, mRNA will immediately begin to express its encoded protein in the cell cytoplasm. The protein will be expressed for a period of time, after which the mRNA is degraded. There is no risk of genetic damage, one of the concerns with plasmid DNA (pDNA) used in traditional gene therapy approaches. Nevertheless, mRNA application in tissue regeneration and regenerative medicine remains limited. In this case, mRNA must overcome its main hurdles: immunogenicity, lack of stability, and intracellular delivery. Research has been done to overcome these limitations, and the future of mRNA seems promising for tissue repair<sup>1,2</sup>. This keynote talk will address questions including: What are the opportunities for mRNA to improve outcomes in musculoskeletal tissue repair, in particular bone and cartilage? What are the key factors and challenges to expediting this technology to patient treatment (beyond COVID-19 vaccination)?

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Recapitulating articulating joint motion *ex vivo* to better understand chondrogenic differentiation**

Martin Stoddart

AO Research Davos, Switzerland

Articulating cartilage experiences a multitude of biophysical cues. Due to its primary function in distributing load with near frictionless articulation, it is clear that a major stimulus for cartilage homeostasis and regeneration is the mechanical load it experiences on a daily basis. While these effects are considered when performing *in vivo* studies, *in vitro* studies are still largely performed under static conditions. Therefore, an increasing complexity of *in vitro* culture models is required, with the ultimate aim to recreate the articulating joint as accurately as possible. We have for many years utilized a complex multiaxial load bioreactor capable of applying tightly regulated compression and shear loading protocols. Using this bioreactor, we have been able to demonstrate the mechanical induction of human bone marrow stromal cell (BMSC) chondrogenesis in the absence of exogenous growth factors. Building on previous bioreactor studies that demonstrated the mechanical activation of endogenous TGF $\beta$ , and subsequent chondrogenesis of human bone marrow derived MSCs, we have been further increasing the complexity of *in vitro* models. For example, the addition of high molecular weight hyaluronic acid, a component of synovial fluid, culture medium leads to reduced hypertrophy and increased glycosaminoglycan deposition. The ultimate aim of all of these endeavors is to identify promising materials and therapies during *in vitro*/ *ex vivo* studies, therefore reducing the numbers or candidates that are finally tested using *in vivo* studies. This 3R approach can improve the opportunities for success while leading to more ethically acceptable product development pathways.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Shedding light on chondrogenesis – the impact of low-level laser on the chondrogenic differentiation**

Sylvia Nürnberger

Medical University of Vienna, Department of Orthopedics and Trauma-Surgery

Photobiomodulation (PBM), the use of light for regenerative purposes, has a long history with first documentations several thousand years ago in ancient Egypt and a Nobel Prize on this topic at the beginning of last century (by Niels Finsen). Nowadays, it is in clinical use for indications such as wound healing, pain relief and anti-inflammatory treatment. Given the rising numbers of in vitro studies, there is increasing evidence for the underlying mechanisms such as wavelength dependent reactive oxygen production and adenosine triphosphate generation. In cartilage regeneration, the use of PBM is controversially discussed with divergent results in clinics and insufficient in vitro studies. As non-invasive therapy, PMB is, though, of particular importance, since a general regenerative stimulus would be of great benefit in the otherwise only surgically accessible tissues. We therefore investigated the influence of different wavelengths - blue (475 nm), green (516 nm) or red (635 nm) of a low-level laser (LLL) - on the chondrogenic differentiation of chondrocytes and adipose derived stromal cells of different human donors and applied the light in different settings (2D, 3D) with cells in a proliferative or differentiating stage. All assessed parameters (spheroid growth, histology, matrix quantification and gene expression) revealed an influence of LLL on chondrogenesis in a donor-, wavelength- and culture-model-dependent manner. Especially encouraging was the finding, that cells with poor chondrogenic potential could be improved by one single 2D treatment. Amongst the three wave lengths, red light was the most promising one with the most positive impact. Although in vivo data are still missing, these in vitro results provide evidence for a proper biofunctional effect of LLL.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Liraglutide's in vitro anti-inflammatory and regenerative properties on inflammatory human osteoarthritic chondrocytes- on behalf of the OA-BIO Consortium**

Eda Ciftci, Sibylle Grad, Mauro Alini, Zhen Li

AO Research Institute Davos, Davos, Switzerland

Osteoarthritis (OA) is the most prevalent degenerative joint disease that is a leading cause of disability worldwide. Existing therapies of OA only address the symptoms. Liraglutide is a well-known anti-diabetic medication that is used to treat type 2 diabetes and obesity. In inflammatory and post-traumatic OA animal models, liraglutide has demonstrated anti-inflammatory, pain-relieving, and cartilage-regenerating effects<sup>1</sup>. The objective of this study is to investigate liraglutide's ability to reduce inflammation and promote anabolism in human OA chondrocytes in vitro. Pellets formed with human OA chondrocytes were cultured with a chondrogenic medium for one week to form cartilage tissue. Afterward, pellets were cultured for another 2 weeks with a chondropermissive medium. The OA group was treated with IL-1 $\beta$  to mimic an inflammatory OA condition. The drug group was treated with 0.5 or 10  $\mu$ M liraglutide. On days 0, 1, and 14, pellets were collected. Conditioned medium was collected over the 2 weeks culture period. The gene and protein expression levels of regenerative and inflammatory biomarkers were evaluated and histological analyzes were performed. Results showed that the nitric oxide release of the OA + 0.5  $\mu$ M liraglutide and OA + 10  $\mu$ M liraglutide groups were lower than the OA group. The DNA content of the OA + 0.5  $\mu$ M liraglutide and OA + 10  $\mu$ M liraglutide groups were higher than the OA group on day 14. The RT-qPCR results showed that the anabolism (ACAN, COMP, and COL2) markers were higher expressed in the OA + 0.5  $\mu$ M liraglutide and OA + 10  $\mu$ M liraglutide groups when compared with the OA group. The inflammation (CCL-2 and IL-8) markers and catabolism markers (MMP-1, MMP-3, ADAMTS4, and ADAMTS5) had lower expression levels in the OA + liraglutide groups compared to the OA group. The histomorphometric analysis (Figure 1) supported the RT-qPCR results. The results indicate that liraglutide has anabolic and anti-inflammatory effects on human OA chondrocyte pellets.

**Reference:** 1 Meurot, C., Martin, C., Sudre, L. et al. Liraglutide, a glucagon-like peptide 1 receptor agonist, exerts analgesic, anti-inflammatory and anti-degradative actions in osteoarthritis. *Sci Rep* 12, 1567 (2022). <https://doi.org/10.1038/s41598-022-05323-7>

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Determining the accuracy of the smart brace using multi-frequency bioimpedance analysis to measure knee swelling

R.M. Gilsing<sup>1</sup>, M. Hoogveen<sup>2</sup>, H. Boers<sup>2</sup>, W. van der Weegen<sup>3</sup>

<sup>1</sup>Orthopedics Biomechanics Department, Technical University Eindhoven, Netherlands; <sup>2</sup>Researcher Health Department, Stichting Imec/Holst centre, Eindhoven, Netherlands; <sup>3</sup>Sports and Orthopaedics Research Center, Anna hospital, Geldrop, Netherlands

Knee swelling is common after injury or surgery, resulting in pain, restricted range of movement and limited mobility. Accurately measuring knee swelling is critical to assess recovery. However, current measurement methods are either unreliable or expensive [1,2]. Therefore, a new measurement method is developed. This wearable (the 'smart brace') has shown the ability to distinguish a swollen knee from a not swollen knee using multi-frequency-bio impedance analysis (MF-BIA) [3].

This study aimed to determine the accuracy of this smart brace. The study involved 25 usable measurements on patients treated for unilateral knee osteoarthritis with a 5mL injection of Lidocaine + DepoMedrol (1:4). MF-BIA measurements were taken before and after the injection, both on the treated and untreated knee. The smart brace accurately measured the effect of the injection by a decrease in resistance of up to 2.6% at 100kHz ( $p < 0.01$ ), where commonly used gel electrodes were unable to measure the relative difference. Remarkably, both the smart brace and gel electrodes showed a time component in the MF-BIA measurements.

To further investigate this time component, 10 participants were asked to lie down for 30 minutes, with measurements taken every 3 minutes using both gel electrodes and the smart brace on both legs. The relative change between each time step was calculated to determine changes over time. The results showed presence of a physiological aspect (settling of knee fluids), and for the brace also a mechanical aspect (skin-electrode interface) [4]. The mechanical aspect mainly interfered with reactance values.

Overall, the smart brace is a feasible method for quantitatively measuring knee swelling as a relative change over time. However, the skin-electrode interface should be improved for reliable measurements at different moments in time. The findings suggest that the smart brace could be a promising tool for monitoring knee swelling during rehabilitation.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Articular chondrocytes from diabetic and non diabetic rats exposed to normo- or hyperglycemia respond differentially to the complement split fragment anaphylatoxin C5a and TNF $\alpha$** N. Fleischmann, T. Braun, A. Reinhardt, T. Schotte, J. Wehrmann, V. Rüdig, C. Gögele, M. Kokozidou, C. Werner, G. Schulze-Tanzil

Institute of Anatomy, Paracelsus Medical University, Salzburg and Nuremberg, Nuremberg, Prof.-Ernst-Nathan Str. 1, 90419 Nuremberg and Salzburg, Nuremberg, Germany

Osteoarthritis (OA) and diabetes mellitus type 2 (DMT2) are pathogenetically linked. Complement dysregulation contributes to OA and could be involved in DMT2. The inflammatory anaphylatoxin C5a is released during complement activation. This study aims to understand the specific responses of chondrocytes isolated from diabetic and non-diabetic rats exposed to C5a and/or the proinflammatory cytokine TNF $\alpha$  in vitro dependent on the glucose supply. Articular chondrocytes of adult Zucker Diabetic Fatty (ZDF) rats (homozygous: fa/fa, diabetic, heterozygous: fa/+, lean controls) were exposed to 10 ng/mL TNF $\alpha$  and 25 ng/mL C5a alone or in combination, both, under normo- (NG, 1 g/L glucose) and hyperglycemic (HG, 4.5 g/L glucose) conditions (4 or 24 h). Chondrocyte survival, metabolic activity and gene expression of collagen type 2, suppressors of cytokine signaling (SOCS)1, -3 and anti-oxidative hemoxygenase-1 (HMOX1) were assessed. The complement regulatory protein CD46 and cell nuclei sizes were analyzed. Chondrocyte vitality remained unaffected by the treatment. Metabolic activity was impaired in chondrocytes of non-diabetic rats under HG conditions. Collagen type 2 transcription was suppressed by TNF $\alpha$  under HG condition in chondrocytes from nondiabetic donors and under both conditions in those of DMT2 rats (24 h)

Except for DMT2 chondrocytes under HG (4 h), HMOX1 was generally induced by TNF $\alpha$  +/- C5a (NG, HG). C5a elevated HMOX1 only in chondrocytes of controls. The SOCS1/3 genes were increased by TNF $\alpha$  (NG, diabetic, non diabetic, 4 and 24 h). This could also be observed in chondrocytes of diabetic, but not of lean rats (24 h, HG). At 4 h, C5a induced SOCS1 only in non diabetic chondrocytes (NG, HG). Cytoprotective CD46 protein was suppressed by TNF $\alpha$  under NG condition. Nuclear volumes of chondrocyte were lower in chondrocytes from DMT2 rats compared to those from controls. The differential response suggests that chondrocytes are irreversibly compromised by DMT2.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## The secretome of AEC: challenges and opportunities in cell free regenerative medicine in tendon disorders

Adrián Cerveró-Varona<sup>1</sup>, Angelo Canciello<sup>1</sup>, Giuseppe Prencipe<sup>1</sup>, Alessia Peserico<sup>1</sup>, Arlette A. Haidar-Montes<sup>1</sup>, Helder A. Santos<sup>2</sup>, Valentina Russo<sup>1</sup>, Barbara Barboni<sup>1</sup>

<sup>1</sup>Unit of Basic and Applied Sciences, Faculty of Biosciences and Agro-Food and Environmental Technologies, University of Teramo, Teramo, Italy; <sup>2</sup>Drug Research Program, Faculty of Pharmacy, University of Helsinki, FI-00014, Helsinki, Finland.

The application of immune regenerative strategies to deal with unsolved pathologies, such as tendinopathies, is getting attention in the field of tissue engineering exploiting the innate immunomodulatory potential of stem cells [1]. In this context, Amniotic Epithelial Cells (AECs) represent an innovative immune regenerative strategy due to their teno-inductive and immunomodulatory properties [2], and because of their high paracrine activity, become a potential stem cell source for a cell-free treatment to overcome the limitations of traditional cell-based therapies. Nevertheless, these immunomodulatory mechanisms on AECs are still not fully known to date. In these studies, we explored standardized protocols [3] to better comprehend the different phenotypic behavior between epithelial AECs (eAECs) and mesenchymal AECs (mAECs), and to further produce an enhanced immunomodulatory AECs-derived secretome by exposing cells to different stimuli. Hence, in order to fulfill these aims, eAECs and mAECs at third passage were silenced for CIITA and Nrf2, respectively, to understand the role of these molecules in an inflammatory response. Furthermore, AECs at first passage were seeded under normal or GO-coated coverslips to study the effect of GO on AECs, and further exposed to LPS and/or IL17 priming to increase the anti-inflammatory paracrine activity. The obtained results demonstrated how CIITA and Nrf2 control the immune response of eAECs and mAECs, respectively, under standard or immune-activated conditions (LPS priming). Additionally, GO exposition led to a faster activation of the Epithelial-Mesenchymal transition (EMT) through the TGF $\beta$ /SMAD signaling pathway with a change in the anti-inflammatory properties. Finally, the combinatory inflammatory stimuli of LPS+IL17 enhanced the paracrine activity and immunomodulatory properties of AECs. Therefore, AECs-derived secretome has emerged as a potential treatment option for inflammatory disorders such as tendinopathies.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Movement data analysis as a novel predictive technology for tendon repair**

Faydaver, Melisa<sup>1</sup>; Russo, Valentina<sup>1</sup>; Di Giacinto, Oriana<sup>1</sup>; El Khatib, Mohammad<sup>1</sup>; Rigamonti, Mara<sup>2</sup>; Rosati, Giorgio<sup>2</sup>; Raspa, Marcello<sup>3</sup>; Scavizzi, Ferdinando<sup>3</sup>; Santos, Helder<sup>4</sup>; Mauro, Annunziata<sup>1</sup>; Barboni, Barbara<sup>1</sup>

<sup>1</sup>Unit of Basic and Applied Biosciences, Department of Biosciences, Agro-Food and Environmental Technologies, University of Teramo, Teramo, Italy; <sup>2</sup>Institute of Biochemistry and Cellular Biology (IBBC), Campus International Development (EMMAINFRACFRONTIER-IMPC), National Research Council (CNR), Monterotondo Scalo, Italy; <sup>3</sup>Tecniplast SpA, Buguggiate, Italy; <sup>4</sup>Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland

Digital Ventilated Cages (DVC) offer an innovative technology to obtain accurate movement data from a single mouse over time [1]. Thus, they could be used to determine the occurrence of a tendon damage event as well as inform on tissue regeneration [2,3]. Therefore, using the mouse model of tendon experimental damage, in this study it has been tested whether the recovery of tissue microarchitecture and of extracellular matrix (ECM) correlates with the motion data collected through this technology.

Mice models were used to induce acute injury in Achilles tendons (ATs), while healthy ones were used as control. During the healing process, the mice were housed in DVC cages (Tecniplast) to monitor animal welfare and to study biomechanics assessing movement activity, an indicator of the recovery of tendon tissue functionality. After 28 days, the AT were harvested and assessed for their histological and immunohistochemical properties to obtain a total histological score (TSH) that was then correlated to the movement data.

DVC cages showed the capacity to distinguish activity patterns in groups from the two different conditions. The data collected showed that the mice with access to the mouse wheel had a higher activity as compared to the blocked wheel group, which suggests that the extra movement during tendon healing improved motion ability. The histological results showed a clear difference between different analyzed groups. The bilateral free wheel group showed the best histological recovery, offering the highest TSH score, thus confirming the results of the DVC cages and the correlation between movement activity and structural recovery.

Data obtained showed a correlation between TSH and the DVC cages, displaying structural and movement differences between the tested groups. This successful correlation allows the usage of DVC type cages as a non-invasive method to predict tissue regeneration and recovery.

**Reference:** 1. Pernold K et.al –PLoS One(2019)14(2):e0211063.

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**Developing regeneration therapies for the intervertebral disc**Benjamin Gantenbein<sup>1</sup><sup>1</sup>University of Bern, Switzerland

Stem cell therapy for the intervertebral disc (IVD) is highly debated but holds great promises. From previous studies, it is known that notochordal cells are highly regenerative and may stimulate other differentiated cells to produce more matrix. Lately, a particular tissue-specific progenitor cell population has been identified in the centre of the intervertebral disc (IVD). The current hope is that these nucleus pulposus progenitor cells (NPPC) could play a particular role in IVD regeneration.

Current evidence confirms the presence of these cells in murine, canine, bovine and in the human fetal/surgical samples. Noteworthy, one of the main markers to identify these cells, i.e., Tie2, is a typical marker for endothelial cells. Thus, it is not very clear what their origin and their role might be in the context of developmental biology. In human surgical specimens, their presence is, even more, obscured depending on the donor's age and the condition of the IVD and other yet unknown factors.

Here, I revisit the recent literature on regenerative cells identified for the IVD in the past decades. Current evidence how these NPPC can be isolated and detected in various species and tissues will be recapitulated. Future directions will be provided on how these progenitor cells could be used for regenerative medicine and tissue engineering.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Therapeutic potential of mesenchymal stem cell-derived bioactive factors: impact on intervertebral disc degeneration**

Graciosa Teixeira<sup>1</sup>

<sup>1</sup>University of Ulm, Germany

Back pain is a leading cause of disability worldwide and it is primarily considered to be triggered by intervertebral disc (IVD) degeneration (IVDD). Current treatments may improve pain and mobility, but carry high costs and fail to address IVD repair or regeneration. As no effective therapeutic approach has been proposed to restore inflamed and degenerated IVDs, there is the urgent need to clarify the key pathomechanism of IVDD, the involvement of inflammation, particularly complement activation in matrix catabolism, and how to target them towards tissue repair/regeneration. Mesenchymal stem cell (MSC)-based therapies have become the focus of several regenerative IVD studies. Although patients in clinical trials reported less pain after cell therapy, the long-term success of cell engraftment is unclear due to the hostile IVD environment. The mechanism-of-action of MSCs is mostly dependent on the secreted soluble factors. Moreover, priming of MSC with interleukin (IL)-1 $\beta$  modulates the secretome content, improving its anti-inflammatory and regenerative effect on IVDD organ culture models. MSC-derived extracellular vesicles (EVs) have also been shown to modulate human IVD cells towards a healthy IVD phenotype in vitro. However, the mechanisms involved in the effect of secretome and EVs, particularly with regard to immunomodulation and matrix metabolism, are not fully understood. Our work investigates the effects of secretome and EVs secreted by IL-1 $\beta$ -primed MSCs to impair IVD matrix degradation and/or improve matrix formation in IVDD.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Simulation of physiological and detrimental loading in whole intervertebral disc organ models**

Sibylle Grad<sup>1</sup>

<sup>1</sup>AO Research Institute, Switzerland

Mechanical loading is important to maintain the homeostasis of the intervertebral disc (IVD) under physiological conditions but can also accelerate cell death and tissue breakdown in a degenerative state. Bioreactor loaded whole organ cultures are instrumental for investigating the effects of the mechanical environment on the IVD integrity and for preclinical testing of new therapies under simulated physiological conditions. Thereby the loading parameters that determine the beneficial or detrimental reactions largely depend on the IVD model and its preparation. Within this symposium we are discussing the use of bovine caudal IVD culture models to reproduce tissue inflammation or matrix degradation with or without bioreactor controlled mechanical loading. Furthermore, the outcome parameters that define the degenerative state of the whole IVD model will be outlined. Besides the disc height, matrix integrity, cell viability and phenotype expression, the tissue secretome can provide indications about potential interactions of the IVD with other cell types such as neurons. Finally, a novel multiaxial bioreactor setup capable of mimicking the six degrees-of-freedom loading environment of IVDs will be introduced that further advances the relevance of preclinical ex-vivo testing.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Comparison of degenerative MRI features of the intervertebral disc between those with and without chronic low back pain. An exploratory study of two large female populations using automated annotation**

Jeremy Fairbank<sup>1</sup>

<sup>1</sup>University of Oxford, United Kingdom

The relationship of degeneration to symptoms has been questioned. MRI detects apparently similar disc degeneration and degenerative changes in subjects both with and without back pain. We aimed to overcome these problems by re-annotating MRIs from asymptomatic and symptomatic groups onto the same grading system.

We analysed disc degeneration in pre-existing large MRI datasets. Their MRIs were all originally annotated on different scales. We re-annotated all MRIs independent of their initial grading system, using a verified, rapid automated MRI annotation system (SpineNet) which reported degeneration on the Pfirrmann (1-5) scale, and other degenerative features (herniation, endplate defects, marrow signs, spinal stenosis) as binary present/absent. We compared prevalence of degenerative features between symptomatics and asymptomatics.

Pfirrmann degeneration grades in relation to age and spinal level were very similar for the two independent groups of symptomatics over all ages and spinal levels. Severe degenerative changes were significantly more prevalent in discs of symptomatics than asymptomatics in the caudal but not the rostral lumbar discs in subjects < 60 years. We found high co-existence of degenerative features in both populations. Degeneration was minimal in around 30% of symptomatics < 50 years.

We confirmed age and disc level are significant in determining imaging differences between asymptomatic and symptomatic populations and should not be ignored. Automated analysis, by rapidly combining and comparing data from existing groups with MRIs and information on LBP, provides a way in which epidemiological and 'big data' analysis could be advanced without the expense of collecting new groups.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Building Vascularized Constructs from the Bottom-Up: Harnessing Microscale Living Materials in Tissue Engineering**Cristina Barrias<sup>1,2,3</sup>

<sup>1</sup>IS - Instituto de Inovação e Investigação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135, Porto, Portugal; <sup>2</sup>INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; <sup>3</sup>ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

Bottom-up tissue engineering (TE) strategies employing microscale living materials as building blocks provide a promising avenue for generating intricate 3D constructs resembling native tissues. These microtissue units exhibit high cell densities and a diverse extracellular matrix (ECM) composition, enhancing their biological relevance. By thoughtfully integrating different cell types, the establishment of vital cell-cell and cell-matrix interactions can be promoted, enabling the recreation of biomimetic micro-niches and the replication of complex morphogenetic processes. Notably, by co-assembling blood vessel-forming endothelial cells with supportive stromal cells, microtissues with stable capillary beds, referred to as vascular units (VUs), can be generated. Through a modular TE approach, these VUs can be further combined with other microtissues and biomaterials to construct large-scale vascularized tissues from the bottom up. Integration of VUs with technologies such as 3D bioprinting and microfluidics allows for the creation of structurally intricate and perfusable constructs. In this presentation, we will showcase examples of VUs and explore their applications in regenerative medicine and tissue modeling.

**Acknowledgements:** This work was supported by project EndoSWITCH (PTDC/BTM-ORG/5154/2020) funded by FCT (Portuguese Foundation for Science and Technology).

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Employing clinically-relevant transgenic animal models – Musculoskeletal regeneration in the PolgA mouse model of aging**Neashan Mathavan<sup>1</sup><sup>1</sup>Institute for Biomechanics, ETH Zurich

Aging impairs the regenerative capacity of musculoskeletal tissues and is associated with poor healing outcomes. PolgA<sup>D257A/D257A</sup> (PolgA) mice present a premature aging phenotype due to the accumulation of mitochondrial DNA (mtDNA) point mutations at rates 3 – 5 fold higher compared to wild type mice. Consequently, PolgA mice exhibit the premature onset of clinically-relevant musculoskeletal aging characteristics including frailty, osteo-sarcopenia, and kyphosis. I will present our recent findings on the use of PolgA mice to investigate the effects of aging on the regenerative capacity of bone. In particular, I will focus on the mechano-sensitivity of the regenerative process in aged bone environments and the opportunities it presents for clinical translation of mechanical intervention therapies.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Omics-based preclinical models of musculoskeletal regeneration**

Esther Wehrle<sup>1</sup>

<sup>1</sup>AO Research Institute Davos and Institute for Biomechanics, ETH Zurich

Despite the major advances in osteosynthesis after trauma, there remains a small proportion of patients (<10%) who exhibit delayed healing and/or eventual progression to non-union. While known risk factors exist, e.g. advanced age or diabetes, the exact molecular mechanism underlying the impaired healing is largely unknown and identifying which specific patient will develop healing complications is still not possible in clinical practice. The talk will cover our novel multimodal approaches in small animals, which have the potential to precisely capture and understand biological changes during fracture healing on an individual basis. Via combining emerging omics technologies with our recently developed femur defect loading equipment in mice, we provide a platform to precisely link mechanical and molecular analyses during fracture healing.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Masquelet technique using a mouse's femur critical-sized bone defect model  
- Characterization of macrophage expression in induced membrane**

Yota Kaneko<sup>1</sup>, Hiroaki Minehara<sup>2</sup>, Tatsuru Sonobe<sup>1</sup>, Takuya Kameda<sup>1</sup>, Miho Sekiguchi<sup>1</sup>, Takashi Matsushita<sup>2</sup>, Shinichi Konno<sup>1</sup>

<sup>1</sup>Department of Orthopaedic Surgery, Fukushima Medical University School of Medicine; <sup>2</sup>Department of Traumatology, Fukushima Medical University School of Medicine

The Masquelet technique is a variable method for treating critical-sized bone defects, but there is a need to develop a technique for promoting bone regeneration. In recent studies of bone fracture healing promotion, macrophage-mesenchymal stem cell (MSC) cross-talk has drawn attention. This study aimed to investigate macrophage expression in the induced membrane (IM) of the Masquelet technique using a mouse critical-sized bone defect model.

The study involved a 3-mm bone defect created in the femur of mice and fixed with a mouse locking plate. The Masquelet (M) group, in which a spacer was inserted, and the Control (C) group, in which the defect was left intact, were established. Additionally, a spacer was inserted under the fascia of the back (B group) to form a membrane due to the foreign body reaction. Tissues were collected at 1, 2, and 4 weeks after surgery (n=5 in each group), and immunostaining (CD68, CD163: M1, M2 macrophage markers) and RT-qPCR were performed to investigate macrophage localization and expression in the tissues.

The study found that CD68-positive cells were present in the IM of the M group at all weeks, and RT-qPCR showed the highest CD68 expression at 1 week. In addition, there was similar localization and expression of CD163. The C group showed lower expression of CD68 and CD163 than the M group at all weeks. The B group exhibited CD68-positive cells in the fibrous capsule and CD163-positive cells in the connective tissue outside the capsule, with lower expression of both markers compared to the M group at all weeks.

Macrophage expression in IM in M group had different characteristics compared to C group and B group. These results suggest that the IM differs from the fibrous capsules due to the foreign body reaction, and the macrophage-MSC cross-talk may be involved in Masquelet technique.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Electrowriting (cell-laden) natural-derived polymer fibers

Miguel Castilho<sup>1,2,3</sup>

<sup>1</sup>Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; <sup>2</sup>Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, the Netherlands; <sup>3</sup>Department of Orthopaedics, University Medical Centre Utrecht, Utrecht, the Netherlands

Orthopaedic soft tissues, such as tendons, ligaments, and articular cartilage, rely on their unique collagen fiber architectures for proper functionality. When these structures are disrupted in disease or fail to regenerate in engineered tissues, the tissues transform into dysfunctional fibrous tissues. Unfortunately, collagen synthesis in regenerating tissues is often slow, and in some cases, collagen fibers do not regenerate naturally after injury, limiting repair options. One of the research focuses of my team is to develop functional fiber replacements that can promote in vivo repair of musculoskeletal tissues throughout the body. In this presentation, I will discuss our recent advancements in electrowriting 3D printing of natural polymers for creating functional fiber replacements. This manufacturing process utilizes electrical signals to control the flow of polymeric materials through an extrusion nozzle, enabling precise deposition of polymeric fibers with sizes that cannot be achieved using conventional extrusion printing methods. Furthermore, it allows for the formation of fiber organizations that surpass the capabilities of conventional electrospinning processes. During the presentation, I will showcase examples of electrowritten microfiber scaffolds using various naturally-derived polymers, such as gelatin (a denatured form of collagen) and silk fibroin. I will discuss the functional properties of silk-based scaffolds and highlight how they exhibit restored  $\beta$ -sheet and  $\alpha$ -helix structures [1]. This restoration results in an elastic response of up to 20% deformation and the ability to withstand cyclic loading without plastic deformation. Additionally, I will present our latest results on the compatibility of this technique with patterning cell-laden fiber structures [2]. This novel biofabrication process allows for the printing of biomimetic microscale architectures with high cell viability, and offers a promising approach to understanding how shear and elongation forces influence cell development of hierarchical (collagen) fibers.

**References:** [1] Viola, et al bioRxiv 2023.06.01.542724; doi: <https://doi.org/10.1101/2023.06.01.542724>; [2] Castilho et al Biomacromolecules 2021, 22, 2, 855–866 <https://doi.org/10.1021/acs.biomac.0c01577>

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Controlling cell organization in wavy electrospun scaffolds for the regeneration of the anterior cruciate ligament**Sandra Camarero-Espinosa<sup>1,2</sup>

<sup>1</sup>BioSmarTE Lab, POLYMAT, University of Basque Country UPV/EHU, Donostia/San Sebastián 20018, Gipuzkoa, Spain; <sup>2</sup>IKERBASQUE, Basque Foundation for Science, 48009, Bilbao, Spain.

The anterior cruciate ligament (ACL) is the connective tissue located at the end of long bones providing stability to the knee joint. After tear or rupture clinical reconstruction of the tissue remains a challenge due to the particular mechanical properties required for proper functioning of the tissue. The outstanding mechanical properties of the ACL are characterized by a viscoelastic behavior responsible of the dissipation of the loads that are transmitted to the bone. These mechanical properties are the result of a very specialized graded extracellular matrix that transitions smoothly between the heterotypic cells, stiffness and composition of the ACL and the adjacent bone. Thus, mimicking the zonal biochemical composition, cellular phenotype and organization are key to reset the proper functioning of the ACL.

We have previously shown how the biochemical composition presented to cells in electrospun scaffolds results in haptokinesis, reverting contact-guidance effects.<sup>[1]</sup> Here, we demonstrate that contact guidance can also be disrupted by structural parameters in aligned wavy scaffolds. The presentation of a wavy fiber arrangement affected the cell organization and the deposition of a specific ECM characteristic of fibrocartilage. Cells cultured in wavy scaffolds grew in aggregates, deposited an abundant ECM rich in fibronectin and collagen II, and expressed higher amounts of collagen II, X and tenomodulin as compared to aligned scaffolds. In-vivo implantation in rabbits of triphasic scaffolds accounting for aligned-wavy-aligned zones showed a high cellular infiltration and the formation of an oriented ECM, as compared to traditional aligned scaffolds.<sup>[2]</sup>

27-29 SEPTEMBER | PORTO, PORTUGAL

## Combining Magnetically-Assisted and Matrix-Assisted 3D Bioprinting for Anisotropic Tissue Engineering

Syeda M. Bakht<sup>1,2</sup> Alberto Pardo<sup>1,2,3</sup>, Rui L. Reis<sup>1,2</sup>, Rui M. A. Domingues<sup>1,2</sup> and Manuela E. Gomes<sup>1,2</sup>

<sup>1</sup>3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciencia e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal; <sup>2</sup>ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal; <sup>3</sup>Colloids and Polymers Physics Group, Particle Physics Department and Health Research Institute, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

A major obstacle in biofabrication is replicating the organization of the extracellular matrix and cellular patterns found in anisotropic tissues within bioengineered constructs. While magnetically-assisted 3D bioprinting techniques have the potential to create scaffolds that mimic natural biological structures, they currently lack the ability to accurately control the dispersion of magnetic substances within the bioinks without compromising the fidelity of the intended composite. To overcome this dichotomy, the concepts of magnetically- and matrix-assisted 3D bioprinting are combined here. This method preserves the resolution of printed structures by keeping low viscosity bioinks uncrosslinked during printing, which allows for the arrangement of magnetically-responsive microfibers without compromising the structural integrity of the design. Solidification is induced after the microfibers are arranged in the desired pattern. Furthermore, the precise design of these magnetic microfillers permits the utilization of low levels of inorganic materials and weak magnetic field strengths, which reduces the potential risks that may be associated with their use. The effectiveness of this approach is evaluated in the context of tendon tissue engineering, and the results demonstrate that combining the tendons like anisotropic fibrous microstructure with remote magneto-mechanical stimulation during in vitro maturation provides both biochemical and biophysical cues that effectively guide human adipose-derived stem cells towards a tenogenic phenotype. In summary, the developed strategy allows the fabrication of anisotropic high-resolution magnetic composites with remote stimulation functionalities, opening new horizons for tissue engineering applications.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Post-printing chondrogenic differentiation of stem cell spheroid constructs for cartilage tissue engineering**Decarli M. C.<sup>1,2</sup>, Seijas-Gamardo, A.<sup>1</sup>, Morgan, F. L. C.<sup>1</sup>, Wieringa, P.<sup>1</sup>, Baker, M. B.<sup>1</sup>, Silva J.V.L.<sup>3</sup>, Moraes A.M.<sup>2</sup>, Lorenzo M.<sup>1</sup>, Mota C.<sup>1</sup>

<sup>1</sup>MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, The Netherlands; <sup>2</sup>School of Chemical Engineering, University of Campinas - UNICAMP, Cidade Universitária "Zeferino Vaz" – Campinas-SP / Brazil; <sup>3</sup>Three-Dimensional Technologies Research Group, CTI Renato Archer, Campinas-SP / Brazil.

Cartilage lesions often undergo irreversible progression due to low self-repair capability of this tissue. Tissue engineered approaches based in extrusion bioprinting of constructs loaded with stem cell spheroids may offer valuable alternatives for the treatment of cartilage lesions. Human mesenchymal stromal cell (hMSC) spheroids can be chondrogenically differentiated faster and more efficiently than single cells. This approach allows obtaining larger tissues in a rapid, controlled and reproducible way. However, it is challenging to control tissue architecture, construct stability, and cell viability during maturation. In this study we aimed at the development of a reproducible bioprinting process followed by post-bioprinting chondrogenic differentiation procedure using large quantities of hMSC spheroids encapsulated in a xanthan gum-alginate hydrogel. Multi-layered constructs were bioprinted, ionically crosslinked, and chondrogenically differentiated for 28 days. The expression of glycosaminoglycan, collagen II and IV were observed. After 56 days in culture, the bioprinted constructs were still stable and show satisfactory cell metabolic activity with profuse extracellular matrix production. These results showed a promising procedure to obtain 3D cartilage-like constructs that could be potential use as stable chondral tissue implants for future therapies.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **The Alliance for Advanced Therapies in Orthopaedics – AtiO**

Tobias Winkler<sup>1</sup>

<sup>1</sup>Charité – Berlin, BIH: Berlin Institute of Health ATiO Foundation

Years ago, we identified the need of a dedicated group and conference for advanced therapies with musculoskeletal indications. We saw a disconnect between high-level science and the criticality of actual medical need, thus creating a gap between research and industry – a gap that needed to be bridged.

To achieve this goal, a vehicle to connect and amplify the expertise of key opinion leaders in advanced therapies in orthopaedics was needed. With that purpose in mind and after years of preparation, the “Advanced Therapies in Orthopaedics Foundation” (ATiO) was established with the aim to create a network consisting of all important stake holders in the field, ranging from clinics & research, to corporates, finance and regulators – an Alliance for Advanced Therapies in Orthopaedics to form the future.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Advanced Therapies in Orthopaedics – A demanding market of the future**

Thomas Kluge<sup>1</sup>

<sup>1</sup>Heraeus Medical, ATiO Foundation, Germany

After initial hesitance and failures, with growing knowledge about advanced products and their characteristics, increasingly more medtech and also pharma companies enter the advanced therapies market. However, due to the specifics of the biology and regulation of advanced therapy products, a lot of new know-how is necessary to be successful in this highly promising field.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **HIPGEN – A phase III study on placental cell therapy for improving muscle regeneration in hip fracture patients**

Tobias Winkler<sup>1</sup>

<sup>1</sup>Charité – Berlin, BIH: Berlin Institute of Health ATiO Foundation

The HIPGEN study funded under EU Horizon 2020 (Grant 7792939) has the aim to investigate the potential of the first regenerative cell therapy for the improvement of recovery after muscle injury in hip fracture patients. For this aim we intramuscularly injected placental derived mesenchymal stromal cells during hip fracture arthroplasty. Despite not having reached the primary endpoint, which was the Short Physical Performance Battery, we could observe an increase in abductor muscle strength and a faster return to balance looking at symmetry in insole measurements during follow up.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Creating a new approach towards bone healing – can we derive advanced products from the immune system?**

Katharina Schmidt-Bleek<sup>1</sup>

<sup>1</sup>Charité – Berlin ATiO Foundation, Germany

Bone regeneration is a complex but very well organized process in which the immune system has a decisive role. The adaptive immune system and its experience level (percentage of effector and memory T cells) has been proven to influence the healing cascade especially in the early healing phases. This opens the possibility of an early intervention to enhance bone healing during the primary clinical treatment. Patients stratified for possible delayed bone healing could benefit from immunomodulatory treatment approaches. In pre-clinical studies cells and signaling molecules have been identified that could represent promising candidates to help patients in need.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **PROTO - Advanced PeRsOnalized Therapies for Osteoarthritis**

Tazio Maleitzke<sup>1</sup>

<sup>1</sup>Charité – Berlin ATiO Foundation, Germany

Osteoarthritis (OA) is the most common joint disease, affecting approximately 16% of the adult population worldwide. The chronic inflammation in the joint leads to the breakdown of cartilage, which leads to permanent pain and limitations in everyday life at an early stage of the disease. To date, there is no therapy that can interrupt the inflammatory state or reverse cartilage damage. The PROTO consortium (funded by the EU Horizon Europe program, Grant 101095635) aims to prevent the development of OA by correcting a pathological biomechanical pattern by a digital training intervention and to treat early stage OA with an innovative allogeneic cell therapy.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Application of Weightbearing CT in Corrective Osteotomies**

Arne Burssens<sup>1</sup>

<sup>1</sup>University Hospital of Ghent, Belgium

Osteotomies in the musculoskeletal system are joint preserving procedures to correct the alignment of the patient. In the lower limb, most of the pre-operative planning is performed on full leg weightbearing radiographs. However, these images contain a 2-dimensional projection of a 3-dimensional deformity, lack a clear visualization of the joint surface and are prone to rotational errors during patient positioning. Weightbearing CT imaging has demonstrated to overcome these shortcomings during the first applications of this device at level of the foot and ankle. Recent advances allow to scan the entire lower limb and novel applications at the level of the knee and hip are on the rise. Here, we will demonstrated the current techniques and 3-dimensional measurements used in supra- and inframalleolar osteotomies around the ankle. Several of these techniques will be transposed to other parts in the lower limb to spark future studies in this field.

**Traditional and novel three-dimensional Measurements using cone-beam CT in Weight-bearing conditions**Claudio Belvedere<sup>1</sup><sup>1</sup>Instituto Ortopedico Rizzoli, Bologna, Italy

3D accurate measurements of the skeletal structures of the foot, in physiological and impaired subjects, are now possible using Cone-Beam CT (CBCT) under real-world loading conditions. In detail, this feature allows a more realistic representation of the relative bone-bone interactions of the foot as they occur under patient-specific body weight conditions. In this context, varus/valgus of the hindfoot under altered conditions or the thinning of plantar tissues that occurs with advancing age are among the most complex and interesting to represent, and numerous measurement proposals have been proposed. This study aims to analyze and compare these measurements from CBCT in weight-bearing scans in a clinical population. Sixteen feet of diabetic patients and ten feet with severe adult flatfoot acquired before/after corrective surgery underwent CBCT scans (Carestream, USA) while standing on the leg of interest. Corresponding 3D shapes of each bone of the shank and hindfoot were reconstructed (Materialise, Belgium). Six different techniques found in the literature were used to calculate the varus/valgus deformity, i.e., the inclination of the hindfoot in the frontal plane of the shank, and the distance between the ground and the metatarsal heads was calculated along with different solutions for the identification of possible calcifications. Starting with an accurate 3D reconstruction of the skeletal structures of the foot, a wide range of measurements representing the same angle of hindfoot alignment were found, some of them very different from each other. Interesting correlations were found between metatarsal height and subject age, significant in diabetic feet for the fourth and fifth metatarsal bones. Finally, CBCT allows 3D assessment of foot deformities under loaded conditions. The observed traditional measurement differences and new measurement solutions suggest that clinicians should consider carefully the anatomical and functional concepts underlying measurement techniques when drawing clinical and surgical conclusions.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Diagnostic applications of Weightbearing CT per Anatomical Area

Jing Li<sup>1</sup>

<sup>1</sup>University Hospital of Ghent, Belgium

Applications of weightbearing computed tomography (WBCT) imaging in the foot and ankle have emerged over the past decade. However, the potential diagnostic benefits are scattered across the literature, and a concise overview is currently lacking. Therefore, we aimed to systematically review all reported diagnostic applications per anatomical region in the foot and ankle. A systematic literature search was performed in the electronic databases PubMed, EMBASE, Cochrane Library, and Web of Science. Search terms consisted of "weightbearing/standing CT and ankle, hind-, mid- or forefoot". English language studies analyzing the diagnostic applications of WBCT were included. Studies were excluded if they simulated weightbearing CT, described normal subjects, included cadaveric samples or samples were case reports. The modified Methodological Index for Non-Randomized Studies (MINORS) was applied for quality assessment. The added value was defined as the review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines and registered in the Prospero database (CRD42019106980). A total of 48 studies (prospective N=8, retrospective N=36, cohort study N=1, diagnostic N=2, prognostic comparative study N=1) were found to be eligible for review. The following diagnostic applications were identified per anatomical area in the foot: ankle (osteoarthritis N=5, ligament injury N=6); hindfoot (deformity N=9); midfoot (Lisfranc injury N=2, flatfoot deformity N=13, osteoarthritis N=1); forefoot (hallux valgus N=12). The identified studies contained diagnostic applications that could not be used on plain radiographs. The mean MINORS equaled 10.1 on a total of 16 (range: 8 to 12). Diagnostic applications of weightbearing CT imaging are most frequently studied in hindfoot deformity, but other area's areas are on the rise. Post-processing of images was identified as the main added value compared to WBRX. However, the findings should be interpreted with caution as the average quality score was moderate. Therefore, future prospective studies are warranted to consolidate the role of WBCT in diagnostic and therapeutic algorithms.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Effect of total ankle replacement on the 3-dimensional subtalar joint alignment in varus ankle osteoarthritis**Kvarda, P.<sup>1</sup>; Siegler, L.<sup>1</sup>; Burssens, A.<sup>2</sup>; Susdorf, R.<sup>1</sup>; Ruiz, R.<sup>1</sup>; Hintermann, B.<sup>1</sup><sup>1</sup>Department of Orthopaedics, Kantonsspital Baselland, Liestal, Switzerland;<sup>2</sup>Department of Orthopaedics, University of Gent, Gent, Belgium; <sup>1</sup>Department of Orthopaedics, Kantonsspital Baselland, Switzerland

Varus ankle osteoarthritis (OA) is typically associated with peritalar instability, which may result in altered subtalar joint position. This study aimed to determine the extent to which total ankle replacement (TAR) in varus ankle OA can restore the subtalar position alignment using 3-dimensional semi-automated measurements on WBCT. Fourteen patients (15 ankles, mean age 61) who underwent TAR for varus ankle OA were retrospectively analyzed using semi-automated measurements of the hindfoot based on pre- and postoperative weightbearing WBCT (WBCT) imaging. Eight 3-dimensional angular measurements were obtained to quantify the ankle and subtalar joint alignment. Twenty healthy individuals were served as a control groups and were used for reliability assessments. All ankle and hindfoot angles improved between preoperative and a minimum of 1 year (mean 2.1 years) postoperative and were statistically significant in 6 out of 8 angles ( $P < 0.05$ ). Values The post-op angles were in a similar range to as those of healthy controls were achieved in all measurements and did not demonstrated statistical difference ( $P > 0.05$ ). Our findings indicate that talus repositioning after TAR within the ankle mortise improves restores the subtalar position joint alignment within normal values. These data inform foot and ankle surgeons on the amount of correction at the level of the subtalar joint that can be expected after TAR. This may contribute to improved biomechanics of the hindfoot complex. However, future studies are required to implement these findings in surgical algorithms for TAR in presence of hindfoot deformity.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Applications of Weighbearing CT in Joint Instability

Matthias Peiffer<sup>1</sup>

<sup>1</sup>Research Fellow FARIL Massachusetts General Hospital Harvard Medical School

Acute syndesmotic ankle injuries continue to impose a diagnostic dilemma and it remains unclear whether weighbearing or external rotation should be exerted during the imaging process. Therefore, we aimed to implement both axial load (weighbearing) and external rotation in the assessment of a clinical cohort of patients with syndesmotic ankle injuries using weightbearing CT imaging. In this retrospective comparative cohort study, patients with an acute syndesmotic ankle injury were analyzed using a WBCT (N= 20; Mean age= 31,64 years; SD= 14,07. Inclusion criteria were an MRI confirmed syndesmotic ankle injury imaged by a bilateral WBCT of the ankle during weightbearing and combined weightbearing-external rotation. Exclusion criteria consisted of fracture associated syndesmotic ankle injuries. Three-dimensional (3D) models were generated from the CT slices. Tibiofibular displacement and Talar Rotation was quantified using automated 3D measurements (Anterior TibioFibular Distance (ATFD), Alpha Angle, Posterior TibioFibular Distance (PTFD) and Talar Rotation (TR) Angle) in comparison to a cohort of non-injured ankles. Results: The difference in neutral-stressed Alpha° and ATFD showed a significant difference between patients with a syndesmotic ankle lesion and healthy ankles (P = 0.046 and P = 0.039, respectively) The difference in neutral-stressed PTFD and TR° did not show a significant difference between patients with a syndesmotic ankle lesion and healthy ankles (P = 0.492 ; P = 0.152, respectively). Conclusion: Application of combined weightbearing-external rotation reveals a dynamic anterior tibiofibular widening in patients with syndesmotic ankle injuries. This study provides the first insights based on 3D measurements to support the potential relevance of applying external rotation during WBCT imaging. However, to what extent certain displacement patterns are associated with syndesmotic instability and thus require operative treatment strategies has yet to be determined in future studies.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Macromolecular crowding and other in vitro microenvironment modulators in tenocyte cultures**Dimitrios I. Zeugolis<sup>1</sup>

<sup>1</sup>Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Charles Institute of Dermatology, Conway Institute of Biomolecular & Biomedical Research and School of Mechanical & Materials Engineering, University College Dublin (UCD), Dublin, Ireland

The term macromolecular crowding is used to describe equilibria and kinetics of biochemical reactions and biological processes that occur via mutual volume exclusion of macromolecules in a highly crowded structureless medium. In vivo, the extracellular space is heavily crowded by a diverse range of macromolecules and thus, biological processes occur rapidly, whilst in vitro, in the absence of macromolecules, the same processes occur very slowly, if they are initiated at all (1-3). This talk will discuss the concept of macromolecular crowding, alone or in combination with other in vitro microenvironment modulators, in tendon engineering context.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Multilineage Differentiation of C2C12 Cells and Monocytes In Vitro within a 6-Bromoindirubin-3'-Oxime Incorporated Chitosan-Based Scaffold for Enhanced Bone Regeneration

Celine J. Agnes<sup>1</sup>, Monzur Murshed<sup>2,3</sup>, Bettina M. Willie<sup>1,2,3</sup>, Maryam Tabrizian<sup>1,2</sup>

<sup>1</sup>Department of Biological and Biomedical Engineering, McGill University, Montreal, Quebec, Canada; <sup>2</sup>Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada; <sup>3</sup>Shriner's Hospital for Children, Montreal, Quebec, Canada

Critical size bone defects deriving from large bone loss are an unmet clinical challenge<sup>1</sup>. To account for disadvantages with clinical treatments, researchers focus on designing biological substitutes, which mimic endogenous healing through osteogenic differentiation promotion. Some studies have however suggested that this notion fails to consider the full complexity of native bone with respect to the interplay between osteoclast and osteoblasts, thus leading to the regeneration of less functional tissue<sup>2</sup>. The objective of this research is to assess the ability of our laboratory's previously developed 6-Bromoindirubin-3'-Oxime (BIO) incorporated guanosine diphosphate crosslinked chitosan scaffold in promoting multilineage differentiation of myoblastic C2C12 cells and monocytes into osteoblasts and osteoclasts<sup>1, 3, 4</sup>. BIO addition has been previously demonstrated to promote osteogenic differentiation in cell cultures<sup>5</sup>, but implementation of a co-culture model here is expected to encourage crosstalk thus further supporting differentiation, as well as the secretion of regulatory molecules and cytokines<sup>2</sup>.

Biocompatibility testing of both cell types is performed using AlamarBlue for metabolic activity, and nucleic acid staining for distribution. Osteoblastic differentiation is assessed through quantification of ALP and osteopontin secretion, as well as osteocalcin and mineralization staining. Differentiation into osteoclasts is verified using SEM and TEM, qPCR, and TRAP staining.

Cellular viability of C2C12 cells and monocytes was maintained when cultured separately in scaffolds with and without BIO for 21 days. Both scaffold variations showed a characteristic increase in ALP secretion from day 1 to 7, indicating early differentiation but BIO-incorporated sponges yielded higher values compared to controls. SEM and TEM imaging confirmed initial aggregation and fusion of monocytes on the scaffold's surface, but BIO addition appeared to result in smoother cell surfaces indicating a change in morphology. Late-stage differentiation assessment and co-culture work in the scaffold are ongoing, but initial results show promise in the material's ability to support multilineage differentiation.

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27-29 SEPTEMBER | PORTO, PORTUGAL

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Calcium phosphates-based nanocoating in orthopaedics: influence of composition and substrate heating on films properties and BM-MSC behavior**

Montesissa M.<sup>1</sup>, Graziani G.<sup>2</sup>, Borciani G.<sup>1</sup>, Boi M.<sup>2</sup>, Rubini K.<sup>3</sup>, Valle F.<sup>4</sup>, Boanini E.<sup>3</sup> and Baldini N.<sup>1,2</sup>

<sup>1</sup>University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy; <sup>2</sup>IRCCS Istituto Ortopedico Rizzoli, Biomedical Science and Technologies and NanoBiotechnologies Lab, Bologna, Italy; <sup>3</sup>University of Bologna, Department of Chemistry "Giacomo Ciamician", Bologna, Italy; <sup>4</sup>Institute of Nanostructured Materials, National Research Council (ISMN-CNR), Bologna, Italy

Calcium phosphates-based (CaPs) nanocoatings on metallic prosthesis are widely studied in orthopedics and dentistry because they mimic the mineral component of native human bone and favor the osseointegration process. Despite the fact that different calcium phosphates have different properties (composition, crystallinity, and ion release), only stoichiometric hydroxyapatite (HA) films have been analyzed in deep. Here, we have realized films of different CaPs (HA, beta-tricalcium phosphate ( $\beta$ -TCP) and brushite (DCPD)) onto Ti6Al4V microrough substrates by Ionized Jet Deposition (IJD). We have implemented the heating of substrates at 400°C during deposition to see the effect on coating properties.

Different film features are evaluated: morphology and topography (FEG-SEM, AFM), physical-chemical composition (FT-IR and EDS), dissolution profile and adhesion to substrate (scratch test), with a focus on how the different CaPs and temperature changed the coating features. After coating optimization, we have studied the in vitro BM-MSC behavior, in term of viability and early adhesion.

We have obtained good transfer of fidelity in composition from target to coating for all CaPs, with nanostructured films formed by globular aggregates (~300 nm diameter), with homogeneous and uniform coverage of the substrate surface, without cracks. The heating during deposition has increased the adhesion of the films to the substrate, with higher stability in medium immersion and wettability, features that can improve the biological behavior of cells. All CaP coatings have showed excellent biocompatibility, with DCPD coating that promote higher cells viability at 14 days respect to HA and  $\beta$ -TCP films. About the early cell adhesion, the BM-MSC have showed switch from a globular to an elongated morphology at 6 hours in all coatings respect to the uncoated titanium, sign of better adhesion.

From these results, the fabrication of different CaP nanocoatings with IJD can be a promising for applications in orthopedics and dentistry.

**27-29 SEPTEMBER | PORTO, PORTUGAL****miRNA-laden magnetic-responsive bioink for tendon and enthesis tissue-engineering**

**Peniche Silva, C.J.**<sup>1</sup>, Dominguez R.<sup>2</sup>, Bakht S.M.<sup>2</sup>, Pardo A.<sup>2</sup>, Gonçalves A.I.<sup>2</sup>, Teixeira S.P.B.<sup>2</sup>, Balmayor E.R.<sup>3</sup>, Gomes M.E.<sup>2</sup>, van Griensven M.<sup>1</sup>

<sup>1</sup>cBITE, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands; <sup>2</sup>3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark - Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal; <sup>3</sup>Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany

Tendons and tendon-to-bone entheses don't usually regenerate after injury, and the hierarchical organization of such tissues makes them challenging sites of study for tissue engineers. In this study, we have tried a novel approach using miRNA and a bioactive bioink to stimulate the regeneration of the enthesis. microRNAs (miRNAs) are short, non-coding sequences of RNA that act as post-transcriptional regulators of gene and protein expression [1]. Mimics or inhibitors of specific miRNAs can be used to restore lost functions at the cell level or improve healing at the tissue level [2,3]. We characterized the healing of a rat patellar enthesis and found that miRNA-16-5p was upregulated in the fibrotic portion of the injured tissue 10 days after the injury. Based on the reported interactions of miRNA-16-5p with the TGF- $\beta$  pathway via targeting of SMAD3, we aimed to explore the effects of miRNA-16-5p mimics on the tenogenic differentiation of adipose-derived stem cells (ASCs) encapsulated in a bioactive bioink [4,5]. Bioinks with different properties are used for the 3D printing of biomimetic constructs. By integrating cells, materials, and bioactive molecules it is possible to tailor the regenerative capacity of the ink to meet the particular requirements of the tissue to engineer [5]. Here we have encapsulated ASCs in a gelatin-methacryloyl (GelMa) bioink that incorporates miR-16-5p mimics and magnetically responsive microfibers (MRFs). When the bioink is crosslinked in the presence of a magnetic field, the MRFs align unidirectionally to create an anisotropic construct with the ability to promote the tenogenic differentiation of the encapsulated ASCs. Additionally, the obtained GelMA hydrogels retained the encapsulated miRNA probes, which permitted the effective 3D transfection of the ASC and therefore, the regulation of gene expression, allowing to investigate the effects of the miR-16-5p mimics on the tenogenic differentiation of the ASCs in a biomimetic scenario.

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## 27-29 SEPTEMBER | PORTO, PORTUGAL

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**Kartogenin-encapsulated coaxial PGS/PCL aligned nanofibers for articular cartilage regeneration**

João C. Silva<sup>1,2</sup>, Ranodhi N. Udangawa<sup>3</sup>, Joaquim M. S. Cabral<sup>1,2</sup>, Frederico Castelo Ferreira<sup>1,2</sup> and Robert J. Linhardt<sup>3</sup>

<sup>1</sup>Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>2</sup>Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>3</sup>Department of Chemistry and Chemical Biology, Biological Sciences, Biomedical Engineering and Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA

Electrospinning is an advantageous technique for cartilage tissue engineering (CTE) applications due to its ability to produce nanofibers recapitulating the size and alignment of the collagen fibers present within the articular cartilage superficial zone. Moreover, coaxial electrospinning allows the fabrication of core-shell fibers able to encapsulate and release bioactive molecules in a sustained manner. Kartogenin (KTG) is a small heterocyclic molecule, which was demonstrated to promote the chondrogenic differentiation of human bone marrow-derived mesenchymal stem/stromal cells (hBMSCs)[1].

In this work, we developed and evaluated the biological performance of core-shell poly(glycerol sebacate)(PGS)/poly(caprolactone)(PCL) aligned nanofibers (core:PGS/shell:PCL) mimicking the native articular cartilage extracellular matrix(ECM) and able to promote the sustained release of the chondroinductive drug KTG[2].

The produced coaxial aligned PGS/PCL scaffolds were characterized in terms of their structure and fiber diameter, chemical composition, thermal properties, mechanical performance under tensile testing and in vitro degradation kinetics, in comparison to monoaxial PCL aligned fibers and respective non-aligned controls. KTG was incorporated into the core PGS solution to generate core-shell PGS-KTG/PCL nanofibers and its release kinetics was studied by HPLC analysis. KTG-loaded electrospun aligned scaffolds capacity to promote hBMSCs chondrogenic differentiation was evaluated by assessing cell proliferation, typical cartilage-ECM production (sulfated glycosaminoglycans(sGAG)) and chondrogenic marker genes expression in comparison to non-loaded controls. All the scaffolds fabricated showed average fiber diameters within the nanometer-scale and the core-shell structure of the fibers was clearly confirmed by TEM. The coaxial PGS-KTG/PCL nanofibers evidenced a more sustained drug release over 21 days. Remarkably, in the absence of the chondrogenic cytokine TGF- $\beta$ 3, KTG-loaded nanofibers promoted significantly the proliferation and chondrogenic differentiation of hBMSCs, as suggested by the increased cell numbers, higher sGAG amounts and up-regulation of the chondrogenic genes COL2A1, Sox9, ACAN and PRG4 expression. Overall, our results highlight the

**27-29 SEPTEMBER | PORTO, PORTUGAL**

potential of core-shell PGS-KTG/PCL aligned nanofibers for the development of novel MSC-based CTE strategies.

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**A Microphysiological System of Inflammation and Fibrosis in Tendon Fibrovascular Scar**Hani Awad<sup>1</sup><sup>1</sup>University of Rochester, USA

Vascular inflammation and activation of myofibroblasts are significant contributors to the progression of fibrosis, which can severely impair tissue function. In various tissues, including tendons, Transforming growth factor beta 1 (TGF- $\beta$ 1) has been identified as a critical driver of adhesion and scar formation. Nevertheless, the mechanisms that underlie fibrotic peritendinous adhesions are still not well comprehended, and human microphysiological systems to help identify effective therapies remain scarce. To address this issue, we developed a novel human Tendon-on-a-Chip (hToC), comprised of an endothelialized vascular compartment harboring circulating monocytes and separated by a 5  $\mu$ m/100 nm dual-scale ultrathin porous membrane from a type I/III collagen hydrogel with primary tendon fibroblasts and tissue-resident macrophages, all under defined serum-free conditions. The hToC models the crosstalk of the various cells in the system leading to the induction of inflammatory and fibrotic pathways including the activation of mTOR signaling. Consistent with phenotypes observed *in vivo* in mouse models and clinical human samples, we observed myofibroblast differentiation and senescence, tissue contraction, excessive extracellular matrix deposition, and monocytes' transmigration and macrophages' secretion of inflammatory cytokines, which were dependent on the presence of the endothelial barrier. This model offers novel insights on the role of vasculature in the pathophysiology of adhesions, which were previously underappreciated. Moreover, in testing whether the hToC could be used to evaluate efficacy of therapeutics, we were able to capture donor-specific variability in the response to Rapamycin treatment, which reduced myofibroblast activation regardless. Thus, our findings demonstrate the value of the hToC as a human microphysiological system for investigating the pathophysiology of fibrotic conditions in the context of peritendinous injury and similar fibrotic conditions, providing an alternative to animal testing.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Octacalcium Phosphate Embedded Hydrogels on 3D Printed Titanium Improve the Corrosion Performance in Simulated Biological Media

Aydin Bordbar Khiabani<sup>1</sup>, Ilijana Kovrlija<sup>2,3</sup>, Janis Locs<sup>2,3</sup>, Dagnija Loca<sup>2,3</sup>, Michael Gasik<sup>1</sup>

<sup>1</sup>Department of Chemical and Metallurgical Engineering, School of Chemical Engineering, Aalto University Foundation, 02150 Espoo, Finland; <sup>2</sup>Rudolfs Cimdinis Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia; <sup>3</sup>Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

Titanium alloys are one of the most used for orthopaedic implants and the fabrication of them by 3D printing technology is a raising technology, which could effectively resolve existing challenges. Surface modification of Ti surfaces is often necessary to improve biocorrosion resistance, especially in inflammatory conditions. Such modification can be made by coatings based on hydrogels, like alginate (Alg) - a naturally occurring anionic polymer. The properties of the hydrogel can be further enhanced with calcium phosphates like octacalcium phosphate (OCP) as a precursor of biologically formed hydroxyapatite. Formed Alg-OCP matrices have a high potential in wound healing, delivery of bioactive agents etc. but their effect on 3D printed Ti alloys performance was not well known.

In this work, Alg-OCP coated 3D printed samples were studied with electrochemical measurements and revealed significant variations of corrosion resistance vs. composition of the coating. The potentiodynamic polarization test showed that the Alg-OCP-coated samples had lower corrosion current density than simple Alg-coated samples. Electrochemical impedance spectroscopy indicated that OCP incorporated hydrogels had also a high value of the Bode modulus and phase angle. Hence Alg-OCP hydrogels could be highly beneficial in protecting 3D printed Ti alloys especially when the host conditions for the implant placement are inflammatory.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Enhancing cartilage tissue formation in GelMA/Alginate-Tyramine Interpenetrated Networks (IPNs) with Low Intensity Pulse Ultrasound Stimulation (LIPUS)**Garazi Larrañaga-Jaurrieta<sup>1,2</sup>, Ander Abarrategui<sup>2,3</sup>, Sandra Camarero-Espinosa<sup>1,3</sup>

<sup>1</sup>BioSmarTE Lab, POLYMAT, University of the Basque Country UPV/EHU, Donostia /San Sebastián 20018, Gipuzkoa, Spain; <sup>2</sup>Regenerative Medicine Lab, CICbiomaGUNE, Donostia/San Sebastián 20014, Gipuzkoa, Spain; <sup>3</sup>IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

In the native articular cartilage microenvironment, chondrocytes are constantly subjected to dynamic physical stimuli that maintains tissue homeostasis. They produce extra cellular matrix (ECM) components such as collagens (type II mainly, 50-75%), proteoglycans (10-30%) and other type of proteins<sup>1</sup>. While collagen offers a large resistance in tension, proteoglycans are the responsible of the viscoelastic response under compression due to the negative charge they confer to the ECM allowing it to entrap a large amount of interstitial fluid. In pathologic states (e.g. osteoarthritis), this ECM is degenerated and the negative charge becomes unbalanced, losing the chondroprotective properties and resulting on an overloaded chondrocytes that further degenerate the matrix.

Low-Intensity Pulsed Ultrasound Stimulation (LIPUS) has been used to generate acoustic (pressure) waves that create bubbles that collapse with cells, inducing a stimulus that can modulate cell response<sup>2</sup>. This mechanical stimulation promotes the expression of type II collagen, type X collagen, aggrecan and TGF- $\beta$ , appearing as a great strategy to regenerate cartilage. However, current strategies make use of extrinsic forces to stimulate cartilage formation overlooking the physico-chemical properties of the degenerated cartilage, resulting in an excessive load-transfer to chondrocytes and the consequent hypertrophy and degeneration.

Here, interpenetrated networks (IPNs) with different compositions were created using methacrylated gelatin (GelMA), to mimic the collagen, and alginate functionalized with tyramine (Alg-tyr) to mimic glycosaminoglycans and to introduce a negative charge in the model. Within the matrix chondrocytes were encapsulated and stimulated under different conditions to identify the ultrasound parameters that enhance tissue formation. Samples with and without stimulation were compared analysing the expression and deposition of collagen II, aggrecan, collagen X and TGF- $\beta$ . The results suggested that the chondrogenic marker expression of the samples stimulated for 10 minutes per day for 28 days, was two times higher overall in all of the cases, which was correlated to the tissue formation detected.

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# EORS 2023

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**Development of photo-crosslinkable decellularized extracellular matrix hydrogels for cartilage tissue engineering**Tosca Roncada<sup>1,2,4</sup>, Daniel J. Kelly<sup>1,2,3,4</sup>

<sup>1</sup>Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland; <sup>2</sup>Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland; <sup>3</sup>Department of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland; <sup>4</sup>Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland

Cartilage lacks the ability to self-repair when damaged, which can lead to the development of degenerative joint disease. Despite intensive research in the field of cartilage tissue engineering, there is still no regenerative treatment that consistently promotes the development of hyaline cartilage. Extracellular matrix (ECM) derived hydrogels have shown to support cell adhesion, growth and differentiation [1,2]. In this study, porcine articular cartilage was decellularized, solubilised and subsequently modified into a photo-crosslinkable methacrylated cartilage ECM hydrogel. Bone marrow derived mesenchymal stem/stromal cells (MSCs) were encapsulated into both methacrylated ECM hydrogels (ECM-MA) and gelatin methacryloyl (GelMA) as control hydrogel, and their chondrogenic potential was assessed using biochemical assays and histological analysis. We found that successful decellularization of the cartilage tissue could be achieved while preserving key ECM components, including collagen and glycosaminoglycans. A live-dead assay demonstrated good viability of MSCs within both GelMA and ECM-MA hydrogels on day 7. Large increases in sGAG accumulation was observed after 21 days of culture in chondrogenic media in both groups. Histological analysis revealed the presence of a more fibrocartilage tissue in the GelMA group, while cells embedded within the ECM-MA showed a round and chondrocytic-like morphology. Both groups stained positively for proteoglycans and collagen, with limited evidence of calcium deposition following Alizarin Red staining. These results show that ECM-MA hydrogels support a hyaline cartilage phenotype and robust cartilaginous matrix production. Future studies will focus on the printability of ECM-MA hydrogels to enable their use as bioinks for the biofabrication of functional tissues.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Near infrared-responsive hydrogels containing adenoviral vectors. Application in bone regeneration

MA Lerma-Juárez<sup>1,2</sup>, C. Escudero-Duch<sup>1,2</sup>, R. Serrano-Yamba<sup>1</sup>, A. Moreno-García<sup>1</sup>, C. Yus<sup>2,3,4</sup>, M. Arruebo<sup>2,3,4</sup>, N. Vilaboa<sup>1,2</sup>

<sup>1</sup>Hospital Universitario La Paz-IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain; <sup>2</sup>Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Spain; <sup>3</sup>Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain; <sup>4</sup>Departamento de Ingeniería Química, Universidad de Zaragoza, Zaragoza, Spain

We have developed plasmonic fibrin-based hydrogels that incorporate gold nanoparticles which transduce incident near-infrared (NIR) light into heat. Human adenovirus serotype type-5 vectors encoding a firefly luciferase (fLuc) coding sequence driven by a heat-inducible promoter were incorporated into the hydrogels. Transmission electronic microscopic analysis revealed that the adenoviral vectors were associated to the fibrin fibers. In vitro experiments in which human cells were cultured with plasmonic hydrogels showed that the adenoviral vectors can diffuse from the hydrogels, transduce the cells, and stimulate heat-induced transgene expression upon NIR irradiation. The hydrogels were implanted in 4.2 mm drill hole defects generated in the humerus of male rabbits. Three days after implantation, the defects were NIR-irradiated. Six h later, the animals were euthanized and samples from the bone defect zone were processed for immunohistochemical analyses using a specific fLuc antibody. The results showed strong expression of fLuc in tissues surrounding the implants of NIR-irradiated rabbits, while non-irradiated animals exhibited negligible expression. We next aimed to use the temperature increase to induce the production of transgenic bone morphogenetic protein 6 (BMP-6), using safe gene switches that can provide tighter control of in vivo transgene expression than heat-inducible promoters. These switches are only activated by heat in the presence of rapamycin and maintain a high level of targeted transgene expression for several days after heat activation. Adenoviral vectors encoding the safe switches that control the expression of BMP-6 were incorporated to the composites. The resulting NIR-responsive hydrogels were implanted in the bone defects generated in rabbits and used as a platform to transduce host cells, generate local hyperthermia and stimulate BMP-6 production.

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**Pathogenetic pathways and possible treatment options for non-union fractures**Martijn van Griensven<sup>1</sup>

<sup>1</sup>dept. cBITE, MERLN Institute, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht, the Netherlands

Bone regeneration is pivotal for the healing of fractures. In case this process is disturbed a non-union can occur. This can be induced by environmental factors such as smoking, overloading etc. Co-morbidities such as diabetes, osteoporosis etc. may be more intrinsic factors besides other disturbances in the process. Those pathways negatively influence the bone regeneration process. Several intrinsic signal transduction pathways (WNT, BMP etc.) can be affected. Furthermore, on the transcriptional level, important mRNA expression can be obstructed by deregulated miRNA levels. For instance, several miRNAs have been shown to be upregulated during osteoporotic fractures. They are detrimental for osteogenesis as they block bone formation and accelerate bone resorption. Modulating those miRNAs may revert the physiological homeostasis. Indeed, physiological fracture healing has a typical miRNA signature. Besides using molecular pathways for possible treatment of non-union fractures, providing osteogenic cells is another solution. In 5 clinical cases with non-union fractures with defects larger than 10 cm, successful administration of a 3D printed PCL-TCP scaffold with autologous bone marrow aspirate concentrate and a modulator of the pathogenetic pathway has been achieved. All patients recovered well and showed a complete union of their fractures within one year after start of the regenerative treatment.

Thus, non-union fractures are a diverse entity. Nevertheless, there seem to be common pathogenetic disturbances. Those can be counteracted at several levels from molecular to cell. Compositions of those may be the best option for future therapies. They can also be used in a more personalized fashion in case more specific measurements such as miRNA signature and stem cell activity are applied.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **The effect of immediate and delayed mechanical stimulation on secondary bone healing**

Jan Barcik<sup>1</sup>, Manuela Ernst<sup>1</sup>, Tim Buchholz<sup>1</sup>, Caroline Constant<sup>1</sup>, Karen Mys<sup>1</sup>, Devakar Epari<sup>2</sup>, Stephan Zeiter<sup>1</sup>, Boyko Gueorguiev<sup>1</sup>, Markus Windolf<sup>1</sup>

<sup>1</sup>AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos, Switzerland; <sup>2</sup>School of Mechanical, Medical and Process Engineering, Faculty of Engineering, Queensland University of Technology, 2 George Street, Brisbane, QLD 4000, Australia

Secondary bone healing is impacted by the extent of interfragmentary motion at the fracture site. It provides mechanical stimulus that is required for the formation of fracture callus. In clinical settings, interfragmentary motion is induced by physiological loading of the broken bone – for example, by weight-bearing. However, there is no consensus about when mechanical stimuli should be applied to achieve fast and robust healing response. Therefore, this study aims to identify the effect of the immediate and delayed application of mechanical stimuli on secondary bone healing. A partial tibial osteotomy was created in twelve Swiss White Alpine sheep and stabilized using an active external fixator that induced well-controlled interfragmentary motion in form of a strain gradient. Animals were randomly assigned into two groups which mimicked early (immediate group) and late (delayed group) weight-bearing. The immediate group received daily stimulation (1000 cycles/day) from the first day post-op and the delayed group from the 22nd day post-op. Healing progression was evaluated by measurements of the stiffness of the repair tissue during mechanical stimulation and by quantifying callus area on weekly radiographs. At the end of the five weeks period, callus volume was measured on the post-mortem high-resolution computer tomography (HRCT) scan. Stiffness of the repair tissue ( $p < 0.05$ ) and callus progression ( $p < 0.01$ ) on weekly radiographs were significantly larger for the immediate group compared to the delayed group. The callus volume measured on the HRCT was nearly 3.2 times larger for the immediate group than for the delayed group ( $p < 0.01$ ). This study demonstrates that the absence of immediate mechanical stimuli delays callus formation, and that mechanical stimulation already applied in the early post-op phase promotes bone healing.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Scoping Review of rotational guided growth in the growing bone**Ahmed Halloum<sup>1</sup>, Søren Kold<sup>1</sup>, Jan D. Rölfing<sup>2</sup>, Ahmed A. Abood<sup>1,3</sup>, Ole Rahbek<sup>1</sup>

<sup>1</sup>Interdisciplinary Orthopaedics, Aalborg University Hospital, Denmark; <sup>2</sup>Children's Orthopaedics and Reconstruction, Aarhus University Hospital, Denmark; <sup>3</sup>Orthopaedic Oncology and Reconstruction, Aarhus University Hospital, Denmark

The aim of this scoping review is to understand the extent and type of evidence in relation to the use of guided growth for correcting rotational deformities of long bones. Guided growth is routinely used to correct angular deformities in long bones in children. It has also been proven to be a viable method to correct rotational deformities, but the concept is not yet fully examined. Databases searched include Medline, Embase, Cochrane Library, Web of Science and Google Scholar.

All identified citations were uploaded into Rayyan.ai and screened by at least two reviewers. The search resulted in 3569 hits. 14 studies were included: 1 review, 3 clinical trials and 10 pre-clinical trials. Clinical trials: a total of 21 children (32 femurs and 5 tibiae) were included. Surgical methods were 2 cannulated screws connected by cable, PediPlates obliquely oriented, and separated Hinge Plates connected by FiberTape. Rotation was achieved in all but 1 child. Adverse effects reported include limb length discrepancy (LLD), knee stiffness and rebound of rotation after removal of tethers. 2 pre-clinical studies were ex-vivo studies, 1 using 8-plates on Sawbones and 1 using a novel z-shaped plates on human cadaver femurs. There were 5 lapine studies (2 using femoral plates, 2 using tibial plates and 1 using an external device on tibia), 1 ovine (external device on tibia), 1 bovine (screws and cable on metacarp) and a case-report on a dog that had an external device spanning from femur to tibia. Rotation was achieved in all studies. Adverse effects reported include implant extrusions, LLD, articular deformities, joint stiffness and rebound. All included studies conclude that guided growth is a viable treatment for rotational deformities of long bones, but there is great variation in models and surgical methods used, and in reported adverse effects.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Thermally disinfected human femoral head as scaffold for osteoinductive substances** **Elyarbek Tashmetov<sup>1</sup>, Dina Saginova<sup>2</sup>, Yevgeniy Kamyshanskiy<sup>3</sup>, Azim Saginov<sup>1</sup>, Amina Koshanova<sup>1</sup>**

<sup>1</sup>Department of surgical diseases, Karaganda Medical University, Karaganda 100000, Kazakhstan; <sup>2</sup>The Center for Applied Scientific Research, National Scientific center of Traumatology and Orthopaedics named after academician N.D.Batpenov, Astana 010000, Kazakhstan; <sup>3</sup>Pathology unit of the University Clinic, Karaganda Medical University, Karaganda 100000, Kazakhstan

Various approaches have been implemented to enhance bone regeneration, including the utilization of autologous platelet-rich plasma and bone morphogenetic protein-2. The objective of this study was to evaluate the impact of Marburg Bone Bank-derived bone grafts in conjunction with platelet-rich plasma (PRP), recombinant human bone morphogenetic protein-2 (rhBMP-2), and zoledronic acid (ZA) on osteogenesis within rabbit bone defects.

**Methodology:** Bone defects (5mm in diameter) were created in the femurs of 96 male rabbits. The animals were allocated into five groups: (1) bone graft + PRP (BG + PRP), (2) bone graft + 5µg rhBMP-2 (BG + rhBMP-2), (3) bone graft + 5µg ZA (BG + ZA), (4) bone graft + 10µg rhBMP-2 + 5µg ZA (BG + rhBMP-2 + ZA), and (5) bone graft (BG). Marburg Bone Bank-processed human femoral head allografts were utilized for bone grafting. The rabbits were euthanized at 14-, 30-, and 60-days post-surgery, and their femurs underwent histopathological and histomorphometric assessments.

**Results:** Histomorphometric analysis revealed significantly enhanced de novo osteogenesis within the bone allografts in the BG + PRP and BG + rhBMP-2 groups compared to the BG, BG + ZA, and BG + rhBMP-2 + ZA groups at 14 and 30 days ( $p < 0.05$ ). However, on day 60, the BG + rhBMP-2 group exhibited elevated osteoclastic activity (early resorption). The local co-administration of ZA with thermally treated grafts impeded both bone graft resorption and new bone formation within the bone defect across all time points. The addition of ZA to BG + rhBMP-2 resulted in diminished osteogenic activity compared to the BG + rhBMP-2 group ( $p < 0.000$ ).

**Conclusion:** The study findings indicated that the combination of PRP and rhBMP-2 with Marburg bone grafts facilitates early-stage osteogenesis in bone defect healing. Incorporating ZA into the thermally treated bone graft hinders both graft resorption and de novo bone formation.

**27-29 SEPTEMBER | PORTO, PORTUGAL****The Correlation between Electrical Impedance and Callus Quality. An In Vivo Study of Tibial Fractures in Rabbits**Markus Winther Frost<sup>1</sup>, Maria Tirta<sup>1</sup>, Ole Rahbek<sup>1</sup>, Laura Amalie Ryttoft<sup>1</sup>, Ming Ding<sup>2</sup>, Ming Shen<sup>3</sup>, Kirsten Duch<sup>4</sup>, Søren Kold<sup>1</sup><sup>1</sup>Department of Orthopaedics, Aalborg University Hospital, Aalborg, Denmark;<sup>2</sup>Department of Orthopaedic Surgery & Traumatology, Odense University Hospital, and Department of Clinical Research, University of Southern Denmark, Odense, Denmark;<sup>3</sup>Department of Electronic Systems, Aalborg University, Denmark, Aalborg, Denmark;<sup>4</sup>Unit of Clinical Biostatistics, Aalborg University Hospital, Aalborg, 9000, Denmark

Healing after bone fracture is assessed by frequent radiographs, which expose patients to radiation and lacks behind biological healing. This study aimed to investigate whether the electrical impedance using electrical impedance spectroscopy correlated to quantitative scores of bone healing obtained from micro-CT and mechanical bending test.

Eighteen rabbits were subjected to tibial fracture that was stabilized with external fixator. Two electrodes were positioned, one electrode placed within the medullary cavity and the other on the lateral cortex, both three millimeters from the fracture site. Impedance was measured daily across the fracture site at a frequency range of 5 Hz to 1 MHz. The animals were divided into three groups with different follow-up time: 1, 3 and 6 weeks for micro-CT (Bone volume/tissue volume (BV/TV, %)) and mechanical testing (maximum stress (MPa), failure energy (kJ/cm<sup>3</sup>), young modulus (Mpa)).

There was a statistically significant correlation between last measured impedance at 5 Hz frequency immediately prior to euthanasia and BV/TV of callus (-0.68, 95%CI: (-0.87; -0.31)). Considering the mechanical testing with three-point bending, no significant correlation was found between last measured impedance at 5 Hz frequency immediately prior to euthanasia and maximum stress (-0.35, 95%CI: (-0.70; 0.14)), failure energy (-0.23, 95%CI: (-0.63; 0.26)), or young modulus (-0.28, 95%CI: (-0.66; 0.22)).

The significant negative correlation between impedance and BV/TV might indicate that impedances correlate with the relative bone volume in the callus site. The lack of correlation between impedance and mechanical parameters when at the same time observing a correlation between impedance and days since operation (0-42 days), might indicate that the impedance can measure biological changes at an earlier time point than rough mechanical testing.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **microRNAs and their relevance in bone regeneration and disease**

Elizabeth Rosado Balmayor<sup>1,2</sup>, Virginie Joris<sup>3</sup>, Martijn van Griensven<sup>2,3</sup>

<sup>1</sup>Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany; <sup>2</sup>Rehabilitation Medicine Research Center, Mayo Clinic, Rochester, MN, USA; <sup>3</sup>cBITE, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

Bone tissue is known to possess an intrinsic regeneration potential. However, in cases of major injury, trauma, and disease, bone loss is present, and the regeneration potential of the tissue is often impaired. The process of bone regeneration relies on a complex interaction of molecules. MicroRNAs (miRNA) are small, non-coding RNAs that inhibit messenger RNAs (mRNA). One miRNA can inhibit several mRNAs and one mRNA can be inhibited by several miRNAs. Functionally, miRNAs regulate the entire proteome via the local inhibition of translation. In fact, miRNA modulation has been shown to be involved in several musculoskeletal diseases<sup>1</sup>. In those pathologies, they modulate the transcriptional activity of mRNAs important for differentiation, tissue-specific activity, extracellular matrix production, etc. Because of their function in inhibiting translation, miRNAs are being researched in many diseases and are already being used for interventional treatment<sup>2</sup>. Bone tissue and its related conditions have been widely investigated up to this day<sup>1,3</sup>. This talk will focus on the relevancy of miRNAs to bone tissue, its homeostasis, and disease. After, examples will be given of how miRNAs can be used in bone regeneration and diseases such as osteoporosis and osteosarcoma. The use of miRNAs in both, detection and therapy will be discussed.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****A novel non-coding RNA-based approach to modulate osteoclasts behavior**Sara R. Moura<sup>1,2,3</sup>, Jacob B. Olesen<sup>4,5</sup>, Mario A. Barbosa<sup>1,2,3</sup>, Kent Soe<sup>4,5</sup>, Maria Inês Almeida<sup>1,2,3</sup>

<sup>1</sup>ICBAS - Instituto de Ciências Biomédica Abel Salazar, University of Porto, Portugal; <sup>2</sup>i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal; <sup>3</sup>INEB – Instituto de Engenharia Biomédica, University of Porto, Portugal; <sup>4</sup>University of Southern Denmark, Denmark; <sup>5</sup>Odense University Hospital, Denmark

Osteoclasts (OCs) are multinucleated cells that play a pivotal role in skeletal development and bone remodeling. Abnormal activation of OCs contributes to the development of bone-related diseases, such as osteoporosis, bone metastasis and osteoarthritis. Restoring the normal function of OCs is crucial for bone homeostasis. Recently, RNA therapeutics emerged as a new field of research for osteoarticular diseases.

The aim of this study is to use non-coding RNAs (ncRNAs) to molecularly engineer OCs and modulate their function. Specifically, we investigated the role of the microRNAs (namely miR-16) and long ncRNAs (namely DLEU1) in OCs differentiation and fusion. DLEU1/DLEU2 region, located at chromosome 13q14, also encodes miR-15 and miR-16. Our results show that levels of these ncRNA transcripts are differently expressed at distinct stages of the OCs differentiation. Specifically, silencing of DLEU1 by small interfering RNAs (siDLEU1) and overexpression of miR-16 by synthetic miRNA mimics (miR-16-mimics) led to a significant reduction in the number of OCs formed per field (OC/field), both at day 5 and 9 of the differentiation stage. Importantly, time-lapse analysis, used to track OCs behavior, revealed a significant decrease in fusion events after transfection with siDLEU1 or miR-16-mimics and an alteration in the fusion mode and partners. Next, we investigated the migration profile of these OCs, and the results show that only miR-16-mimics-OCs, but not siDLEU-OCs, have a lower percentage of immobile cells and an increase in cells with mobile regime, compared with controls. No differences in cell shape were found. Moreover, mass-spectrometry quantitative proteomic analysis revealed independent effects of siDLEU1 and miR-16-mimics at the protein levels. Importantly, DLEU1 and miR-16 act by distinct processes and pathways. Collectively, our findings support the ncRNAs DLEU1 and miR-16 as therapeutic targets to modulate early stages of OCs differentiation and, consequently, to impair OC fusion, advancing ncRNA-therapeutics for bone-related diseases.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **MicroRNAs 125b, 199a-5p and 214 as modulators of bone homeostasis**

Virginie Joris<sup>1</sup>, Elizabeth R. Balmayor<sup>2</sup>, Martijn van Griensven<sup>1</sup>

<sup>1</sup>Dept. cBITE, MERLN Institute, Maastricht University, Maastricht, the Netherlands;

<sup>2</sup>Experimental Orthopaedics and Trauma Surgery, Clinic for Orthopaedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen, Germany

Bone homeostasis is a highly regulated process involving pathways in bone as WNT, FGF or BMP, but also requiring support from surrounding tissues as vessels and nerves. In bone diseases, the bone-vessel-nerve triad is impacted. Recently, new players appeared as regulators of bone homeostasis: microRNAs (miRNA). Five miRNAs associated with osteoporotic fractures are already known, among which miR-125b is decreasing bone formation by downregulating human mesenchymal stem cells (hMSCs) differentiation. Other miRNAs, as miR-214 (in cluster with miR-199a), are secreted by osteoclasts to regulate osteoblasts and inhibit bone formation. This forms a very complex regulatory network.

hMSCs and osteoblasts (n=3) were transfected with mimic/antagomiR of miR-125b, miR-199a-5p or miR-214, or with a scrambled miRNA (negative control) in osteogenic differentiation calcium-enriched medium (Ca<sup>++</sup>). Mineralization was assessed by Alizarin Red/CPC staining, miRNA expression by qPCR and protein by western blotting. Exposure of hMSCs or osteoblasts to Ca<sup>++</sup> increased mineralization compared to basal medium. hMSCs transfected with miR-125b mimic in Ca<sup>++</sup> presented less mineralization compared to scramble. This correlated with decreased levels of BMPR2 and RUNX2. hMSCs transfected with miR-125b inhibitor presented higher mineralization. Interestingly, hMSCs transfected with miR-214 mimic in Ca<sup>++</sup> presented no mineralization while miR-214 inhibitor increased mineralization. No differences were observed in hMSCs transfected with miR-199a-5p modulators. On the contrary, osteoblasts transfected with miR-199a-5p mimic present less mineralization than scrambled-transfected and same was observed for miR-214 and miR-125b mimics.

We highlight that miR-125b and miR-214 decrease mineralization of hMSCs in calcium-enriched medium. We noticed that miR-199a-5p is able to regulate mineralization in osteoblasts but not in hMSCs suggesting that this effect is cell-specific. Interestingly, the cluster miR-199a/214 is known as modulator of vascular function and could thus contribute to bone remodeling via different ways. With this work we slightly open the door to possible therapeutic approaches for bone diseases.

**Transfection of hMSCs with chemically modified mRNA coding for BMP-7 enhances osteogenesis**

Claudia Del Toro Runzer<sup>1</sup>, Joanna Sadowska<sup>2</sup>, Christian Plank<sup>3</sup>, Fergal J. O'Brien<sup>2</sup>, Martijn van Griensven<sup>1,4</sup>, Elizabeth R. Balmayor<sup>4,5</sup>

<sup>1</sup>Dept. cBITE, MERLN Institute, Maastricht University, Maastricht, the Netherlands; <sup>2</sup>Tissue Engineering Research Group, dept. of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland; <sup>3</sup>Ethris GmbH, Planegg, Germany; <sup>4</sup>Musculoskeletal Gene Therapy Group, Mayo Clinic, Rochester, MN, USA; <sup>5</sup>Experimental Orthopaedics and Trauma Surgery, RWTH Aachen University Hospital, Aachen, Germany

Bone morphogenetic proteins (BMPs) have been widely investigated for treating non-healing fractures. They participate in bone reconstruction by inducing osteoblast differentiation, and osteoid matrix production.<sup>1</sup> The human recombinant protein of BMP-7 was among the first growth factors approved for clinical use. Despite achieving comparable results to autologous bone grafting, severe side effects have been associated with its use.<sup>2</sup> Furthermore, BMP-7 was removed from the market.<sup>3</sup> These complications are related to the high doses used (1.5-40 milligrams per surgery)<sup>2</sup> compared to the physiological concentration of BMP in fracture healing (in the nanogram to picogram per milliliter range).<sup>4</sup> In this study, we use transcript therapy to deliver chemically modified mRNA (cmRNA) encoding BMP-7. Compared to direct use of proteins, transcript therapy allows the sustained synthesis of proteins with native conformation and true post-translational modifications using doses comparable to the physiological ones.<sup>5</sup> Moreover, cmRNA technology overcomes the safety and affordability limitations of standard gene therapy i.e. pDNA.<sup>6</sup> BMP-7 cmRNA was delivered using Lipofectamine™ MessengerMAX™ to human mesenchymal stromal cells (hMSCs). We assessed protein expression and osteogenic capacity of hMSCs in monolayer culture and in a house-made, collagen hydroxyapatite scaffold. Using fluorescently-labelled cmRNA we observed an even distribution after loading complexes into the scaffold and a complete release after 3 days. For both monolayer and 3D culture, BMP-7 production peaked at 24 hours post-transfection, however cells transfected in scaffolds showed a sustained expression. BMP-7 transfected hMSCs yielded significantly higher ALP activity and Alizarin red staining at later timepoints compared to the untransfected group. Interestingly, BMP-7 cmRNA treatment triggered expression of osteogenic genes like OSX, RUNX-2 and OPN, which was also reflected in immunostainings. This work highlights the relevance of cmRNA technology that may overcome the shortcomings of protein delivery while circumventing issues of traditional pDNA-based gene therapy for bone regeneration.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Precision Magnetoplexes for microRNA Delivery Targeting Tendon Inflammation**Ana F. Almeida<sup>1</sup>, Margarida S. Miranda<sup>1</sup>, Lindsay A.N. Crowe<sup>2</sup>, Moeed Akbar<sup>2</sup>, Márcia T. Rodrigues<sup>1</sup>, Neal L. Millar<sup>2</sup>, Manuela E. Gomes<sup>1</sup>

<sup>1</sup>3B's Research Group, 13Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal; ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal; <sup>2</sup>School of Infection and Immunity, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow, UK.

MicroRNA (miR) delivery to regulate chronic inflammation hold extraordinary promise, with new therapeutic possibilities emanating from their ability to fine-tune multiple target gene regulation pathways which is an important factor in controlling aberrant inflammatory reactions in complex multifactorial disease. However, several hurdles have prevented advancements in miR-based therapies. These include off-target effects of miRs, limited trafficking, and inefficient delivery. We propose a magnetically guided nanocarrier to transport therapeutically relevant miRs to assist self-resolving inflammation processes at injury sites and reduce the impact of chronic inflammation-related diseases such as tendinopathies. The high prevalence, significant socio-economic burden and increasing recognition of dysregulated immune mediated pathways in tendon disease provide a compelling rationale for exploring inflammation-targeting strategies as novel treatments in this condition. By combining cationic polymers, miR species (e.g., miR 29a, miR155 antagonist), and magnetic nanoparticles in the form of magnetoplexes with highly efficient magnetofection procedures, we developed inexpensive, easy-to-fabricate, and biocompatible systems with competent miR-binding and fast cellular uptake into different types of human cells, namely macrophages and tendon-derived cells. The system was shown to be cell-compatible and to successfully modulate the expression and production of inflammatory markers in tendon cells, with evidence of functional pro-healing changes in immune cell phenotypes. Hence, magnetoplexes represent a simple, safe, and non-viral nanoplatform that enables contactless miR delivery and high-precision control to reprogram cell profiles toward improved pro-regenerative environments.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Leveraging the Hedgehog Signaling Pathway to Improve Tendon-to-Bone Integration

Kamalitdinov T<sup>1</sup>, Fujino K<sup>1</sup>, Jiang X<sup>1</sup>, Madi R<sup>1</sup>, Marcelin J<sup>1</sup>, Kuntz A<sup>1</sup>, Dyment N<sup>1</sup>

<sup>1</sup>McKay Orthopaedic Research Laboratory, Dept. of Orthopaedic Surgery, University of Pennsylvania, USA

Despite extensive research aimed at improving surgical outcomes of enthesis injuries, re-tears remain a common problem, as the repairs often lead to fibrovascular scar as opposed to a zonal enthesis. Zonal enthesis formation involves anchoring collagen fibers, synthesizing proteoglycan-rich fibrocartilage, and mineralizing this fibrocartilage [1]. During development, the hedgehog signaling pathway promotes the formation and maturation of fibrocartilage within the zonal tendon-to-bone enthesis [1-4]. However, whether this pathway has a similar role in adult zonal tendon-to-bone repair is not known. Therefore, we developed a murine anterior cruciate ligament (ACL) reconstruction model [5] to better understand the zonal tendon-to-bone repair process and perturb key developmental regulators to determine the extent to which they can promote successful repair in the adult. In doing so, we activated the hedgehog signaling pathway both genetically using transgenic mice and pharmacologically via agonist injections. We demonstrated that both treatments improved the formation of zonal attachments and tunnel integration strength [6]. These improved outcomes were due in part to hedgehog signaling's positive role in proliferation of the bone marrow stromal cell (bMSC) progenitor pool and subsequent fibrocartilage production of bMSC progeny cells that form the attachments. These results suggest that, similar to growth and development, hedgehog signaling promotes the production and maturation of fibrocartilage during tendon-to-bone integration in adults. Lastly, we developed localized drug delivery systems to further improve the treatment of these debilitating injuries in future translational studies.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Does Leucocyte Rich and Poor Platelet Rich Plasma differ by their Metabolites?**

Bilge Basak Fidan<sup>1</sup>, Ilayda Demirdis<sup>2</sup>, Emine Çiftçi<sup>3</sup>, Hakan Aydınli<sup>4</sup>, Ozan Kaplan<sup>1</sup>, Mustafa Çelebier<sup>1</sup>, Özge Boyacioglu<sup>5</sup>, Petek Korkusuz<sup>6</sup>, Yigitcan Karanfil<sup>3</sup>, Feza Korkusuz<sup>3</sup>

<sup>1</sup>Hacettepe University Faculty of Pharmacy, Department of Analytical Chemistry; <sup>2</sup>Institute of Sciences, Department of Biology and Molecular Biology; <sup>3</sup>Faculty of Medicine, Department of Sports Medicine; <sup>4</sup>Faculty of Medicine; <sup>5</sup>Graduate School of Science and Engineering, Department of Bioengineering and Atilim University, Faculty of Medicine, Department of Medical Biochemistry; <sup>6</sup>Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, Ankara 06230, Turkey

Platelet Rich Plasma (PRP), either rich (L-PRP) or poor (P-PRP) of leukocytes, is frequently used as an anti-inflammatory and regenerative tool in osteoarthritis (OA). PRP contains proteins but not genes as it is derived from megakaryocytes. Proteomics but not metabolomics of PRP was recently studied. Metabolomics is a field of 'omics' research involved in comprehensive portrayal of the small molecules, metabolites, in the metabolome. These small molecules can be endogenous metabolites or exogenous compounds found in an organism (1). Our aim was to determine the difference between L-PRP and P-PRP.

A cross-sectional clinical study was designed in six recreational male athletes between the ages of 18 and 35 years. 3 mL P-PRP and 3 mL -LPRP was prepared from 60 mL of venous blood after treating with 9 mL of sodium citrate and centrifugation at 2.700 rpm for 10 min. Half of the prepared PRP's were frozen at -20°C for a week. Fresh and frozen samples were analyzed at the Q-TOF LC/MS device after thawing to room temperature.

Untargeted metabolomic results revealed that the metabolomic profile of the L-PRP and P-PRP were significantly different from each other. A total of 33.438 peaks were found. Statistically significant ( $p < 0.05$ ) peaks were uploaded to the MetaboAnalyst 5.0 platform. Exogenous out of 2.308 metabolites were eliminated and metabolites found significant for our study were subjected to pathway analysis. Steroid biosynthesis, sphingolipid metabolism and metabolism of lipid pathways were affected. In the L-PRP samples, Nicotinamide riboside (FC: 2.2), MHPG (FC: 3.0), estrone sulfate (FC: 7.5), thiamine diphosphate (FC: 2.0), leukotriene E4 (FC: 7.5), PC(18:1 (9Z)e/2:0) (FC: 9.8) and Ap4A (FC: 2.1) were higher compared to P-PRP. C24 sulfatide (FC: -11.8), 3-hexaprenyl-4,5-dihydroxybenzoic acid (FC: -2.8) metabolites were furthermore lower in P-PRP. Clinical outcomes of PRP application should consider these metabolic pathways in future studies (2).

# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**The metabolic role of IL-4 and IL-10 in Intervertebral Disc Degeneration**

Paola Bermudez-Lekerika<sup>1,2</sup>, Sofia Tseranidou<sup>3</sup>, Exarchos Kanelis<sup>4,5</sup>, Katherine B. Crump<sup>1,2</sup>, Christine Le Maitre<sup>6</sup>, Karin Wuertz-Kozak<sup>7</sup>, Leonidas G. Alexopoulos<sup>4,5</sup>  
Jérôme Noailly<sup>3</sup> and Benjamin Gantenbein<sup>1,2</sup>

<sup>1</sup>Tissue Engineering for Orthopaedics and Mechanobiology, Bone & Joint Program, Department for BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, Switzerland; <sup>2</sup>Department of Orthopaedic Surgery & Traumatology, Inselspital, University of Bern, Switzerland. <sup>3</sup>BCN MedTech (Universitat Pompeu Fabra), Spain <sup>4</sup>Protavio Ltd, Agia Paraskevi, Greece; <sup>5</sup>School of Mechanical Engineering, National Technical University of Athens, Zografou, Greece; <sup>6</sup>Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, United Kingdom; <sup>7</sup>Department of Biomedical Engineering, Rochester Institute of Technology, Rochester, United States

Intervertebral disc (IVD) degeneration is a pathological process often associated with chronic back pain and considered a leading cause of disability worldwide<sup>1</sup>. During degeneration, progressive structural and biochemical changes occur, leading to blood vessel and nerve ingrowth and promoting discogenic pain<sup>2</sup>. In the last decades, several cytokines have been applied to IVD cells *in vitro* to investigate the degenerative cascade. Particularly, IL-10 and IL-4 have been predicted as important anabolic factors in the IVD according to a regulatory network model based in silico approach<sup>3</sup>. Thus, we aim to investigate the potential presence and anabolic effect of IL-10 and IL-4 in human NP cells (*in vitro*) and explants (*ex vivo*) under hypoxia (5% O<sub>2</sub>) after a catabolic induction.

Primary human NP cells were expanded, encapsulated in 1.2% alginate beads (4 x 10<sup>6</sup> cells/ml) and cultured for two weeks in 3D for phenotype recovery while human NP explants were cultured for five days. Afterwards, both alginate and explant cultures were i) cultured for two days and subsequently treated with 10 ng/ml IL-10 or IL-4 (single treatments) or ii) stimulated with 0.1 ng/ml IL-1 $\beta$  for two days and subsequently treated with 10 ng/ml IL-10 or IL-4 (combined treatments).

The presence of IL-4 receptor, IL-4 and IL-10 was confirmed in human intact NP tissue (Fig 1). Additionally, IL-4 single and combined treatments induced a significant increase of proinflammatory protein secretion *in vitro* (Fig. 2A-C) and *ex vivo* (Fig. 2D and E). In contrast, no significant differences were observed in the secretome between IL-10 single and combined treatments compared to control group.

Overall, IL-4 containing treatments promote human NP cell and explant catabolism in contrast to previously reported IL-4 anti-inflammatory performance<sup>4</sup>. Thus, a possible pleiotropic effect of IL-4 could occur depending on the IVD culture and environmental condition.

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# EORS 2023

31st Annual Meeting of the  
European Orthopaedic Research Society

**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Chronic High-Fat Diet Consumption Accelerates Osteoblast Dysfunction and Osteoporosis by Gut Microbial Metabolite Trimethylamine-N-Oxide**Yu-Han Lin<sup>1</sup>, Wei-Shiung Lian<sup>1,2</sup>, Yu-Shan Chen<sup>2</sup>, Holger Jahr<sup>3</sup>, Feng-Sheng Wang<sup>1,2</sup>

<sup>1</sup>Center for Mitochondrial Research and Medicine, Kaohsiung Chang Gung Memorial Hospital, Taiwan; <sup>2</sup>Core Laboratory for Phenomics and Diagnostic, Kaohsiung Chang Gung Memorial Hospital, Taiwan; <sup>3</sup>Department of Anatomy and Cell Biology, University Hospital RWTH Aachen, Germany

Obesity is correlated with the development of osteoporotic diseases. Gut microbiota-derived metabolite trimethylamine-n-oxide (TMAO) accelerates obesity-mediated tissue deterioration. This study was aimed to investigate what role TMAO may play in osteoporosis development during obesity.

Mice were fed with high-fat diet (HFD; 60 kcal% fat) or chow diet (CD; 10 kcal% fat) or 0.2% TMAO in drinking water for 6 months. Body adiposity and bone microstructure were investigated using  $\mu$ CT imaging. Gut microbiome and serum metabolome were characterized using 16S rRNA sequencing and liquid chromatography-tandem mass spectrometry. Osteogenic differentiation of bone-marrow mesenchymal cells was quantified using RT-PCR and von Kossa staining. Cellular senescence was evaluated by key senescence markers p16, p21, p53, and senescence association  $\beta$ -galactosidase staining.

HFD-fed mice developed hyperglycemia, body adiposity and osteoporosis signs, including low bone mineral density, sparse trabecular microarchitecture, and decreased biomechanical strength. HFD consumption induced gut microbiota dysbiosis, which revealed a high Firmicutes/Bacteroidetes ratio and decreased  $\alpha$ -diversity and abundances of beneficial microorganisms Akkermansiaceae, Lactobacillaceae, and Bifidobacteriaceae. Serum metabolome uncovered increased serum L-carnitine and TMAO levels in HFD-fed mice. Of note, transplantation of fecal microbiota from CD-fed mice compromised HFD consumption-induced TMAO overproduction and attenuated loss in bone mass, trabecular microstructure, and bone formation rate. TMAO treatment inhibited trabecular and cortical bone mass and biomechanical characteristics; and repressed osteogenic differentiation capacity of bone-marrow mesenchymal cells. Mechanistically, TMAO accelerated mitochondrial dysfunction and senescence program, interrupted mineralized matrix production in osteoblasts.

Gut microbial metabolite TMAO induced osteoblast dysfunction, accelerating the development of obesity-induced skeletal deterioration. This study, for the first time, conveys a productive insight into the catabolic role of gut microflora metabolite TMAO in regulating osteoblast activity and bone tissue integrity during obesity.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **STAT3 activation in the synovial tissues from the hip joint in the early stage of rapidly destructive coxopathy**

Tadashi Yasuda<sup>1</sup>, Shigeo Hara<sup>1</sup>, Shinnosuke Yamashita<sup>1</sup>, Sadaki Mitsuzawa<sup>1</sup>, Yoshihiro Tsukamoto<sup>1</sup>, Hisataka Takeuchi<sup>1</sup>, Satoshi Ota<sup>1</sup>, and Eijiro Onishi<sup>1</sup>

<sup>1</sup>Kobe City Medical Center General Hospital, Kobe, Japan

The interleukin-6/gp130-associated Janus Kinases/STAT3 axis is known to play an important role in mediating inflammatory signals, resulting in production of matrix metalloproteinase-3 (MMP-3). The hip joints with rapidly destructive coxopathy (RDC) demonstrate rapid chondrolysis, probably by increased production of MMP-3 observed in the early stage of RDC. In the recent study, no apparent activation of STAT3 has been shown in the synovial tissues obtained from the osteoarthritic joint at operation. However, no data are currently available on STAT3 activation in the synovial tissues in the early stage of RDC. This study aimed to elucidate STAT3 activation in the synovial tissues in the early stage of RDC. Synovial tissues within 7 months from the disease onset were obtained from four RDC patients with femoral head destruction and high serum levels of MMP-3. RDC synovial tissues showed the synovial lining hyperplasia with an increase of CD68-positive macrophages and CD3-positive T lymphocytes. STAT3 phosphorylation was found in the synovial tissues by immunohistochemistry using anti-phospho-STAT3 antibody. The majority of phospho-STAT3-positive cells were the synovial lining cells and exhibited negative expression of macrophage or T cell marker. Treatment with tofacitinib, a Janus Kinase inhibitor, resulted in a decrease in phospho-STAT3-positive cells, especially with high intensity, indicating effective suppression of STAT3 activation in RDC synovial tissues. Inhibitory effect of tofacitinib could act through the Janus Kinase/STAT3 axis in the synovial tissues in the early stage of RDC. Therefore, STAT3 may be a potential therapeutic target for prevention of joint structural damage in RDC.

**Acknowledgements:** This study was supported by Katakami Foundation for Clinical Research.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Impact of Mincing Techniques on Chondrocyte Viability in Bovine Articular Cartilage: A Comparative Study between Commercially Available Shavers and Scalpel Mincing**  
**C. Bauer<sup>1</sup>, L. Moser<sup>2</sup>, A. Otahal<sup>1</sup>, D. Kern<sup>1</sup>, D. Dammerer<sup>2</sup>, T. Zantop<sup>3</sup>, S. Nehrer<sup>1</sup>**

<sup>1</sup>University for Continuing Education Krems, Center for Regenerative Medicine, Dr.-Karl-Dorrek-Strasse 30, 3500 Krems, Austria; <sup>2</sup>University Hospital Krems, Department of Orthopedics, Mitterweg 10, 3500 Krems, Austria; <sup>3</sup>University for Continuing Education Krems, Center for Health Sciences and Medicine, Dr.-Karl-Dorrek-Strasse 30, 3500 Krems, Austria

Mincing cartilage with commercially available shavers is increasingly used for treating focal cartilage defects. This study aimed to compare the impact of mincing bovine articular cartilage using different shaver blades on chondrocyte viability.

Bovine articular cartilage was harvested using a scalpel or three different shaver blades (2.5 mm, 3.5 mm, or 4.2 mm) from a commercially available shaver. The cartilage obtained with a scalpel was minced into fragments smaller than 1 mm<sup>3</sup>. All four conditions were cultivated in a culture medium for seven days. After Day 1 and Day 7, metabolic activity, RNA isolation, and gene expression of anabolic (COL2A1, ACAN) and catabolic genes (MMP1, MMP13), Live/Dead staining and visualization using confocal microscopy, and flow cytometric characterization of minced cartilage chondrocytes were measured.

The study found that mincing cartilage with shavers significantly reduced metabolic activity after one and seven days compared to scalpel mincing ( $p < 0.001$ ). Gene expression of anabolic genes was reduced, while catabolic genes were increased after day 7 in all shaver conditions. The MMP13/COL2A1 ratio was also increased in all shaver conditions. Confocal microscopy revealed a thin line of dead cells at the lesion site with viable cells below for the scalpel mincing and a higher number of dead cells diffusely distributed in the shaver conditions. After seven days, there was a significant decrease in viable cells in the shaver conditions compared to scalpel mincing ( $p < 0.05$ ). Flow cytometric characterization revealed fewer intact cells and proportionally more dead cells in all shaver conditions compared to the scalpel mincing.

Mincing bovine articular cartilage with commercially available shavers reduces the viability of chondrocytes compared to scalpel mincing. This indicates that mincing cartilage with a shaver should be considered a matrix rather than a cell therapy. Further experimental and clinical studies are required to standardize the mincing process with a shaver.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Psychological and clinical issues in octogenarians and nonagenarians patients addressing elective total hip arthroplasty**

A. Camera<sup>1</sup>, S. Biggi<sup>1</sup>, A. Capuzzo<sup>1</sup>, G. Cattaneo<sup>1</sup>, R. Tedino<sup>1</sup>, G. Bolognesi<sup>2</sup>

<sup>1</sup>Clinica Città di Alessandria – Policlinico di Monza; <sup>2</sup>Clinica Ortopedica – Università di Genova

Elective orthopaedic procedures, and particularly total hip arthroplasty (THA), in octogenarians and nonagenarians patients are burdened of several implications. Besides the comorbidities and the anesthesiological issues, legal and ethical implications are present. Some literature data show the clinical improvement of THA in elderly patient but the psychological aspects are not yet evaluated. Aim of this study is to evaluate the clinical aspects and the psychological impact in daily living in octogenarians and nonagenarians patients addressing THA.

We conducted a retrospective evaluation of 81 THA in 81 patients of age more than 85 years with a minimum follow-up of 6 months. Clinical aspects were evaluated using the Hip disability and Osteoarthritis Outcome Score (HOOS). The psychological issues were evaluated with the Short Form 12 (SF-12) using both the Physical Component Summary (PCS) and the Mental Component Summary (MCS). From the starter cohort of 81 patients, 8 patients were died for causes unrelated to surgery, 13 were lost to follow-up, 1 patient was revised for periprosthetic fracture; 59 patients composed the final cohort. Mean HOOS rased from  $18,07 \pm 17,81$  to  $92,36 \pm 5,74$  with statistically significant distribution both in the global score than in all of the different subscales. The PCS raised from  $26,81 \pm 10,81$  to  $51,86 \pm 4,45$  and The MCS raised from  $34,84 \pm 10,81$  to  $56,70 \pm 5,04$ , but none of them showed a statistically significant distribution. THA in octogenarians and nonagenarians patients could be a safe procedure with positive results for clinical and psychological aspects.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Dual mobility articulation confers lower dislocation and revision rates: a study using review of reviews methodology**S. Mehta<sup>2</sup>, A. Goel<sup>1</sup>, U. Mahajan<sup>1</sup>, N.R. Reddy<sup>1</sup>, D. Bhaskar<sup>1</sup><sup>1</sup>Glan Clwyd Hospital, Rhyl, North Wales, LL18 5UJ, UK.; <sup>2</sup>Airedale Hospital NHS trust, Keighley, BD20 6TD, UK.

Dislocation post THA confers a higher risk of re-dislocation (Kotwal et al, 2009). The dual mobility (DM) cup design (1974) was aimed at improving the stability by increasing the femoral head to neck ratio (Cuthbert et al., 2019) combining the ideas of low friction arthroplasty with increased jump distance associated with a big head arthroplasty.

Understand the dislocation rates, rates of aseptic loosening, infection rate and revision rates between the 2 types of constructs to provide current and up-to date evidence.

Medline, pubmed, embase and Cochrane databases were used based on PRISMA guidelines. RevMan software was used for the meta-analysis. Studies (English literature) which used DM construct with atleast 6 months follow-up used as intervention and non DM construct as control were included. 2 independent reviewers conducted the review with a third reviewer in case of difference in opinion regarding eligibility. Primary outcome was dislocation rate and secondary outcome was rate of revision.

564 articles identified out of which 44 articles were screened for full texts and eventually 4 systematic review articles found eligible for the study. Thus, study became a review of systematic reviews. From the 4 systematic reviews, another 35 studies were identified for data extraction and 13 papers were used for meta-analysis. Systematic reviews evaluated, projected an average follow up of 6-8 years with significantly lower dislocation rates for DM cups. The total number of patients undergoing DM cup primary THA were 30,559 with an average age 71 years while the control group consisted of 218,834 patients with an average age of 69 years. DM group had lower rate of dislocation ( $p < 0.00001$ ), total lower rate of cup revision ( $p < 0.00001$ ), higher incidence of fracture ( $p > 0.05$ ).

DM THA is a viable alternative for conventional THA. The long-term results of DM cups in primary THA need to be further evaluated using high quality prospective studies and RCTs.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Comparing the risk assessments of iatrogenic peroneal nerve injury in all-inside lateral meniscal repair between standard knee MRIs and simulated actual arthroscopic lateral meniscal repair position MRIs**

Chaiwat Chuaychoosakoon<sup>1</sup>, Tanarat Boonriong<sup>1</sup>, Wachiraphan Parinyakhup<sup>1</sup>

<sup>1</sup>Department of Orthopedics, Faculty of Medicine, Prince of Songkla University, Thailand.

Several studies have evaluated the risk of peroneal nerve (PN) injuries in all-inside lateral meniscal repair using standard knee magnetic resonance imaging (MRI) with the 30 degrees flexed knee position which is different from the knee position during actual arthroscopic lateral meniscal repair. The point of concern is “Can the risk of PN injury using standard knee MRIs be accurately determined”.

To evaluate and compare the risk of PN injury in all-inside lateral meniscal repair in relation to both borders of the popliteus tendon (PT) using MRIs of the two knee positions in the same patients.

Using axial MRI studies with standard knee MRIs and figure-of-4 with joint fluid dilatation actual arthroscopic lateral meniscal repair position MRIs, direct lines were drawn simulating a straight all-inside meniscal repair device from the anteromedial and anterolateral portals to the medial and lateral borders of the PT. The distance from the tip of each line to the PN was measured. If a line touched or passed the PN, a potential risk of iatrogenic injury was noted and a new line was drawn from the same portal to the border of the PN. The danger area was measured from the first line to the new direct line along the joint capsule.

In 28 adult patients, the closest distances from each line to the PN in standard knee MRI images were significantly shorter than arthroscopic position MRI images (all p-values < 0.05). All danger areas assessed in the actual arthroscopic position MRIs were included within the danger areas as assessed by the standard knee MRIs.

We found that the standard knee MRIs can be used to determine the risk of peroneal nerve injury in arthroscopic lateral meniscal repair, although the risks are slightly overestimated.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **ORTHOPOD: Predicting injury proportionality from Neck of Femur Fractures**

Morris, T.<sup>1</sup>; Fouweather, F.<sup>2</sup>; Walshaw, T.<sup>1</sup>; Baldock, T.<sup>1</sup>; Wei, N.<sup>1</sup>; Eardley, W.<sup>1</sup>

<sup>1</sup>Orthopaedic Surgery Department, James Cook University Hospital, Marton Road, Middlesbrough, England, TS4 3BW; <sup>2</sup>Newcastle University, Newcastle-upon-Tyne, England, NE1 7RU

The need to accurately forecast the injury burden has never been higher. With an aging, ever expanding trauma population and less than half of the beds available compared to 1990, the National Health Service (NHS) is stretched to breaking point<sup>1,2</sup>. We utilised a dataset of 22,585 trauma patients across the four countries of the United Kingdom (UK) admitted to 83 hospitals between 22/08/22 – 16/10/22 to determine whether it is possible to predict the proportionality of injuries treated operatively within orthopaedic departments based on their number of Neck of Femur fracture (NOF) patients.

More operations were performed for elderly hip fractures alone than for the combined totals of the next four most common fractures: ankle, distal radius, tibial shaft and forearm (6387 vs 5922). Conversely, 10 out of the 13 fracture types were not encountered by at least one hospital and 93% of hospitals encountered less than 2 fractures of a certain type.

60% trauma is treated within Trauma Units (TUs) however, per unit, Major Trauma Centres (MTCs) treat approximately 43% more patients.

After excluding NOF, lower limb fractures accounted for approximately 57% of fractures in all countries and ankle and distal radius fracture combined comprised more than 50% in 74% of regions.

The number of hip fractures seen on average by an individual unit remains relatively consistent as does the regional variation of any given fracture; resultantly, it is possible to predict injury proportionality based off a unit's hip fracture numbers. This powerful tool could transform both resource allocation and recruitment.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Adjunct CPM therapy does not appear improve the ROM achieved after MUA for stiffness in Total Knee Arthroplasty.**

A. Firth<sup>1</sup>, K. Lee<sup>1</sup>, B.H. van Duren<sup>1</sup>, R. Berber<sup>1</sup>, H.E. Matar<sup>1</sup>, B.V. Bloch<sup>1</sup>

<sup>1</sup> Nottingham Elective Orthopaedic Services, Nottingham University Hospitals NHS Trust, Nottingham

Stiffness is reported in up to 16% of patients after total knee replacement (TKR)<sup>1</sup>. Treatment of stiffness after TKR remains a challenge. Manipulation under anaesthesia (MUA) accounts for between 6%-36% of readmissions following TKR<sup>2,3</sup>. The outcomes of MUA remain variable/unpredictable. Post-operative CPM is used as an adjuvant to MUA, potentially offering improved ROM, however, remains the subject of debate. We report a retrospective study comparing MUA with and without post-operative CPM.

In our institution patients undergoing MUA to receive CPM post-operatively. Owing to the COVID-19 pandemic hospital admissions were limited. During this period MUA procedures were undertaken without CPM. Two cohorts were included: 1) MUA + post-operative CPM 2) Daycase MUA. Patients' demographics, pre-manipulation ROM, post-MUA ROM, and ROM at final follow-up were recorded.

Between 2017-2022 126 patients underwent MUA and were admitted for CPM and 42 had daycase MUA. The median Age was 66.5 and 64% were female. 57% had extension deficit (>5°), 70% had flexion deficit (< 90°), and 37% had both. The mean Pre-operative ROM was 72.3°(SD:18.3°) vs. 68.5°(19.0°), ROM at MUA was 95.5°(SD:20.7°) vs 108.3°(SD:14.1°) [p< 0.01], and at final follow-up 87.4°(SD:21.9°) vs. 92.1°(SD:18.2°) for daycase and CPM groups respectively. At final follow-up for the daycase and CPM groups respectively 10% vs. 7% improved, 29% vs. 13% maintained, and 57% vs. 79% regressed from the ROM achieved at MUA. The mean percentage of ROM gained at MUA maintained at final follow-up was 92%(SD:17) and 85%(SD:14)[p=0.03] for daycase and CPM groups respectively.

There was no significant difference in ROM achieved at final follow-up despite the significantly greater improvement in ROM achieved at MUA for the CPM group. The CPM group lost a greater ROM after MUA (15% vs. 8%). We conclude that post-operative CPM does not improve ROM achieved after MUA.

**References:** 1. E. Carlos Rodríguez-Merchán, The stiff total knee arthroplasty: causes, treatment modalities and results, EFORT Open Rev. 2019 Oct; 4(10): 602–610.; 2. Husted H, Otte KS, Kristensen BB, Orsnes T, Kehlet H (2010) Readmissions after fast-track hip and knee arthroplasty. Arch Orthop Trauma Surg 130(9):1185–1191; 3.Zmistowski B, Restrepo C, Hess J, Adibi D, Cangoz S, Parvizi J (2013) Unplanned readmission after total joint arthroplasty: rates, reasons, and risk factors. J Bone Joint Surg Am 95(20):1869–1876

**27-29 SEPTEMBER | PORTO, PORTUGAL****Safe use of intra-operative tourniquet, does orthopaedic practice need guidance to prevent rare complications?**Amr Elbahi<sup>1</sup>, Mohamed Wasim<sup>1</sup>, Karshe Yusuf<sup>1</sup>, Michael Thilagarajah<sup>1</sup><sup>1</sup>Dartford and Gravesham NHS Foundation Trust

Tourniquet is a commonly used tool in orthopaedic practice. Incidence of complications is low but if any develops, it is devastating. Transient nerve damage, ischemia or skin burns are the possible tourniquet related complications. There is big variation in practice regarding the limb occlusion pressure.

51 procedures in 50 patients were reviewed retrospectively in our district general hospital. We looked at quality of documentation guided by the BOAST standard (The Safe Use of Intraoperative Tourniquets, published in October 2021). Limb occlusion pressure and ischemic time were analysed. Intra-operative and post-operative notes were reviewed to assess quality of documentation and post-operative complications. Although limb occlusion pressure was above the recommended range in more than 75% of cases, there were no significant complications observed. Two cases only developed transient neuropraxia in common peroneal nerve and median nerve following tibial plateau ORIF and trapeziectomy simultaneously. Tibial ORIF fixation case had prolonged ischemic time (more than 120 minutes) and the limb occlusion pressure for the hand case was above the recommended range. Both have recovered within few days with no long-term consequences. Minimum documentation threshold was not met with regarding tourniquet site condition, method of skin isolation and padding, and exsanguination method.

This relatively new standard with no previous similar guidance needs time until it is followed by the health care professionals especially when there is no high incidence of complications related to the use of the tourniquet. However, it is crucial to increase the theatre staff awareness of such standards. This will prevent devastating complications specifically in vulnerable patients. Adjustments to theatre checklist have been suggested to improved documentation. Additionally, local teaching sessions will be delivered to theatre personnel aiming at improving our compliance to this standard.

27-29 SEPTEMBER | PORTO, PORTUGAL

## Extracellular Vesicle-functionalized fibrinogen and magnesium scaffolds to promote bone regeneration

Cardona-Timoner M<sup>1,2</sup>, Bessa-Gonçalves M<sup>1,3,4</sup>, Nogueira F<sup>1,3,4</sup>, Barbosa MS<sup>1,3,4</sup>, Santos SG<sup>1,3</sup>

<sup>1</sup>3S - Instituto de Investigação e Inovação em Saúde, da Universidade do Porto, Portugal; <sup>2</sup>Facultat de Medicina, Universitat de Barcelona, Spain; <sup>3</sup>INEB - Instituto Nacional de Engenharia Biomédica; <sup>4</sup>ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal.

Bone defects and fractures, caused by injury, trauma or tumour resection require hospital treatment and temporary loss of mobility, representing an important burden for societies and health systems worldwide. Autografts are the gold standard for promoting new bone formation, but these may provide insufficient material and lead to donor site morbidity and pain. We previously showed that Fibrinogen (Fg) scaffolds promote bone regeneration *in vivo* (1), and that modifying them with 10mM of Magnesium (Mg) ions modulates macrophage response *in vitro* and *in vivo* (2). Also, we showed that Extracellular Vesicles (EV) secreted by Dendritic Cells (DC) recruit Mesenchymal Stem/Stromal Cells (MSC)(3).

Herein, we aim to functionalize FgMg scaffolds with DC-EV, to promote recruitment and osteogenic differentiation of MSC.

Scaffolds were produced by freeze-drying (2). Ethical permission was sought for all studies. Primary human peripheral blood monocyte-derived DC were cultured, their secreted EV were isolated by differential (ultra)-centrifugation and characterised by transmission electron microscopy and nanoparticle tracking analysis (3). Bone marrow MSC were used to determine the impact of EV-functionalized scaffolds through migration assays and their osteogenic differentiation was assessed by Alizarin Red staining.

Fg and FgMg scaffolds functionalized with EV were characterized. Fg and FgMg scaffolds functionalized with DC-secreted EV were more efficient at recruiting MSC than scaffolds alone. MSC cultured on FgMg scaffolds showed significantly increased calcium deposits, in comparison with those cultured on Fg scaffolds.

Fg scaffold modification by Mg promotes MSC osteogenic differentiation, while their functionalization with DC-secreted EV acts to promote MSC recruitment. This renders the FgMg-EV functionalized scaffolds an attractive material to promote new bone formation.

**References:** 1) Vasconcelos DM, et al. *Biomaterials*. 2016; 111:163-178; 2) Bessa-Gonçalves M, et al. *Acta Biomater* 2023; 155: 667-683; 3) Silva AM, et al. *SciRep* 2017; 7:1667

# EORS 2023

31st Annual Meeting of the  
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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **The induced membrane technique improves the health-related quality of life in patients with a post-traumatic long bone non-union**

L. van der Broeck<sup>1</sup>, J. Geurts<sup>2</sup>, S. Qiu<sup>3</sup>, M. Poeze<sup>1,4</sup>, T.J. Blokhuis<sup>1,4</sup>

<sup>1</sup>Department of Trauma Surgery, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands; <sup>2</sup>Department of Orthopaedics, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands; <sup>3</sup>Department of Plastic Surgery, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands; <sup>4</sup>Department of Surgery, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, Maastricht, The Netherlands.

The optimal treatment strategy for post-traumatic long bone non-unions is subject of an ongoing discussion. At the Maastricht University Medical Center (MUMC+) the induced membrane technique is used to treat post-traumatic long bone non-unions. This technique uses a multimodal treatment algorithm involving bone marrow aspirate concentrate (BMAC), the reamer-irrigator-aspirator (RIA) and P-15 bioactive peptide (iFactor, Cerapedics). Bioactive glass (S53P4 BAG, Bonalive) is added when infection is suspected. This study aims to objectify the effect of this treatment algorithm on the health-related quality of life (HRQoL) of patients with post-traumatic long bone non-unions. We hypothesized that HRQoL would improve after treatment. From January 2020 to March 2023, consecutive patients who were referred to a multidisciplinary (trauma, orthopaedic and plastic surgery) non-union clinic at the MUMC+, The Netherlands, were evaluated using the Non-Union Scoring System (NUSS). The EQ-5D-5L questionnaire and the Lower Extremity Functional Scale (LEFS) were employed to obtain HRQoL outcomes both prior to and subsequent to surgery, with a follow-up at 6, 18 and 35 weeks.

Seventy-six patients were assessed at baseline (T0), with a mean NUSS of 40 ( $\pm$  13 SD). Thirty-eight patients had their first follow-up, six weeks after surgery (T1). Thirty-one patients had a second follow-up at 18 weeks (T2), and twenty patients had the third follow-up at 35 weeks (T3). The EQ-5D index mean at baseline was 0.480, followed by an index of 0.618 at T1, 0.636 at T2, and 0.702 at T3. A significant difference was found in the HRQoL score between T0 and T1, as well as T2 and T3 ( $p < 0.001$ ;  $p = 0.011$ ). The mean LEFS significantly increased from 26 before intervention to 34, 39, and 43 after treatment ( $p < 0.001$ ;  $p = 0.033$ ;  $p = 0.016$ ).

This study demonstrated a significant improvement in the health-related quality of life of patients with post-traumatic long bone non-unions after the standardized treatment algorithm following the induced membrane technique.

**Tendon health & disease: Exploring the interfascicular niche**Screen HRC<sup>1</sup><sup>1</sup>Queen Mary University of London

Tendon injury is debilitating and recalcitrant. With limited knowledge of disease aetiology we have are lacking in effective treatments for this prevalent musculoskeletal complaint.

This presentation will outline our findings over the past few years in which we have demonstrated the importance of the interfascicular matrix (IFM) niche in maintaining healthy tendon function and driving disease progression<sup>1,2</sup>. It will also continue to describe our progress in developing both in vivo and in vitro models to interrogate disease progression.

We have developed and validated a rat Achilles tendon overload model, in order to explore the impact of loading on IFM and fascicle structure, and the resulting cell response. Data highlights that structural disruption and inflammatory response both initiate in the IFM region, and can be seen in the absence of demonstrable changes to animal gait, indicating a sub-injury response in the tendon which we hypothesis may drive increased matrix turnover and repair<sup>3</sup>.

We are now looking to interrogate the pathways driving this inflammatory behaviour in an organ-chip model, exploring the interplay between IFM cells and cells within fascicles. We have demonstrated phenotypic distinction of cells from the two niche environments, localized the progenitor phenotype to the IFM region and demonstrated significant mechanosensitivity in the IFM cell population<sup>4</sup>. We are currently building appropriate niche environments to maintain cell phenotype in our in vitro models, to explore the metabolic changes associated with disease progression.

**References:** 1. Patel et al (2021) *Acta Biomaterialia*. doi: 10.1016/j.actbio.2021.07.019; 2. Zamboulis et al (2020) *E Life*. doi: 10.7554/eLife.58075; 3. Gains et al (2023) *J Biomech*. doi: 10.1016/j.jbiomech.2023.111546; 4. Zamboulis et al (2023) *Biorxiv*. doi.org/10.1101/2023.01.04.522701

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Multi-cellular spheroid engineering using bioinspired materials for osteochondral tissue regeneration**

Heungsoo Shin<sup>1</sup>

<sup>1</sup>Department of Bioengineering, Hanyang University, 04763, Republic of Korea

Recently, technologies to culture one or more cell types in three dimensions have attracted a great deal of attention in tissue engineering. Particularly, the improved viability, self-renewal capacity, and differentiation potential have been reported for stem cell spheroids. However, it is crucial to modulate spheroid functions with instructive signals to use multi-cellular spheroids in tissue engineering. We have been developing ECM-mimicking fibrous materials decorated with cell-instructive cues, which were incorporated within 3D stem cell spheroids to fine-tune their functions as modular building blocks for bottom-up tissue-engineering applications. In particular, we created composite spheroids of human adipose-derived stem cells (hADSCs) incorporating nanofibers coated with instructive signal of either transforming growth factor- $\beta$ 3 or bone morphogenetic growth factor-2 for chondrogenesis or osteogenesis of stem cells, respectively. The bilayer structure of osteochondral tissue was subsequently mimicked by cultivating each type of spheroid inside 3D-printed construct. The *in vitro* chondrogenic or osteogenic differentiation of hADSCs within the biphasic construct under general media was locally regulated by each inductive component. More importantly, hADSCs from each spheroid proliferated and sprouted to form the integrated tissue with interface of bone and cartilage tissue. This approach may be applied to engineer complex tissue with hierarchically organized structure.

**27-29 SEPTEMBER | PORTO, PORTUGAL****New generation superior single plating versus low-profile dual mini-fragment plating of diaphyseal clavicle fractures – a biomechanical study**Tatjana Pastor<sup>1,2</sup>, Ivan Zderic<sup>1</sup>, Till Berk<sup>3</sup>, Firas Souleiman<sup>4</sup>, Esther Vögelin<sup>2</sup>, Frank J P Beeres<sup>5</sup>, Boyko Gueorguiev<sup>1</sup>, Torsten Pastor<sup>5</sup>

<sup>1</sup>AO Research Institute Davos, Davos, Switzerland; <sup>2</sup>Department of Hand Surgery, Bern University Hospital, University of Bern, Bern, Switzerland; <sup>3</sup>Department of Trauma, University Hospital Zurich, Zurich, Switzerland; <sup>4</sup>Department of Orthopaedics, Trauma and Plastic Surgery, University Hospital Leipzig, Leipzig, Germany; <sup>5</sup>Department of Orthopaedic and Trauma Surgery, Lucerne Cantonal Hospital, Lucerne, Switzerland

Recently, a new generation of superior clavicle plates was developed featuring the variable-angle locking technology for enhanced screw positioning and optimized plate-to-bone fit design. On the other hand, mini-fragment plates used in dual plating mode have demonstrated promising clinical results. However, these two bone-implant constructs have not been investigated biomechanically in a human cadaveric model. Therefore, the aim of the current study was to compare the biomechanical competence of single superior plating using the new generation plate versus dual plating with low-profile mini-fragment plates.

Sixteen paired human cadaveric clavicles were assigned pairwise to two groups for instrumentation with either a 2.7 mm Variable Angle Locking Compression Plate placed superiorly (Group 1), or with one 2.5 mm anterior plate combined with one 2.0 mm superior matrix mandible plate (Group 2). An unstable clavicle shaft fracture AO/OTA15.2C was simulated by means of a 5 mm osteotomy gap. All specimens were cyclically tested to failure under craniocaudal cantilever bending, superimposed with bidirectional torsion around the shaft axis and monitored via motion tracking.

Initial stiffness was significantly higher in Group 2 ( $9.28 \pm 4.40$  N/mm) compared to Group 1 ( $3.68 \pm 1.08$  N/mm),  $p=0.003$ . The amplitudes of interfragmentary motions in terms of craniocaudal and shear displacement, fracture gap opening and torsion were significantly bigger over the course of 12500 cycles in Group 1 compared to Group 2;  $p \leq 0.038$ . Cycles to 2 mm shear displacement were significantly lower in Group 1 ( $22792 \pm 4346$ ) compared to Group 2 ( $27437 \pm 1877$ ),  $p=0.047$ .

From a biomechanical perspective, low-profile 2.5/2.0 dual plates demonstrated significantly higher initial stiffness, less interfragmentary movements, and higher resistance to failure compared to 2.7 single superior variable-angle locking plates and can therefore be considered as a useful alternative for diaphyseal clavicle fracture fixation especially in unstable fracture configurations.

27-29 SEPTEMBER | PORTO, PORTUGAL

## The relation between microstructure and mechanical response in the human meniscus

Maria Pierantoni<sup>1</sup>, Hector Dejea<sup>1,2</sup>, Lisa Geomini<sup>1</sup>, Martin Abrahamsson<sup>1</sup>, Stefan Gsthöhl<sup>3</sup>, Christian M. Schlepütz<sup>3</sup>, Martin Englund<sup>1</sup>, Hanna Isaksson<sup>1</sup>

<sup>1</sup>Lund University, SE, <sup>2</sup>MAX IV Laboratory, SE, <sup>3</sup>Swiss Light Source, Paul Scherrer Institute, CH

To characterize the microstructural organization of collagen fibers in human medial menisci and the response to mechanical loading in relation to age. We combine high resolution imaging with mechanical compression to visualize the altered response of the tissue at the microscale. Menisci distribute the load in the knee and are predominantly composed of water and specifically hierarchically arranged collagen fibers. Structural and compositional changes are known to occur in the meniscus during aging and development of osteoarthritis. However, how microstructural changes due to degeneration affect mechanical performance is still largely unknown [1].

Fresh frozen 4 mm Ø plugs of human medial menisci (n=15, men, 20-85 years) with no macroscopic damage nor known diseases from the MENIX biobank at Skåne University Hospital were imaged by phase contrast synchrotron tomography at the TOMCAT beamline (Paul Scherrer Institute, CH). A rheometer was implemented into the beamline to perform in-situ stress relaxation (2 steps 15% and 30% strain) during imaging (21 keV, 2.75µm pixel size). 40s scans were acquired before and after loading, while 14 fast tomographs (5s acquisitions) were taken during relaxation. The fiber 3D orientations and structural changes during loading were determined using a structure tensor approach (adapting a script from [1]). The 3D collagen fiber orientation in menisci revealed alternating layers of fibers. Two main areas are shown: surfaces and bulk. The surface layers are a mesh of randomly oriented fibers. Within the bulk 2-3 layers of fibers are visible that alternate about 30° to each other. Structural degeneration with age is visible and is currently being quantified. During stress-relaxation all menisci show a similar behavior, with samples from older donors being characterized by larger standard deviation. Furthermore, the behavior of the different layers of fibers is tracked during relaxation showing how fibers with different orientation respond to the applied loading.

**References:** [1] Einarsson and Pierantoni et al. OA Cartilage. 2022, 30:1222–33.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Recent advances in guided bone growth for deformity correction**

Rahbek O<sup>1,2</sup>, Halloum A<sup>2,3</sup>, Rolfing J, Kold S, Abood A<sup>3</sup>

<sup>1</sup>Interdisciplinary Orthopedics, Aalborg Universityhospital; <sup>2</sup>Danish Pediatric Orthopaedic Research; <sup>3</sup>Department of Orthopedics, Aarhus Universityhospital

The concept of guided growth was proposed by Andry in 1741. In the last decades the concept has been widely used as implants has been introduced that can modulate the growth of the bone and pediatric longitudinal and angular deformities is widely treated by this technique. However, there is there is a huge variation in techniques and implants used and high-quality clinical trials is still lacking. Recently implants correcting rotational bony deformities have been proposed and clinical case series have been published.

The current status of guided growth will be presented in this narrative review and preliminary experiences with rotational guided growth will be shared. Is guided growth to be considered a safe treatment at this time point?

27-29 SEPTEMBER | PORTO, PORTUGAL

## Novel Mineralized Electroactive PAN/PEDOT:PSS Nanofibers for Bone Tissue Engineering

Frederico Barbosa<sup>1,2</sup>, João C. Silva<sup>1,2</sup>, Fábio F. F. Garrudo<sup>1,2,3</sup>, Joaquim M. S. Cabral<sup>1,2</sup>, Jorge Morgado<sup>3</sup>, Frederico Castelo Ferreira<sup>1,2</sup>

<sup>1</sup>Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>2</sup>Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>3</sup>Department of Bioengineering and Instituto de Telecomunicações, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

Bone defects can result from different incidents such as acute trauma, infection or tumor resection. While in most instances bone healing can be achieved given the tissue's innate ability of self-repair, for critical-sized defects spontaneous regeneration is less likely to occur, therefore requiring surgical intervention. Current clinical procedures have failed to adequately address this issue. For this reason, bone tissue engineering (BTE) strategies involving the use of synthetic grafts for replacing damaged bone and promoting the tissue's regeneration are being investigated. The electrical stimulation (ES) of bone defects using direct current has yielded very promising results, with neo tissue formation being achieved in the target sites *in vivo*. Electroactive implantable scaffolds comprised by conductive biomaterials could be used to assist this kind of therapy by either directing the ES specifically to the damaged site or promoting the integration of electrodes within the bone tissue as a coating. In this study, we developed novel conductive heat-treated polyacrylonitrile/poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PAN/PEDOT:PSS) nanofibers via electrospinning capable of mimicking key native features of the bone tissue's extracellular matrix (ECM) and providing a platform for the delivery of exogenous ES. The developed scaffolds were doped with sulfuric acid and mineralized in Simulated Body Fluid to mimic the inorganic phase of bone ECM. As expected, the doped PAN/PEDOT:PSS nanofibers exhibited electroconductive properties and were able to preserve their fibrous structure. The addition of PEDOT:PSS was found to improve the bioactivity of the scaffolds, with a more significant *in vitro* mineralization being obtained. By seeding the scaffolds with MG-63 osteoblasts and human mesenchymal stem/stromal cells, an increased cell proliferation was observed for the mineralized PAN/PEDOT:PSS nanofibers, which also registered an increased expression of key osteogenic markers (e.g Osteopontin). Our findings appear to corroborate the promising potential of the generated nanofibers for future ES-based BTE applications.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Technical Note For The Novel Osteopore® Wedge In Medial Opening Wedge High Tibial Osteotomy

Soon Yaw Walter Wong, MBChB<sup>1</sup>, Kong Hwee Lee, MBBS, MMed (Ortho), FRCSEd (Orth)<sup>2</sup>, Hamid Rahmatullah Bin Abd Razak, MBBS, MMed (Ortho), FRCSEd (Orth)<sup>1,3</sup>

<sup>1</sup>Department of Orthopaedic Surgery, Sengkang General Hospital, 110 Sengkang East Way, Singapore 544886; <sup>2</sup>Department of Orthopaedic Surgery, Singapore General Hospital, 31 Third Hospital Ave, Singapore 168753; <sup>3</sup>SingHealth Duke-NUS Musculoskeletal Sciences Academic Clinical Programme 20 College Road, Academia Level 4 Singapore 169865

Medial opening wedge high tibial osteotomy (MOWHTO) is the workhorse procedure for correcting varus malalignment of the knee. There have been recent developments in the synthetic options to fill the osteotomy gap. The current gold standard for filling this osteotomy gap is autologous bone graft which is associated with donor site morbidity. We would like to introduce and describe the process of utilizing the novel Osteopore® 3D printed, honeycomb structured, Polycaprolactone and  $\beta$ -Tricalcium Phosphate wedge for filling the gap in MOWHTO. In the advent of additive manufacturing and the quest for more biocompatible materials, the usage of the Osteopore® bone wedge in MOWHTO is a promising technique that may improve the biomechanical stability as well the healing of the osteotomy gap.

**27-29 SEPTEMBER | PORTO, PORTUGAL****A novel selective prostaglandin-receptor EP4-agonist promotes spinal fusion in rats**

Corina Vater<sup>1,2</sup>, Xinggui Tian<sup>1,2</sup>, Lisa Findeisen<sup>1,2</sup>, Deepak Bushan Raina<sup>3</sup>, Hannes Kern<sup>1,2</sup>, Julia Bolte<sup>1,2</sup>, Luisa Straßburger<sup>1,2</sup>, Lucas-Maximilian Matuszewski<sup>1,2</sup>, Niels Modler<sup>4</sup>, Robert Gottwald<sup>4</sup>, Anja Winkler<sup>4</sup>, Klaus-Dieter Schaser<sup>1,2</sup>, Alexander C. Disch<sup>1,2</sup>, Stefan Zwingenberger<sup>1,2</sup>

<sup>1</sup>University Center of Orthopaedic, Trauma and Plastic Surgery, University Hospital Carl Gustav Carus at Technische Universität Dresden, 01307 Dresden, Germany; <sup>2</sup>Center for Translational Bone, Joint and Soft Tissue Research, University Hospital Carl Gustav Carus at Technische Universität Dresden, 01307 Dresden, Germany; <sup>3</sup>Lund University, Faculty of Medicine, Department of Clinical Sciences Lund, Orthopaedics, Lund 22185, Sweden; <sup>4</sup>Institute of Lightweight Engineering and Polymer Technology at Technische Universität Dresden, Germany

A novel EP4 selective agonist (KMN-159) was developed [1] and has been proven that it can act as an osteopromotive factor to repair critical-size femoral bone defects in rats at a dose-dependent manner [2]. Based on its osteopromotive properties, we hypothesized that KMN-159 could also aid in bone formation for spinal fusion. Therefore, the aim of this study was to investigate its spinal fusion effect in a dorsolateral spinal fusion model in rats. This study was performed on 192, 10-week-old male Wistar rats. The rats were randomized into 8 groups (n = 12 per group): 1) SHAM (negative control), 2) MCM (scaffold only), 3) MCM + 20 µg BMP-2 (positive control), 4-8) MCM + 0.2, 2, 20, 200 or 2000 µg KMN-159. A posterolateral intertransverse process spinal fusion at L4 to L5 was performed bilaterally by implanting group dependent scaffolds (see above) or left empty in the SHAM group (protocol no. 25-5131/474/38). Animals were euthanized after 3 weeks and 6 weeks for µCT and biomechanical testing analysis. The results showed that KMN-159 promoted new bone formation in a dose-dependent manner at 3 weeks and 6 weeks as verified by µCT. The biomechanical testing showed that the dose of 20, 200 and 2000 µg KMN-159 groups obtained comparable strength with BMP-2 group, which higher than SHAM, MCM and lower doses of 0.2 and 2 µg KMN-159 groups. In conclusion, KMN-159 could be a potential replacement of BMP-2 as a novel osteopromotive factor for spinal fusion.

**References:** [1]. Barrett et al., Med Chem 2019, 62 (9), 4731-4741; [2]. Vater et al., Biomedicines 2021, 9 (11), 1712.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **The knee-hip-spine trilemma in patients with severe congenital dysplasia of the hip undergoing total hip arthroplasty. 77 hips followed-up for a minimum of five years.**

Eduardo García-Rey<sup>1,2</sup>, Enrique Gómez-Barrena<sup>1,2</sup>

<sup>1</sup>Orthopedic Surgery and Trauma Service, La Paz University Hospital, Madrid; <sup>2</sup>IdiPaz, La Paz University Hospital, Madrid

Pelvic bone defect in patients with severe congenital dysplasia of the hip (CDH) lead to abnormalities in lumbar spine and lower limb alignment that can determine total hip arthroplasty (THA) patients' outcome. These variables may be different in uni- or bilateral CDH.

We compared the clinical outcome and the spinopelvic and lower limb radiological changes over time in patients undergoing THA due to uni- or bilateral CHD at a minimum follow-up of five years.

Sixty-four patients (77 hips) undergoing THA due to severe CDH between 2006 and 2015 were analyzed: Group 1 consisted of 51 patients with unilateral CDH, and group 2, 113 patients (26 hips) with bilateral CDH. There were 32 females in group 1 and 18 in group 2 ( $p=0.6$ ). The mean age was 41.6 years in group 1 and 53.6 in group 2 ( $p<0.001$ ). We compared the hip, spine and knee clinical outcomes. The radiological analysis included the postoperative hip reconstruction, and the evolution of the coronal and sagittal spinopelvic parameters assessing the pelvic obliquity (PO) and the sacro-femoro-pubic (SFP) angles, and the knee mechanical axis evaluating the tibio-femoral angle (TFA).

At latest follow-up, the mean Harris Hip Score was 88.6 in group 1 and 90.7 in group 2 ( $p=0.025$ ). Postoperative leg length discrepancy of more than 5 mm was more frequent in group 1 ( $p=0.028$ ). Postoperative lumbar back pain was reported in 23.4% of the cases and knee pain in 20.8%, however, there were no differences between groups. One supracondylar femoral osteotomy and one total knee arthroplasty were required. The radiological reconstruction of the hip was similar in both groups. The PO angle improved more in group 1 ( $p=0.01$ ) from the preoperative to 6-weeks postoperative and was constant at 5 years. The SFP angle improved in both groups but there were no differences between groups ( $p=0.5$ ). 30 patients in group 1 showed a TFA less than  $10^\circ$  and 17 in group 2 ( $p=0.7$ ).

Although the clinical outcome was better in terms of hip function in patients with bilateral CDH than those with unilateral CDH, the improvement in low back and knee pain was similar. Patients with unilateral dysplasia showed a better correction of the PO after THA. All spinopelvic and knee alignment parameters were corrected and maintained over time in most cases five years after THA.

**27-29 SEPTEMBER | PORTO, PORTUGAL****A fetal-based injectable biomaterial to treat Intervertebral Disc degeneration**Morena F. Fiordalisi<sup>1,2,3</sup>, Inês Sousa<sup>1</sup>, Mário A. Barbosa<sup>1,2,3</sup>, Raquel M. Gonçalves<sup>1,2,3</sup>,  
Joana Caldeira<sup>1,2</sup>

<sup>1</sup>i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; <sup>2</sup>INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal; <sup>3</sup>ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal

Intervertebral disc (IVD) degeneration is the most frequent cause of Low Back Pain (LBP) affecting nearly 80% of the population [1]. Current treatments fail to restore a functional IVD or to provide a long-term solution, so, there is an urgent need for novel therapeutic strategies. We have defined the IVD extracellular matrix (ECM) profile, showing that the pro-regenerative molecules Collagen type XII and XIV, are uniquely expressed during fetal stages [2]. Now we propose the first fetal injectable biomaterial to regenerate the IVD.

Fetal decellularized IVD scaffolds were recellularized with adult IVD cells and further implanted in vivo to evaluate their anti-angiogenic potential. Young decellularized IVD scaffolds were used as controls. Finally, a large scale protocol to produce a stable, biocompatible and easily injectable fetal IVD-based hydrogel was developed.

Fetal scaffolds were more effective at promoting Aggrecan and Collagen type II expression by IVD cells. In a Chorioallantoid membrane assay, only fetal matrices showed an anti-angiogenic potential. The same was observed in vivo when the angiogenesis was induced by human NP cells. In this context, human NP cells were more effective in GAG synthesis within a fetal microenvironment. Vacuum-assisted perfusion decellularized IVDs were obtained, with high DNA removal and sGAG retention. Hydrogel pre-solution passed through 21-30G needles. IVD cells seeded on the hydrogels initially decreased metabolic activity, but increased up to 70% at day 7, while LDH assay revealed cytotoxicity always below 30%.

This study will open new avenues for the establishment of a disruptive treatment for IVD degeneration with a positive impact on the angiogenesis associated with LBP, and on the improvement of patients' quality of life.

**References:** [1] Colombier P *et al.* Joint Bone Spine. 2014; 81:125-129; [2] Caldeira J *et al.*, Sci Rep. 2017, 7:11629-11644.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Cell-derived extracellular matrix tailoring for intervertebral disc regeneration

Catarina Milheiro<sup>1,2</sup>, Raquel M. Gonçalves<sup>1,2</sup>, Mario Amendola<sup>3,4</sup>, Mário Barbosa<sup>1,2</sup>, Joana Caldeira<sup>2</sup>

<sup>1</sup>ICBAS- Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; <sup>2</sup>3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; <sup>3</sup>Généthon, Évry, France; <sup>4</sup>Integrare Research Unit UMR\_S951, Université Paris-Saclay, Univ Evry, Inserm, Généthon, Évry, France.

Intervertebral disc (IVD) degeneration is characterized by tissue loss of function and major structural changes, which can lead to Low Back Pain. Current treatments fail to tackle the disc's hindered function and degenerative alterations. Our goal is to promote IVD regeneration, through the recapitulation of a fetal-like microenvironment based on a tailored cell-derived matrix (CDM), rich in two fetal exclusive collagens – COLXII and COLXIV. We hypothesize that this engineered CDM will have a pro-regenerative potential and allow IVD functional restoration. Immortalized mesenchymal stromal/stem cells (iMSCs) were transduced with the CRISPR/dCas9-VP64 lentiviral system, targeting *COL12A1* or *COL14A1*. Gene and protein expression were evaluated by qRT-PCR, Western Blot and immunofluorescence. Simultaneously, optimization of CDM decellularization protocol was performed. Process efficacy was validated by immunofluorescence. DNA content was quantified using the Quant-iT™ PicoGreen™ Kit. Successful iMSCs engineering was confirmed by an average increase of mRNA relative expression for both collagens (2.12-fold for *COL12A1* and 11.7-fold for *COL14A1*). Protein level assessment by western blot and immunofluorescence confirmed increased COLXII and COLXIV. Decellularized CDM production was optimized. Cell removal was demonstrated by nuclear and actin staining reduction, when compared with the non-decellularized control. DNA content showed a marked decrease in both NH4OH- and SDS-based protocols. Matrix preservation after decellularization was confirmed by fibronectin staining only in the NH4OH-based decellularization. Preliminary proteomic characterization data of engineered iMSCs as shown some functional impact namely on Sonic Hedgehog (Shh) *pathway and repression of pain by DREAM (Downstream regulator element antagonist modulator)*. In conclusion, we were able to establish a CRISPR activating system for *COL12A1* and *COL14A1*, allowing the use of iMSCs as biofactories for a IVD fetal-tailored CDM. The use of a customized ECM and fetal microenvironment recapitulations is an innovative strategy for IVD functional restoration, and its versatility opens new avenues in the tissue regeneration field.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Advanced bioreactor studies of region-specific response in the intervertebral disc to compression, flexion/extension and torsion**Šećerović A<sup>1</sup>, Ristaniemi A<sup>1</sup>, Crivelli F<sup>2</sup>, Heub S<sup>3</sup>, Weder G<sup>3</sup>, Ferguson SJ<sup>4</sup>, Ledroit D<sup>3</sup>, Grad S<sup>1</sup><sup>1</sup>AO Research Institute, Davos, Switzerland; <sup>2</sup>CSEM, Alpnach, Switzerland; <sup>3</sup>CSEM, Neuchatel, Switzerland; <sup>4</sup>ETH, Zurich, Switzerland

Intervertebral disc (IVD) degeneration is inadequately understood due to the lack of *in vitro* systems that fully mimic the mechanical and biological complexity of this organ. We have recently made an advancement by developing a bioreactor able to simulate physiological, multiaxial IVD loading and maintain the biological environment in *ex vivo* IVD models [1].

To validate this new bioreactor system, we simulated natural spine movement by loading 12 bovine IVDs under a combination of static compression (0.1 MPa), cyclic flexion/extension ( $\pm 3^\circ$ ,  $\pm 6^\circ$  or  $0-6^\circ$ ) and cyclic torsion ( $\pm 2^\circ$ ,  $\pm 4^\circ$  or  $0-4^\circ$ ) for more than 10'000 (0.2 Hz) or 100'000 (1 Hz) cycles over 14 days. A higher number of cycles increased the release of glycosaminoglycans and nitric oxide, as an inflammation marker, whereas fewer cycles maintained these two factors at physiological levels. All applied protocols upregulated the expression of *MMP13* in the outermost annulus fibrosus (AF), indicating a collagen degradation response. This was supported by fissures observed in the AF after a longer loading duration. Increasing loading cycles induced high cell death in the nucleus pulposus and inner AF, while with fewer cycles, high cell viability was maintained in all IVD regions, irrespective of the magnitude of rotation.

Less frequent multiaxial loading maintains IVD homeostasis while more frequent loading initiates an IVD degenerative profile. Specifically, the morphological and molecular changes were localized in the AF, which can be associated with combined flexion/extension and torsion. More loading cycles induced region-specific cell death and a higher release of extracellular matrix molecules from the innermost IVD regions, likely associated with longer exposure to static compression. Altogether, we demonstrated the advantages of the multiaxial bioreactor to study region-specific response in the IVD, which will allow a more profound investigation of IVD degeneration under different combinations of motions.

**References:** 1. ACS BiomaterSciEng, 2022. **8**(9):3969-3976.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Growth factor signaling interactions required for bone regeneration**

Kurt D. Hankenson<sup>1</sup>

<sup>1</sup>University of Michigan Medical School, USA

Growth factors produced by inflammatory cells and mesenchymal progenitors are required for proper bone regeneration. Signaling pathways activated downstream of these proteins work in concert and synergistically to drive osteoblast and/or chondrocyte differentiation. While dysregulation can result in abnormal healing, activating these pathways in the correct spatiotemporal context can enhance healing. Bone morphogenetic protein (BMP) signaling is well-recognized as being required for bone regeneration, and BMP is used clinically to enhance bone healing. However, it is imperative to develop new therapeutics that can be used alone or in conjunction with BMP to drive even more robust healing. Notch signaling is another highly conserved signaling pathway involved in tissue development and regeneration. Our work has explored Notch signaling during osteoblastogenesis and bone healing using both in vitro studies with human primary mesenchymal progenitor cells and in vivo studies with genetically modified mouse models. Notch signaling is required and sufficient for osteoblast differentiation, and is required for proper bone regeneration. Indeed, intact Notch signaling through the Jagged-1 ligand is required for BMP induced bone formation. On-going work continues to explore the intersection between BMP and Notch signaling, and determining cell types that express Notch receptors and Notch ligands during bone healing. Our long-term objective is to develop Notch signaling as a clinical therapy to repair bone.

**27-29 SEPTEMBER | PORTO, PORTUGAL****In vivo posterior stabilized total knee kinematics: it is not all about the implant design**

Lenka Stroobant<sup>1</sup>, Matthias Verstraete<sup>1</sup>, Stefaan Van Onsem<sup>1</sup>, Jan Victor<sup>1</sup>, Amélie Chevalier<sup>1,2</sup>

<sup>1</sup>Ghent University, Department of Human Structure and Repair, Ghent University Hospital, 9000 Gent, Belgium; <sup>2</sup>University of Antwerp, Department of Electromechanics, CosysLab and AnSyMo/Cosys Flandersmake, the strategic research center for the manufacturing industry.

Numerous papers present in-vivo knee kinematics data following total knee arthroplasty (TKA) from fluoroscopic testing. Comparing data is challenging given the large number of factors that potentially affect the reported kinematics. This paper aims at understanding the effect of following three different factors: implant geometry, performed activity and analysis method.

A total of 30 patients who underwent TKA were included in this study. This group was subdivided in three equal groups: each group receiving a different type of posterior stabilized total knee prosthesis. During single-plane fluoroscopic analysis, each patient performed three activities: open chain flexion extension, closed chain squatting and chair-rising. The 2D fluoroscopic data were subsequently converted to 3D implant positions and used to evaluate the tibiofemoral contact points and landmark-based kinematic parameters.

Significantly different anteroposterior translations and internal-external rotations were observed between the considered implants. In the lateral compartment, these differences only appeared after post-cam engagement. Comparing the activities, a significant more posterior position was observed for both the medial and lateral compartment in the closed chain activities during mid-flexion. A strong and significant correlation was found between the contact-points and landmarks-based analyses method. However, large individual variations were also observed, yielding a difference of up to 25% in anteroposterior position between both methods.

In conclusion, all three evaluated factors significantly affect the obtained tibiofemoral kinematics. The individual implant design significantly affects the anteroposterior tibiofemoral position, internal-external rotation and timing of post-cam engagement. Both kinematics and post-cam engagement additionally depend on the activity investigated, with a more posterior position and associated higher patella lever arm for the closed chain activities. Attention should also be paid to the considered analysis method and associated kinematics definition: analyzing the tibiofemoral contact points potentially yields significantly different results compared to a landmark-based approach.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Optimising the Tibial Keel Slot for the Oxford Unicompartmental Knee Replacement** Lachlan Arthur<sup>1</sup>, Xiaoyi Min<sup>1</sup>, Shihfan Jack Tu<sup>1</sup>, Stefano Campi<sup>1</sup>, Stephen Mellon<sup>1</sup>, David Murray<sup>1</sup>

<sup>1</sup>Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences, University of Oxford

Tibial periprosthetic fracture is an important complication of the Oxford Unicompartmental Knee Replacement (OUKR). Primary fixation of cementless OUKR tibial components relies on the interference-fit of the 'keel' and a slot in the proximal tibia. Clinically used double blade keel saws (DKS) create slots with two grooves, generating stress concentrations where fractures may initiate. This study aimed to investigate slot factors that may influence incidence of tibial periprosthetic fractures. Slots were made in PCF20 polyurethane foam using the DKS plus/minus adjuvant rasping, single blade keel saw (SKS), and rasp-only. Round and square slots were machined with milling cutters. Compact tensile tests were conducted per ASTM E399 to determine tensile load to fracture (TLTF) and results were validated using bovine tibia. Cementless OUKR components were implanted into slots in custom polyurethane blocks and compressed to failure to determine anatomical load to fracture (ALTF). A custom MATLAB program calculated slot roundness from cross-sectional images.

Round slots had higher TLTF (29.5N, SD=2.7) than square (25.2N, SD=1.7,  $p<0.05$ ) and DKS slots (23.3N, SD=2.7,  $p<0.0001$ ). Fractures occurred at the round slot apices, square slot corners, and deepest DKS slot grooves. ALTF was not significantly different between square and round slots. Adjuvant rasping made DKS slots significantly rounder, resulting in significantly higher TLTF, but rasping did not increase ALTF. ALTF was significantly higher for SKS (850N, SD=133,  $p<0.01$ ) and rasp-only (912N, SD=100,  $p<0.001$ ) slots compared to standard DKS slots (703N, SD=81).

Round keel slots minimise stress concentrations and increase TLTF but do not increase ALTF. The SKS and rasp-only slots retain material at slot ends and have significantly higher ALTF. Future studies should assess saw blades that retain material and round slot ends to evaluate if their use may significantly reduce the incidence of tibial periprosthetic fracture.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Incidence and surgical treatment of component breakage after unicompartmental and total knee arthroplasty. A single centre long-term experience**A. Camera<sup>1</sup>, S. Biggi<sup>1</sup>, A. Capuzzo<sup>1</sup>, G. Cattaneo<sup>1</sup>, R. Tedino<sup>1</sup>, G. Bolognesi<sup>1</sup><sup>1</sup> Clinica Città di Alessandria – Policlinico di Monza

Fractures of the prosthetic components after total knee arthroplasty (TKA) are rare but dangerous complications, sometimes difficult to diagnose and to manage. Aim of this study is to evaluate the incidence of component breakage and its treatment in our single institution's experience. We retrospectively review our institution registry. From 605 revision knee arthroplasties since 2000 to 2018, we found 8 cases of component breakage, of these 3 belonged to UKA, and 5 belonged to TKA. The UKA fractures were all on the metal tibial component; while 4 TKA fractures were ascribed to the liner (2 Posterior-Stabilized designs and 2 constrained designs) and only one case was on the femoral component. For every patient a revision procedure was performed, in two cases a tibial tubercle osteotomy was performed, while in one case (where the fracture was of the post cam) an arthroscopy was performed prior to the arthrotomy.

All of the UKA fractures were treated with a standard revision implant. As regard the TKA, 2 liner fractures were treated with the only liner exchange, while the other 2 liner fractures and the fracture of the metallic component were treated with total knee revision. No intra- and post-operative complications were found. Component breakage after TKA is a serious complication. Its treatment, always surgical, can hide pitfalls, especially if the timing is not correct; indeed apart from the revision of one or more components, the surgeons must address any issues of management of bone defect and ligamentous stability.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Radiosynoviorthesis is effective in patients with knee replacement and chronic synovitis**

Liepe K<sup>1</sup>, Baehr M<sup>1</sup>

<sup>1</sup>Klinikum Frankfurt (Oder), Dept. of Nuclear Medicine, Müllroser Chaussee 7, 15236 Frankfurt (Oder), Germany

After knee replacement, therapy resistant, chronic synovitis is common and leads to effusion and pain.

A cohort of 55 patients with 57 knee replacements and chronic synovitis underwent radiosynoviorthesis. In summary, 101 joints were treated using  $182 \pm 9$  MBq of <sup>90</sup>Y-citrate. The number of radiosynoviorthesis ranged from 1 to 4 (53%, 21%, 23%, and 4%). Every patient received a <sup>99m</sup>Tc-MDP scintigraphy before and three months after every radiosynoviorthesis. Follow-up ranged from 5.7 to 86.7 months. For qualitative analysis, a four steps scoring was used (0 = no response or worsening, 1 = slight, 2 = good, 3 = excellent response). For quantification, the uptake was determined within the <sup>99m</sup>Tc-MDP scintigraphy soft tissue phase before and after therapy.

At the end of long-term follow-up 27% of patients have an excellent, 24% good, 30% slight and 20% no response. The duration of response was  $7.5 \pm 8.3$  months (maximum 27 months). In repeated treatment, the effect after the first therapy was lesser than in patients who received a single treatment in total. However, three months after the last radiosynoviorthesis, patients with repeated treatment showed a similar effectiveness than single treated patients. At the end of long-term follow-up, patients with repeated radiosynoviorthesis had a higher effectiveness at similar duration response. In the <sup>99m</sup>Tc-MDP scan 65% of patients showed a reduction of uptake. When comparing subjective and objective response 78% of patients showed a concordance in both, symptoms and scintigraphy. Pilot histological analysis revealed that the synovitis is triggered by small plastic particles.

Radiosynoviorthesis is effective in patients with knee replacement and chronic synovitis. It shows good subjective and objective response rates and long response duration. Repeated treatment leads to a stronger long-time response. The chronic synovitis is caused by plastic particles, which result from the abrasion of the polymeric inlay of endoprosthesis.

**Targeting hypertrophic chondrocytes in osteoarthritis – the role of inflammation and the use of a multi-model approach in search of pharmacological treatments**

Gerjo J.V.M. van Osch

Erasmus MC, University Medical Center Rotterdam, the Netherlands  
Delft University of Technology, Delft, the Netherlands

In osteoarthritis, chondrocytes acquire a hypertrophic phenotype that contributes to matrix degradation. Inflammation is proposed as trigger for the shift to a hypertrophic phenotype. Using *in vitro* culture of human chondrocytes and cartilage explants we could not find evidence for a role of inflammatory signalling activation. We found, however, that tissue repair macrophages may contribute to the onset of hypertrophy (doi: 10.1177/19476035211021907) Intra-articularly injected triamcinolone acetonide to inhibit inflammation in a murine model of collagenase-induced osteoarthritis, increased synovial macrophage numbers and osteophytosis, confirming the role of macrophages in chondrocyte hypertrophy occurring in osteophyte formation (doi: 10.1111/bph.15780).

In search of targets to inhibit chondrocyte hypertrophy, we combined existing microarray data of different cartilage layers of murine growth plate and murine articular cartilage after induction of collagenase-induced osteoarthritis. We identified common differentially expressed genes and selected those known to be associated to inflammation. This revealed EPHA2, a tyrosine kinase receptor, as a new target. Using *in silico*, *in vitro* and *in vivo* models we demonstrated that inhibition of EPHA2 might be a promising treatment for osteoarthritis.

Recently, single cell RNA-seq. has revealed detailed information about different populations of chondrocytes in articular cartilage during osteoarthritis. We re-analysed a published scRNA-seq data set of healthy and osteoarthritic cartilage to obtain the differentially expressed genes in the population of hypertrophic chondrocytes compared to the other chondrocytes, applied pathway analyses and then used drug databases to search for upstream inhibitors of these pathways. This drug repurposing approach led to the selection of 6 drugs that were screened and tested using several *in vitro* models with human chondrocytes and cartilage explants. In this lecture I will present this sequence of studies to highlight different approaches and models that can be used in the quest for a disease modifying drug for osteoarthritis.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **A comparative study of three different bioactive glass scaffolds tailored for cartilage tissue Engineering**

Clemens Gögele<sup>1</sup>, Silvana Müller<sup>2</sup>, Sven Wiltzsch<sup>2</sup>, Armin Lenhart<sup>2</sup>, Kerstin Schäfer-Eckart<sup>3</sup>, Gundula Schulze-Tanzil<sup>1</sup>

<sup>1</sup>Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg, Germany; <sup>2</sup>Faculty of Materials Science, TH Nürnberg Georg Simon Ohm (Nuremberg); <sup>3</sup>Bone marrow Transplantation Unit, Medizinische Klinik 5, (Nürnberg)

The regenerative capacity of hyaline cartilage is greatly limited. To prevent the onset of osteoarthritis, cartilage defects have to be properly treated. Cartilage, tissue engineered by mean of bioactive glass (BG) scaffolds presents a promising approach. Until now, conventional BGs have been used mostly for bone regeneration, as they are able to form a hydroxyapatite (HA) layer and are therefore, less suited for cartilage reconstruction. The aim of this study is to compare two BGs based on a novel BG composition tailored specifically for cartilage (CAR12N) and patented by us with conventional BG (BG1393) with a similar topology. The highly porous scaffolds consisting of 100% BG (CAR12N, CAR12N with low Ca<sup>2+</sup>/Mg<sup>2+</sup> and BG1393) were characterized and dynamically seeded with primary porcine articular chondrocytes (pACs) or primary human mesenchymal stem cells (hMSCs) for up to 21 days. Subsequently, cell viability, DNA and glycosaminoglycan contents, cartilage-specific gene and protein expression were evaluated. The manufacturing process led to a comparable high (over 80%) porosity in all scaffold variants. Ion release and pH profiles confirmed bioactivity for them. After both, 7 and 21 days, more than 60% of the total surfaces of all three glass scaffold variants was densely colonized by cells with a vitality rate of more than 80%. The GAG content was significantly higher in BG1393 colonized with pACs. In general, the GAG content was higher in pAC colonized scaffolds in comparison to those seeded with hMSCs. The gene expression of cartilage-specific collagen type II, aggrecan, SOX9 and FOXO1 could be detected in all scaffold variants, irrespectively whether seeded with pACs or hMSCs. Cartilage-specific ECM components could also be detected at the protein level. In conclusion, all three BGs allow the maintenance of the chondrogenic phenotype or chondrogenic differentiation of hMSCs and thus, they present a high potential for cartilage regeneration.

**Could a decellularization protocol be validated without talking about HLA?**

Julie Manon<sup>1,4</sup>, Robin Evrard<sup>1,4</sup>, Lies Fievé<sup>2</sup>, Daela Xhema<sup>3</sup>, Louis Maistriaux<sup>2</sup>, Thomas Schubert<sup>1,4</sup>, Benoît Lengelé<sup>2</sup>, Catherine Behets<sup>2</sup>, Olivier Cornu<sup>1,4</sup>

<sup>1</sup>Pôle de Neuro-Musculo-Squelettique (NMSK), IREC, UCLouvain, Bruxelles ; <sup>2</sup>Pôle de Morphologie (MORF), IREC, UCLouvain, Bruxelles ; <sup>3</sup>Pôle de Chirurgie Expérimentale et Transplantation (CHEX), IREC, UCLouvain, Bruxelles; <sup>4</sup>Centre de Thérapie Cellulaire et Tissulaire Locomoteur (UTTICAL), Cliniques Universitaires Saint-Luc, Bruxelles

Decellularization techniques have advanced to reduce the risk of immune rejection in transplantation. Validation of these protocols typically relies on Crapo's criteria<sup>1</sup>, which include the absence of visible nuclei and low DNA content. In our study, five decellularization protocols were compared to determine the optimal approach for human fascia lata (HFL) samples. However, our findings raised questions as to why recipients can still develop immunity despite meeting validation criteria.

HFL samples were decellularized using four protocols with SDS-Triton X100-DNase (D1 to D4-HFL) and one protocol using solvent-detergent-based baths (D5-HFL). The decellularized samples (D-HFL) were compared to native samples (N-HFL) using histology, and DNA content was measured. The human leukocyte antigen (HLA) content within the matrix was assessed using western blot analysis. Both D-HFL and N-HFL samples, along with negative control patches, were implanted in the backs of 28 Wistar rats. Anti-human IgG serum levels were evaluated after one month.

H&E and Hoechst staining revealed the absence of residual cells in all decellularization protocols. DNA content was consistently below the critical threshold ( $p < 0.05$ ). All implanted D-HFL samples resulted in significantly lower anti-human IgG levels compared to N-HFL ( $p < 0.01$ ). However, 2.5 out of 4 rats developed immunity after being implanted with D1 to D4-HFL, with varying levels of anti-human IgG. Only rats implanted with D5-HFL showed undetectable levels of IgG and were considered non-immunized. Western blot analysis indicated that only D5-HFL had a residual HLA content below 1%.

The literature on decellularization has primarily relied on Crapo's criteria, which do not consider the role of HLA mismatch in acute immune rejection. Our results suggest that a residual HLA content below 1% should also be considered to prevent immunization, even if other validation criteria are met. Further research is needed to evaluate the impact of residual HLA levels on human allotransplantation outcomes.

**References:** 1. Crapo, P. M., Gilbert, T. W. & Badylak, S. F. An overview of tissue and whole organ decellularization processes. *Biomaterials* **32**, 3233–3243 (2011).

27-29 SEPTEMBER | PORTO, PORTUGAL

## A New Tissue-Engineered Product Indicated For Bone Reconstruction: A Proof-Of-Concept

Randy Buzisa Mbuku\*<sup>1,2</sup>, Christelle Sanchez\*<sup>3</sup>, Robin Evrard<sup>1,2</sup>, Alexandre Englebort<sup>1,2</sup>, Julie Manon<sup>1,2</sup>, Valentin Henri<sup>4</sup>, Gregory Nolens<sup>4</sup>, Khanh Tran Duy<sup>5</sup>, Thomas Schubert<sup>2</sup>, Yves Henrotin<sup>3,6,7</sup>, Olivier Cornu<sup>1,2</sup>

<sup>1</sup>Neuro Musculo SKEletal lab, IREC, UCLouvain, Brussels, Belgium; <sup>2</sup>Orthopedic Surgery and trauma Department, Cliniques Universitaires Saint-Luc, Brussels, Belgium; <sup>3</sup>MusculoSKEletal Innovative research Lab, University of Liège, Center for Interdisciplinary Research on Medicines, Liège, Belgium; <sup>4</sup>Cerhum SA, Liège, Belgium <sup>5</sup>3D-SIDE SA, Mont-Saint-Guibert, Belgium; <sup>6</sup>Physical Therapy and Rehabilitation department, Princess Paola Hospital, Vivalia, Marche-en-Famenne, Belgium; <sup>7</sup>Artialis SA, Liège, Belgium

\* These authors share first authorship

To design slow resorption patient-specific bone graft whose properties of bone regeneration are increased by its geometry and composition and to assess it in in-vitro and in-vivo models.

A graft composed by hydroxyapatite (HA) and  $\beta$ -TCP was designed as a cylinder with 3D gyroid porosities and 7 mm medullary space based on swine's anatomy. It was produced using a stereolithography 3D-printing machine (V6000, Prodways).

Sterile bone grafts impregnated with or without a 10 $\mu$ g/mL porcine BMP-2 (pBMP-2) solution were implanted into porcine femurs in a bone loss model. Bone defect was bi-weekly evaluated by X-ray during 3 months. After sacrifice, microscanner and non-decalcified histology analysis were conducted on biopsies.

Finally, osteoblasts were cultured inside the bone graft or in monolayer underneath the bone graft. Cell viability, proliferation, and gene expression were assessed after 7 and 14 days of cell culture (n=3 patients).

3D scaffolds were successfully manufactured with a composition of 80% HA and 20%  $\beta$ -TCP  $\pm$ 5% with indentation compressive strength of 4.14 MPa and bending strength of 11.8MPa.

In vivo study showed that bone regeneration was highly improved in presence of pBMP-2. Micro-CT shows a filling of the gyroid sinuses of the implant (*Figure 1*).

*In vitro*, the presence of BMP2 did not influence the viability of the osteoblasts and the mortality remained below 3%. After 7 days, the presence of BMP2 in the scaffold significantly increased by 85 and 65% the COL1A1 expression and by 8 and 33-fold the TNAP expression by osteoblasts in the monolayer or in the scaffold, respectively. This BMP2 effect was transient in monolayer and did not modify gene expression at day 14.

**27-29 SEPTEMBER | PORTO, PORTUGAL**

**BMP2-impregnated bone graft is a promising patient-personalized 3D-printed solution for bone defect regeneration, by promoting neighboring host cells recruitment and solid new bone formation.**

27-29 SEPTEMBER | PORTO, PORTUGAL

## **Bone tissue regeneration induced by local delivery of bone morphogenetic protein 2 from pro-angiogenic near infrared-responsive hydrogels**

Escudero-Duch C<sup>1,2</sup>, Serrano-Yamba R<sup>2</sup>, Sánchez-Casanova S<sup>2,1</sup>, Falguera-Uceda M<sup>2</sup>, Yus C<sup>3,4,1</sup>, Lerma- Juárez MA<sup>2,1</sup>, Arruebo M<sup>3,4,1</sup>, Vilaboa N<sup>2,1</sup>

<sup>1</sup>Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Spain; <sup>2</sup>Hospital Universitario La Paz-IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain; <sup>3</sup>Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain; <sup>4</sup>Departamento de Ingeniería Química, Universidad de Zaragoza, Zaragoza, Spain

In this work, we combined tissue engineering and gene therapy technologies to develop a therapeutic platform for bone regeneration. We have developed photothermal fibrin-based hydrogels that incorporate degradable CuS nanoparticles (CuSNP) which transduce incident near-infrared (NIR) light into heat. A heat-activated and rapamycin-dependent transgene expression system was incorporated into mesenchymal stem cells to conditionally control the production of bone morphogenetic protein 2 (BMP-2). Genetically engineered cells were entrapped in the photothermal hydrogels. In the presence of rapamycin, photoinduced mild hyperthermia induced the release of BMP-2 from the NIR responsive cell constructs. Transcriptome analysis of BMP-2 expressing cells showed a signature of induced genes related to stem cell proliferation and angiogenesis. We next generated 4 mm diameter calvarial defects in the left parietal bone of immunocompetent mice. The defects were filled with NIR-responsive hydrogels entrapping cells that expressed BMP-2 under the control of the gene circuit. After one and eight days, rapamycin was administered intraperitoneally followed by irradiation with an NIR laser. Ten weeks after implantation, the animals were euthanized and samples from the bone defect zone were processed for histological analysis using Masson's trichrome staining and for immunohistochemistry analyses using specific CD31 and CD105 antibodies. Samples from mice that were only administered rapamycin or vehicle or that were only NIR-irradiated showed the persistence of fibrous tissue bridging the defect. In animals that were treated with rapamycin, NIR irradiation of implants resulted in the formation of new mineralized tissue with a high degree of vascularization, thus indicating the therapeutic potential of the approach.

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**HIF-1 $\alpha$  initiates vascular recruitment and irreversible fibrosis in tendon**Greta Moschini<sup>1</sup>

<sup>1</sup>University Hospital Balgrist, University of Zurich; Institute for Biomechanics, ETH Zurich

Tendinopathy is the most common form of chronic tendon disorders, accounting for up to 30% of all musculoskeletal clinic visits [1]. In tendon disease, the largely avascular tendon tissue often becomes hypervascularized and fibrotic [2]. As blood vessel growth and angiogenic signaling molecules are often induced by the lack of adequate nutrients and oxygen, hypoxic signaling is speculated to be a root cause of tendon neovascularization and tendinopathy [3,4,5]. However, how the vascular switch is initiated in tendons, and how vascularization contributes to tendon pathology remains unknown. In this talk, we provide evidence that HIF-1 $\alpha$  is implicated in tendon disease and HIF-1 $\alpha$  stabilization in human tendon cells induces vascular recruitment of endothelial cells via VEGFa secretion. More interestingly, HIF-1 $\alpha$  stabilization in tendon cells *in vivo*, seems to recapitulate all main features of fibrotic human tendon disease, including vascular ingrowth, matrix disorganization, changes in tissue mechanics, cell proliferation and innervation. Surprisingly, *in vivo* knock-out of VEGFa rescued angiogenesis in the tendon core but it did not affect tendon mechanical properties and tissue pathophysiological changes, suggesting that blood vessels ingrowth might not be a primary cause but a consequence of HIF-1 $\alpha$  activation.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Tenocytes through space and time – determining the fate of tendon healing

Jessica Ackerman<sup>1</sup>

<sup>1</sup>University of Oxford

Tendon injuries present a major clinical challenge, as they necessitate surgical intervention and are prone to fibrotic progression. Despite advances in physical therapy and surgical technique, tendons fail to return to full native functioning, underlining the need for a biological therapeutic to improve tendon healing. Myofibroblasts are activated fibroblasts that participate in the proliferative and remodeling phases of wound healing, and while these matrix-producing cells are essential for proper healing, they are also linked to fibrotic initiation. A subset of tenocytes has been shown to give rise to the myofibroblast fate, and potentially contribute to fibrotic tendon healing. A viable anti-fibrotic therapy in other tissues has been reprogramming the fibroblast-myofibroblast differentiation route, avoiding a more pro-fibrotic myofibroblast phenotype. Thus, defining the molecular programs that underlie both physiological and pathological tendon healing is critical for the development of potential pharmacologic treatments. Towards that end, we have taken advantage of spatial transcriptomics, using the tenocyte marker *Scleraxis* as a tool, and have outlined three major spatiotemporally distinct tenocyte differentiation trajectories (synthetic, proliferative, and reactive) following acute tendon injury in mouse FDL. We have further outlined key transcriptional controls that may be manipulated to alter the differentiation process and influence the resulting myofibroblast phenotype, thereby promoting regenerative tendon healing.

**27-29 SEPTEMBER | PORTO, PORTUGAL****SPARC - a modulator of tendon homeostasis and healing**Renate Gehwolf<sup>1</sup><sup>1</sup>Paracelsus Medical University; Institute of Tendon and Bone Regeneration

Tendons are characterised by an inferior healing capacity when compared to other tissues, ultimately resulting in the formation of a pathologically altered extracellular matrix structure. Although our understanding of the underlying causes for the development and progression of tendinopathies remains incomplete, mounting evidence indicates a coordinated interplay between tendon-resident cells and the ECM is critical. Our recent results demonstrate that the matricellular protein SPARC (Secreted protein acidic and rich in cysteine) is essential for regulating tendon tissue homeostasis and maturation by modulating the tissue mechanical properties and aiding in collagen fibrillogenesis [1,2]. Consequently, we speculate that SPARC may also be relevant for tendon healing.

In a rat patellar tendon window defect model, we investigated whether the administration of recombinant SPARC protein can modulate tendon healing. Besides the increased mRNA expression of collagen type 1 and the downregulation of collagen type 3, a robust increase in the expression of pro-regenerative fibroblast markers in the repair tissue after a single treatment with rSPARC protein was observed. Additionally, pro-fibrotic markers were significantly decreased by the administration of rSPARC. Determination of structural characteristics was also assessed, indicating that the ECM structure can be improved by the application of rSPARC protein. Therefore, we believe that SPARC plays an important role for tendon healing and the application of recombinant SPARC to tendon defects has great potential to improve functional tendon repair.

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27-29 SEPTEMBER | PORTO, PORTUGAL

## **Towards Functional Patient Derived Organoids as Models for Soft Tissue Joint Disease**

Nicole Dvorak<sup>1</sup>

<sup>1</sup>University of Oxford

*In-vitro* models of disease are valuable tools for studying disease and analysing response to therapeutics. Recently, advances in patient-derived organoid (PDO) models have been shown to faithfully recapitulate structure, function, and therapeutic response for a wide range of tissues. Frozen shoulder is a rare example of a chronic inflammatory fibrotic disease which is self-limiting, unlike many other soft tissue fibrotic disorders. As no *in-vitro* 3D models or *in-vivo* animal models exist for frozen shoulder, establishing an organoid model which recapitulates core disease features may give insight into fibrosis resolution. Consequently, using biocompatible hydrogels, primary capsular fibroblasts, monocyte-derived macrophages and HUVEC cells, we generated stable PDO cultures which exhibited key disease phenotypes, including vascularization, increased stiffness, and an expanded lining layer over 21 days of culture. Through further investigation of cell-matrix and cell-cell interactions in the organoid model, we intend to unpack the differences between resolving and non-resolving fibrotic disease and uncover clinically relevant therapeutic targets for fibrosis.

**Unmet clinical needs and challenges in orthopaedic implants**Gianluca Vadalà<sup>1</sup><sup>1</sup>Campus Biomedico University, Rome, Italy

Infections are among the most diffused complications of the implantation of medical devices. In orthopedics, they pose severe societal and economic burden and interfere with the capability of the implants to integrate in the host bone, significantly increasing failure risk. Infection is particularly severe in the case of comorbidities and especially bone tumors, since oncologic patients are fragile, have higher infection rate and impaired osteoregenerative capabilities. For this reason, prevention of infection is to be preferred over treatment.

This is even more important in the case of spine surgery, since spine is among the main site for tumor metastases and because incidence of post operative surgical-site infections is significant (up to 15-20%) and surgical options are limited by the need of avoiding damaging the spinal cord.

Functionalization of the implant surfaces, so as to address infection and, possibly, co-adjuvate anti-tumor treatments, appears as a breakthrough innovation. Unmet clinical needs in infection and tumors is presented, with a specific focus on the spine, then, new perspectives are highlighted for their treatment.

**27-29 SEPTEMBER | PORTO, PORTUGAL****Double doped calcium phosphate coatings with antimicrobial efficacy for biodegradable metal biomedical implants**Julietta V. Rau<sup>1</sup><sup>1</sup>Italian National Research Council, Institute of the Structure of Matter, Rome, Italy

Over the last decades, biodegradable metals emerged as promising materials for various biomedical implant applications, aiming to reduce the use of permanent metallic implants and, therefore, to avoid additional surgeries for implant removal. However, among the important issue to be solved is their fast corrosion - too high to match the healing rate of the bone tissue. The most effective way to improve this characteristic is to coat biodegradable metals with substituted calcium phosphates. Tricalcium phosphate ( $\beta$ -TCP) is a resorbable bioceramic widely used as synthetic bone graft. In order to modulate and enhance its biological performance, the substitution of  $\text{Ca}^{2+}$  by various metal ions, such as strontium ( $\text{Sr}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), iron ( $\text{Fe}^{2+}$ ) etc., can be carried out. Among them, copper ( $\text{Cu}^{2+}$ ), manganese ( $\text{Mn}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ) etc. could add antimicrobial properties against implant-related infections. Double substitutions of TCP containing couples of  $\text{Cu}^{2+}/\text{Sr}^{2+}$  or  $\text{Mn}^{2+}/\text{Sr}^{2+}$  ions are considered to be the most perspective based on the results of our study. We established that single phase  $\text{Ca}_{3-2x}(\text{M}'\text{M}'')_x(\text{PO}_4)_2$  solid solutions are formed only at  $x \leq 0.286$ , where  $\text{M}'$  and  $\text{M}''$ —divalent metal ions, such as  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ , and that in case of double substitutions, the incorporation of  $\text{Sr}^{2+}$  ions allows one to extend the limit of solid solution due to the enlargement of the unit cell structure. We also reported that antimicrobial properties depend on the substitution ion occupation of  $\text{Ca}^{2+}$  crystal sites in the  $\beta$ -TCP structure. The combination of two different ions in the  $\text{Ca}_5$  position, on one side, and in the  $\text{Ca}_1$ ,  $\text{Ca}_2$ ,  $\text{Ca}_3$ , and  $\text{Ca}_4$  positions, on another side, significantly boosts antimicrobial properties. In the present work, zinc-lithium (Zn-Li) biodegradable alloys were coated with double substituted  $\text{Mn}^{2+}/\text{Sr}^{2+}$   $\beta$ -TCP and double substituted  $\text{Cu}^{2+}/\text{Sr}^{2+}$   $\beta$ -TCP, with the scope to promote osteoinductive effect (due to the  $\text{Sr}^{2+}$  presence) and to impart antimicrobial properties (thanks to  $\text{Cu}^{2+}$  or  $\text{Mn}^{2+}$  ions). The Pulsed Laser Deposition (PLD) method was applied as the coating's preparation technique. It was shown that films deposited using PLD present good adhesion strength and hardness and are characterized by a nanostructured background with random microparticles on the surface. For coatings characterization, Fourier Transform Infrared Spectroscopy, X-ray Diffraction, and Scanning Electron Microscopy coupled with Energy Dispersive X-ray and X-ray Photoelectron Spectroscopy were applied. The microbiology tests on the prepared coated Zn-Li alloys were performed with the Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (*Salmonella typhimurium*, *Escherichia coli*) bacteria strains and *Candida albicans* fungus. The antimicrobial activity tests showed that  $\text{Mn}^{2+}/\text{Sr}^{2+}$   $\beta$ -TCP-coated and  $\text{Cu}^{2+}/\text{Sr}^{2+}$   $\beta$ -TCP coated Zn-Li alloys were able to inhibit

**27-29 SEPTEMBER | PORTO, PORTUGAL**

the growth of all five microorganisms. The prepared coatings are promising in improving the degradation behavior and biological properties of Zn-Li alloys, and further studies are necessary before a possible clinical translation.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **New zinc-bone apatite films show anti-tumor efficacy against bone metastases *in vitro***

Gabriela Graziani<sup>1</sup>

<sup>1</sup>Rizzoli Orthopaedic Institute, Bologna, Italy

Decreasing the chance of local relapse or infection after surgical excision of bone metastases is a main goal in orthopedic oncology. Indeed, bone metastases have high incidence rate (up to 75%) and important cross-relations with infection and bone regeneration. Even in patients with advanced cancer, bone gaps resulting from tumor excision must be filled with bone substitutes. Functionalization of these substitutes with antitumor and antibacterial compounds could constitute a promising approach to overcome infection and tumor at one same time. Here, for the first time, we propose the use of nanostructured zinc-bone apatite coatings having antitumor and antimicrobial efficacy. The coatings are obtained by Ionized Jet Deposition from composite targets of zinc and bovine-derived bone apatite.

Antibacterial and antibiofilm efficacy of the coatings is demonstrated *in vitro* against *S. Aureus* and *E. Coli*. Anti-tumor efficacy is investigated against MDA-MB-231 cells and biocompatibility is assessed on L929 and MSCs.

A microfluidic based approach is used to select the optimal concentration of zinc to be used to obtain antitumor efficacy and avoid cytotoxicity, exploiting a custom gradient generator microfluidic device, specifically designed for the experiments. Then, coatings capable of releasing the desired amount of active compounds are manufactured. Films morphology, composition and ion-release are studied by FEG-SEM/EDS, XRD and ICP. Efficacy and biocompatibility of the coatings are verified by investigating MDA, MSCs and L929 viability and morphology by Alamar Blue, Live/Dead Assay and FEG-SEM at different timepoints. Statistical analysis is performed by SPSS/PC + Statistics TM 25.0 software, one-way ANOVA and post-hoc Sheffé test. Data are reported as Mean  $\pm$  standard Deviation at a significance level of  $p < 0.05$ .

**Results and Discussion.** Coatings have a nanostructured surface morphology and a composition mimicking the target. They permit sustained zinc release for over 14 days in medium. Thanks to these characteristics, they show high antibacterial ability (inhibition of bacteria viability and adhesion to substrate) against both the gram + and gram - strain.

The gradient generator microfluidic device permits a fine selection of the concentration of zinc to be used, with many potential perspectives for the design of biomaterials.

For the first time, we show that zinc and zinc-based coatings have a selective efficacy against MDA cells. Upon mixing with bone apatite, the efficacy is maintained and cytotoxicity is avoided. For the first time, new antibacterial metal-based films are

**27-29 SEPTEMBER | PORTO, PORTUGAL**

proposed for addressing bone metastases and infection at one same time. At the same time, a new approach is proposed for the design of the coatings, based on a microfluidic approach. We demonstrated the efficacy of Zn against the MDA-MB-231 cells, characterized for their ability to form bone metastases *in vivo*, and the possibility to use nanostructured metallic coatings against bone tumors. At the same time, we show that the gradient-generator approach is promising for the design of antitumor biomaterials.

Efficacy of Zn films must be verified *in vivo*, but the dual-efficacy coatings appear promising for orthopedic applications.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **New high throughput methods for antibacterial validation of metallic thin films: different metals for different microbial communities**

Daniele Ghezzi<sup>1,2</sup>, Maria Sartori<sup>3</sup>, Marco Boi<sup>2</sup>, Matteo Montesissa<sup>4</sup>, Enrico Sassoni<sup>5</sup>, Milena Fini<sup>6</sup>, Nicola Baldini<sup>2,4</sup>, Martina Cappelletti<sup>1</sup>, Gabriela Graziani<sup>2,7</sup>

<sup>1</sup>University of Bologna, Department of Pharmacy and Biotechnology, via Irnerio 42, 40126, Bologna, Italy; <sup>2</sup>IRCCS Istituto Ortopedico Rizzoli, Biomedical Science and Technologies and Nanobiotechnology Lab, via di Barbiano 1/10, 40136, Bologna, Italy; <sup>3</sup>IRCCS Istituto Ortopedico Rizzoli, Surgical Sciences and Technologies, via di Barbiano 1/10, 40136, Bologna, Italy; <sup>4</sup>University of Bologna, Department of Biomedical and Neuromotor Sciences, via Massarenti 9, 40128, Bologna, Italy; <sup>5</sup>University of Bologna, Department of Civil, Chemical, Environmental and Materials Engineering, via Terracini 28, 40131, Bologna, Italy; <sup>6</sup>IRCCS Istituto Ortopedico Rizzoli, Scientific Direction, via di Barbiano 1/10, 40136, Bologna, Italy; <sup>7</sup>Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "G. Natta", Via Luigi Mancinelli 7, 20131, Milan, Italy

Prosthetic joint infections represent complications connected to the implantation of biomedical devices, they have high incidence, interfere with osseointegration, and lead to a high societal burden. The microbial biofilm, which is a complex structure of microbial cells firmly attached to a surface, is one of the main issues causing infections. Biofilm-forming bacteria are acquiring more and more resistances to common clinical treatments due to the abuse of antibiotics administration. Therefore, there is increasing need to develop alternative methods exerting antibacterial activities against multidrug-resistant biofilm-forming bacteria. In this context, metal-based coatings with antimicrobial activities have been investigated and are currently used in the clinical practice. However, traditional coatings exhibit some drawbacks related to the insufficient adhesion to the substrate, scarce uniformity and scarce control over the toxic metal release reducing their efficacy. Here, we propose the use of antimicrobial silver-based nanostructured thin films to discourage bacterial infections. Coatings are obtained by Ionized Jet Deposition, a plasma-assisted technique that permits to manufacture films of submicrometric thickness having a nanostructured surface texture, allow tuning silver release, and avoid delamination. To mitigate interference with osseointegration, here silver composites with bone apatite and hydroxyapatite were explored. The antibacterial efficacy of silver films was tested *in vitro* against gram-positive and gram-negative species to determine the optimal coatings characteristics by assessing reduction of bacterial viability, adhesion to substrate, and biofilm formation. Efficacy was tested in an *in vivo* rabbit model, using a multidrug-resistant strain of *Staphylococcus aureus* showing significant reduction of the bacterial load on the silver prosthesis both when coated with the metal only (>99% reduction) and when in combination with bone apatite (>86% reduction). These

**27-29 SEPTEMBER | PORTO, PORTUGAL**

studies indicate that IJD films are highly tunable and can be a promising route to overcome the main challenges in orthopedic prostheses.

27-29 SEPTEMBER | PORTO, PORTUGAL

## **In vitro 3D study of the effect of uniaxial loading on naïve MSC differentiation fate**

Priscilla Fülleemann, Thomas Jörimann, Martin Stoddart, Romano Matthys<sup>2</sup>, [Sophie Verrier](#)

<sup>1</sup>AO Research Institute Davos, Switzerland, <sup>2</sup>RISystem AG, Landquart, Switzerland

Bone healing outcome is highly dependent on the initial mechanical fracture environment [1]. In vivo, direct bone healing requires absolute stability and an interfragmentary strain (IFS) below 2% [2]. In the majority of cases, however, endochondral ossification is engaged where frequency and amplitude of IFS are key factors. Still, at the cellular level, the influence of those parameters remains unknown. Understanding the regulation of naïve hMSC differentiation is essential for developing effective bone healing strategies.

Human bone-marrow-derived MSC (KEK-ZH-NR: 2010-0444/0) were embedded in 8% gelatin methacryol. Samples (5mm Ø x 4mm) were subjected to 0, 10 and 30% compressive strain (5sec compression, 2hrs pause sequence for 14 days) using a multi-well uniaxial bioreactor (RISystem) and in presence of chondro-permissive medium (CP, DMEM HG, 1% NEAA, 10 µM ITS, 50 µg/mL ascorbic acid, and 100 mM Dex). Cell differentiation was assessed by qRT-PCR and histo-/immunohistology staining. Experiments were repeated 5 times with cells from 5 donors in duplicate. ANOVA with Tukey post-hoc correction or Kurskal-Wallis test with Dunn's correction was used.

Data showed a strong upregulation of hypertrophic related genes COMP, MMP13 and Type 10 collagen upon stimulation when compared to chondrogenic SOX9, ACAN, Type 2 collagen or to osteoblastic related genes Type 1 Collagen, Runx2. When compared to chondrogenic control medium, cells in CP with or without stimulation showed low proteoglycan synthesis as shown by Safranin-O-green staining. In addition, the cells were significantly larger in 10% and 30% strain compared to control medium with 0% strain. Type 1 and 10 collagens immunostaining showed stronger Coll 10 expression in the samples subjected to strain compared to control.

Uniaxial deformation seems to mainly promote hypertrophic-like chondrocyte differentiation of MSC. Osteogenic or potentially late hypertrophic related genes are also induced by strain.

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**27-29 SEPTEMBER | PORTO, PORTUGAL****Ultrasound counteracts inflammation and induces chondrogenic differentiation of ASCs in 3D piezoelectric hydrogels**

Cristina Manferdini<sup>1</sup>, Elena Gabusi<sup>1</sup>, Paolo Dolzani<sup>1</sup>, Diego Trucco<sup>2,3</sup>, Enrico Lenzi<sup>1</sup>, Giovanni D'Atri<sup>1</sup>, Lorenzo Vannozzi<sup>2,3</sup>, Andrea Cafarelli<sup>2,3</sup>, Leonardo Ricotti<sup>2,3</sup>, Gina Lisignoli<sup>1</sup>

<sup>1</sup>IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale Bologna, Italy; <sup>2</sup>The BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy; <sup>3</sup>Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, Pisa, Italy

In cartilage tissue engineering (TE), new solutions are needed to effectively drive chondrogenic differentiation of mesenchymal stromal cells in both normal and inflammatory milieu. Ultrasound waves represent an interesting tool to facilitate chondrogenesis. In particular, low intensity pulsed ultrasound (LIPUS) has been shown to regulate the differentiation of adipose mesenchymal stromal cells. Hydrogels are promising biomaterials capable of encapsulating MSCs by providing an instructive biomimetic environment, graphene oxide (GO) has emerged as a promising nanomaterial for cartilage TE due to its chondroinductive properties when embedded in polymeric formulations, and piezoelectric nanomaterials, such as barium titanate nanoparticles (BTNPs), can be exploited as nanoscale transducers capable of inducing cell growth/differentiation. The aim of this study was to investigate the effect of dose-controlled LIPUS in counteracting inflammation and positively committing chondrogenesis of ASCs embedded in a 3D piezoelectric hydrogel.

ASCs at  $2 \times 10^6$  cells/mL were embedded in a 3D VitroGel RGD<sup>®</sup> hydrogel without nanoparticles (Control) or doped with 25 µg/ml of GO nanoflakes and 50 µg/ml BTNPs. The hydrogels were exposed to basal or inflammatory milieu (+IL1β 10ng/ml) and then to LIPUS stimulation every 2 days for 10 days of culture. Hydrogels were chondrogenic differentiated and analyzed after 2, 10 and 28 days. At each time point cell viability, cytotoxicity, gene expression and immunohistochemistry (COL2, aggrecan, SOX9, COL1) and inflammatory cytokines were evaluated.

Ultrasound stimulation significantly induced chondrogenic differentiation of ASCs loaded into 3D piezoelectric hydrogels under basal conditions: COL2, aggrecan and SOX9 were significantly overexpressed, while the fibrotic marker COL1 decreased compared to control samples. LIPUS also has potent anti-inflammatory effects by reducing IL6 and IL8 and maintaining its ability to boost chondrogenesis.

These results suggest that the combination of LIPUS and piezoelectric hydrogels promotes the differentiation of ASCs encapsulated in a 3D hydrogel by reducing the inflammatory milieu, thus representing a promising tool in the field of cartilage TE.

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# EORS 2023

31st Annual Meeting of the  
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**27-29 SEPTEMBER | PORTO, PORTUGAL**

**ADMAIORA (AdvanceD nanocomposite MAterials for in situ treatment and ultRASound-mediated management of osteoarthritis).**

**Mechanical stimulation of piezoelectric scaffolds promotes the cells osteogenic differentiation**Nikoleta Natalia Tavernaraki <sup>1</sup>, Varvara Platania <sup>1</sup>, Maria Chatzinikolaidou <sup>1,2</sup>

<sup>1</sup>Department of Materials Science and Technology, University of Crete, Heraklion, Greece; <sup>2</sup>Foundation for Research and Technology Hellas (FO.R.T.H)-IESL, Heraklion, Greece

Bone is a dynamic tissue that undergoes continuous mechanical forces. Mechanical stimuli applied on scaffolds resembling a part of the human bone tissue affects the osteogenesis [1]. Poly(3,4-ethylenedioxythiophene) (PEDOT) is a piezoelectric material that responds to mechanical stimulation producing an electrical signal, which in turn promotes the osteogenic differentiation of bone-forming cells by opening voltage-gated calcium channels [2]. In this study we examined the biological behavior of pre-osteoblastic cells seeded onto lyophilized piezoelectric PEDOT-containing scaffolds applying uniaxial compression.

Two different concentrations of PEDOT (0.10 and 0.15% w/v) were combined with a 5% w/v poly(vinyl alcohol) (PVA) and 5% w/v gelatin, casted into wells, freeze dried and crosslinked with 2% v/v (3-glycidyloxypropyl)trimethoxysilane and 0.025% w/v glutaraldehyde. The scaffolds were physicochemically characterized by FTIR, measurement of the elastic modulus, swelling ratio and degradation rate. The cell-loaded scaffolds were subjected to uniaxial compression with a frequency of 1 Hz and a strain of 10% for 1 h every second day for 21 days. The loading parameters were selected to resemble the in vivo loading situation [3]. Cell viability and morphology on the PEDOT/PVA/gelatin scaffolds was determined. The alkaline phosphatase (ALP) activity, the collagen and calcium production were determined.

The elastic modulus of PEDOT/PVA/gelatin scaffolds ranged between 1 and 5 MPa. The degradation rate indicates a mass loss of 15% after 21 days. The cell viability assessment displays excellent biocompatibility, while SEM images display well-spread cells. The ALP activity at days 3, 7 and 18 as well as the calcium production are higher in the dynamic culture compared to the static one. Moreover, energy dispersive spectroscopy analysis revealed the presence of calcium phosphate in the extracellular matrix after 14 days. The results demonstrate that PEDOT/PVA/gelatin scaffolds promote the adhesion, proliferation, and osteogenic differentiation of pre-osteoblastic cells under mechanical stimulation, thus favoring bone regeneration.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**Electrical stimulation combined with additive manufactured 3D conductive scaffolds towards improved bone tissue engineering strategies**

João C. Silva<sup>1,2</sup>, Fábio F.F. Garrudo<sup>1,2,3</sup>, João Meneses<sup>4</sup>, Pedro Marcelino<sup>1,2,4</sup>, Frederico Barbosa<sup>1,2</sup>, Carla S. Moura<sup>3,5</sup>, Nuno M. Alves<sup>4</sup>, Paula Pascoal-Faria<sup>4</sup> and Frederico C. Ferreira<sup>1,2</sup>

<sup>1</sup>Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>2</sup>Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>3</sup>Department of Bioengineering and Instituto de Telecomunicações, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; <sup>4</sup>CDRSP – Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Rua de Portugal-Zona Industrial, Marinha Grande, Portugal; <sup>5</sup>Polytechnic Institute of Coimbra, Applied Research Institute, Rua da Misericórdia, Lagar dos Cortiços – S. Martinho do Bispo, 3045-093 Coimbra, Portugal

The growing number of non-union fractures in an aging population has increased the clinical demand for tissue-engineered bone. Electrical stimulation (ES) has been described as a promising strategy for bone regeneration treatments in several clinical studies. However the underlying mechanism by which ES augments bone formation is still poorly understood and its use in bone tissue engineering (BTE) strategies is currently underexplored. Additive manufacturing (AM) technologies (Fused Deposition Modeling/3D Printing) have been widely used in BTE due to their ability to fabricate scaffolds with a high control over their structural and mechanical properties in a reproducible and scalable manner. Thus, in this work, we combined AM methods with conductive biomaterials and ES to enhance the osteogenic differentiation of human bone marrow-derived mesenchymal stem/stromal cells (hBMSCs) envisaging improved BTE strategies.

First, we started by developing AM-based electro-bioreactor devices containing medical-grade electrodes (stainless steel and Ti6Al4V) to apply ES to monolayer 2D cultures and 3D cell-seeded scaffolds. Computer modeling(Finite Element Analysis-FEA) was employed to predict the magnitude/distribution of electrical fields within the ES devices and along the different conductive scaffolds. Prior to scaffold culture, 5 different ES protocols were tested in terms of their ability to promote hBMSCs proliferation and osteogenic differentiation in 2D cultures. The best performance ES protocol was then used in two different AM-based BTE strategies: 1) Two different conductive scaffolds (conductive poly lactic acid (PLA) and titanium) were seeded with hBMSCs and cultured for 21 days under osteogenic medium conditions with and without ES and their biological performance was evaluated in comparison to non-conductive standard PLA scaffolds; 2) Different PEDOT:PSS-based coating solutions were screened to obtain PEDOT:PSS/Gelatin-coated 3D polycaprolactone (PCL)

**27-29 SEPTEMBER | PORTO, PORTUGAL**

scaffolds with a high( $11 \text{ S.cm}^{-1}$ ) and stable electroconductivity. When cultured under ES, PEDOT:PSS/Gelatin-PCL scaffolds enhanced significantly hBMSCs osteogenic differentiation and mineralization(calcium deposition), highlighting their potential for BTE applications.

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**Bone and its interaction with other organ systems in health and disease**Martijn van Griensven<sup>1</sup>

<sup>1</sup>dept. cBITE, MERLN Institute, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht, the Netherlands

Anatomically, bone consists of building blocks called osteons, which in turn comprise a central canal that contains nerves and blood vessels. This indicates that bone is a highly innervated and vascularized tissue. The function of vascularization in bone (development) is well-established: providing oxygen and nutrients that are necessary for the formation, maintenance, and healing. As a result, in the field of bone tissue engineering many research efforts take vascularization into account, focusing on engineering vascularized bone. In contrast, while bone anatomy indicates that the role of innervation in bone is equally important, the role of innervation in bone tissue engineering has often been disregarded.

For many years, the role of innervation in bone was mostly clear in physiology, where innervation of a skeleton is responsible for sensing pain and other sensory stimuli. Unraveling its role on a cellular level is far more complex, yet more recent research efforts have unveiled that innervation has an influence on osteoblast and osteoclast activity. Such innervation activities have an important role in the regulation of bone homeostasis, stimulating bone formation and inhibiting resorption. Furthermore, due to their anatomical proximity, skeletal nerves and blood vessels interact and influence each other, which is also demonstrated by pathways cross-over and joint responses to stimuli.

Besides those closely connected systems, the immune system plays also a pivotal role in bone regeneration. Certain cytokines are important to attract osteogenic cells and (partially) inhibit bone resorption. Several leukocytes also play a role in the bone regeneration process.

Overall, bone interacts with several systems. Aberrations in those systems affect the bone and are important to understand in the context of bone regeneration. This crosstalk has become more evident and is taken more into consideration. This leads to more complex tissue regeneration, but may recapitulate better physiological situations.

27-29 SEPTEMBER | PORTO, PORTUGAL

## The interplay of angiogenesis and osteogenesis for bone regeneration

Banfi A.<sup>1</sup>

<sup>1</sup>Regenerative Angiogenesis Lab, Department of Biomedicine, Basel University Hospital, Basel, CH

Bone regeneration is an area of acute medical need, but its clinical success is hampered by the need to ensure rapid vascularization of osteogenic grafts. Vascular Endothelial Growth Factor (VEGF) is the master regulator of vascular growth and during bone development angiogenesis and osteogenesis are physiologically coupled through so-called angiocrine factors produced by blood vessels. However, how to exploit this process for therapeutic bone regeneration remains a challenge (1).

Here we will describe recent work aiming at understanding the cross-talk between vascular growth and osteogenesis under conditions relevant for therapeutic bone regeneration. To this end we take advantage of a unique platform to generate controlled signalling microenvironments, by the covalent decoration of fibrin matrices with tunable doses and combinations of engineered growth factors. The combination of human osteoprogenitors and hydroxyapatite in these engineered fibrin matrices provides a controlled model to investigate how specific molecular signals regulate vascular invasion and bone formation *in vivo*. In particular, we found that:

1) Controlling the distribution of VEGF protein in the microenvironment is key to recapitulate its physiologic function to couple angiogenesis and osteogenesis (2);

2) Such coupling is exquisitely dependent on VEGF dose and on a delicate equilibrium between opposing effects. A narrow range of VEGF doses specifically activates Notch1 signaling in invading blood vessels, inducing a pro-osteogenic functional state called Type H endothelium, that promotes differentiation of surrounding mesenchymal progenitors. However, lower doses are ineffective and higher ones paradoxically inhibit both vascular invasion and bone formation (Figure 1) (3);

3) Semaphorin3a (Sema3a) acts as a novel pro-osteogenic angiocrine factor downstream of VEGF and it mediates VEGF dose-dependent effects on both vascular invasion and osteogenic progenitor stimulation.

In conclusion, vascularization of osteogenic grafts is not simply necessary in order to enable progenitor survival. Rather, blood vessels can actively stimulate bone regeneration in engineered grafts through specific molecular signals that can be harnessed for therapeutic purposes.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## Functional activity of mCRP in intervertebral disc

Clara Ruiz-Fernández<sup>1,2</sup>, Djedjiga Ait Eldjoudi<sup>1</sup>, María Gonzalez-Rodríguez<sup>1</sup>, Alfonso Cordero Barreal<sup>1</sup>, Yousof Farrag<sup>1</sup>, Ali Mobasher<sup>3</sup>, Jesús Pino<sup>1</sup>, Daisuke Sakai<sup>2</sup>, Oreste Gualillo<sup>1</sup>

<sup>1</sup>University Clinical Hospital of Santiago de Compostela and IDIS (Health Research Institute of Santiago de Compostela), NEIRID Lab (Neuroendocrine Interactions in Rheumatology and Inflammatory Diseases), Santiago de Compostela, Spain; <sup>2</sup>Intervertebral Disc Research Unit, Department of Orthopedic Surgery, Tokai University School of Medicine, Isehara, Japan; <sup>3</sup>Research Unit of Medical Imaging, Physics, and Technology, Faculty of Medicine, University of Oulu, Finland

Monomeric C reactive protein (mCRP) presents important proinflammatory effects in endothelial cells, leukocytes, or chondrocytes. However, CRP in its pentameric form exhibits weak anti-inflammatory activity. It is used as a biomarker to follow severity and progression in infectious or inflammatory diseases, such as intervertebral disc degeneration (IVDD). This work assesses for the first time the mCRP effects in human intervertebral disc cells, trying to verify the pathophysiological relevance and mechanism of action of mCRP in the etiology and progression of IVD degeneration.

We demonstrated that mCRP induces the expression of multiple proinflammatory and catabolic factors, like nitric oxide synthase 2 (NOS2), cyclooxygenase 2 (COX2), matrix metalloproteinase 13 (MMP13), vascular cell adhesion molecule 1 (VCAM1), interleukin (IL)-6, IL-8, and lipocalin 2 (LCN2), in human annulus fibrosus (AF) and nucleus pulposus (NP) cells. We also showed that nuclear factor- $\kappa$ B (NF- $\kappa$ B), extracellular signal-regulated kinase 1/2 (ERK1/2), and phosphoinositide 3-kinase (PI3K) are at play in the intracellular signaling of mCRP.

Our results indicate that the effect of mCRP is persistent and sustained, regardless of the proinflammatory environment, as it was similar in healthy and degenerative human primary AF cells. This is the first article that demonstrates the localization of mCRP in intravertebral disc cells of the AF and NP and that provides evidence for the functional activity of mCRP in healthy and degenerative human AF and NP disc cells.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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27-29 SEPTEMBER | PORTO, PORTUGAL

## The Bone Morphogenetic Protein L51P Enhances Spinal Fusion in Combination with BMP2 - An *In Vivo* Rat Spinal Fusion Model of the Elderly

Benjamin Gantenbein<sup>1,2</sup>, Katharina A. C. Oswald<sup>1</sup>, Sebastian F. Bigdon<sup>1</sup>, Georg F. Erbach<sup>1</sup>, Andreas S. Croft<sup>2</sup>, Paola Bermudez-Lekerika<sup>2</sup>, Franziska Strunz<sup>2</sup>, Niklas Rutsch<sup>1</sup>, Christoph E. Albers<sup>1</sup>

<sup>1</sup>Department of Orthopaedic Surgery & Traumatology, Medical Faculty, Inselspital, University of Bern, Switzerland; <sup>2</sup>Tissue Engineering for Orthopaedics and Mechanobiology, Bone & Joint Program, Department for BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, Switzerland

Non-union and pseudoarthrosis remain major complications after spinal fusion surgery, resulting in unsatisfactory outcomes and high socio-economic costs. Several biomaterials and osteo-biologics have been used to improve spinal fusion, including bone morphogenetic protein (BMP) 2. However, its necessary high dose application often leads to adverse effects. L51P, a BMP-2 analogue and inhibitor of BMP antagonists, has been shown to augment BMP-induced bone formation and lower the required doses. The current study therefore aimed to demonstrate the effects of L51P and BMP-2 on spinal fusion *in vivo*. 46 elderly Wistar rats (~12 months, 52% female, 423±78g) underwent a two-step spinal fusion surgery [1]. Firstly, a custom external fixator was applied in the proximal tail. Secondly, discectomy and disc replacement with a  $\beta$  tri-calcium-phosphate ( $\beta$ -TCP) carrier were conducted. Carriers were loaded with the study compounds based on random and blinded allocation into seven groups: Phosphate-buffered-saline (PBS) as material control, 1 $\mu$ g and 10 $\mu$ g BMP-2, 10 $\mu$ g L51P, 1 $\mu$ g BMP-2 + 1/5/10 $\mu$ g L51P. Digital X-rays were performed on day zero, at six weeks, and twelve weeks postoperatively. After twelve weeks, high-resolution  $\mu$ CT scans and histology were obtained. *Results.* At twelve weeks, 10 $\mu$ g BMP-2, 1 $\mu$ g BMP-2 + 5 $\mu$ g L51P and 1 $\mu$ g BMP-2 + 10 $\mu$ g L51P showed significantly higher fusion rates compared to the PBS control in X-ray analysis.  $\mu$ CT analysis showed significantly higher fusion rates for all groups compared to the control group. 1 $\mu$ g BMP-2 + 1 $\mu$ g L51P demonstrated significantly higher fusion rates than 1 $\mu$ g BMP2 alone and equivalent ossification compared to 10 $\mu$ g BMP-2; higher doses of L51P did not lead to a better fusion outcome. Histological analysis confirmed the radiographical results. Combining low doses of L51P and BMP-2 enhances spinal fusion equivalent to high-dose BMP-2 and may reduce BMP-2 doses and side effects at similar to higher efficacy in clinical application.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Patients Perspectives on Orthobiologics for Osteoarthritis**

Feza Korkusuz<sup>1</sup>

<sup>1</sup>Hacettepe University Medical Faculty

Disease modifying approaches are commonly applied in OA patients. An aging society with better life expectancies is increasing in Europe and the globe. Orthobiologics cover intraarticular hyaluronan injections and also cellular therapies. Cellular therapies range from platelet rich plasma (PRP) applications to exosomes. Short term follow-up of limited number of patients revealed favorable results in clinical cellular therapies. Most of these studies evaluated decrease of pain and increase in function. Recent basic science studies focused on the action mechanism of orthobiologic therapies however patient perspective is less studied. Our research team has recently performed a qualitative study on the patient perspective of hyaluronan injection of the knee joint. Findings of that study will be shared and future patient knowledge based options on orthobiologics will be discussed.

**Cellular and Molecular Endotypes for Osteoarthritis**Girish Pattappa<sup>1</sup>

<sup>1</sup>Experimental Trauma Surgery, Department of Trauma Surgery, University Regensburg Medical Centre, Regensburg, Germany

The biological understanding for the disease progression osteoarthritis (OA) has uncovered specific biomarkers from either synovial fluid, articular chondrocytes or synoviocytes that can be used to diagnose the disease. Examples of these biomarkers include interleukin-1 $\beta$  (IL-1 $\beta$ ) or collagen II fragments (1, 2). In parallel, isolation of chondrocytes or bone marrow derived mesenchymal stromal cells (MSCs) has yielded cell-based strategies that have shown long- term beneficial effects in a specific cohort of patients, specifically in traumatic cartilage lesions (2). This latter finding shows that patient stratification of OA is an important tool to both match patients for a specific treatment and to develop novel therapies, especially disease modifying drugs. In order to create disease stage specific therapies, the use of next generation analysis tools such as RNAseq and metabolomics, has the potential to decipher specific cellular and molecular endotypes. Alongside greater understanding of the clinical phenotype (e.g. imaging, pain, co- morbidities), therapies can be designed to alleviate the symptoms of OA at specific points of the disease in patients. This talk will outline the current biological understanding of OA and discuss how patient stratification could assist in the design of innovative therapies for the disease.

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

## **Translational Models for OA: Speeding up from Bench to Bedside**

Sylvia Nürnberger<sup>1</sup>

<sup>1</sup>Medical University Vienna

Translational models for OA have used a variety of small (mouse, rat) and large (sheep, pig) animal models to evaluate the efficacy of a specific therapy. Clinical trials based on the results of these animal models have yielded mixed results with respect to the treatment of the disease. Due to greater stringency in EU regulations in the use of animal models for research, ex vivo models of OA (e.g. cartilage explants, bioreactors) are being developed to mimic human joint motion as well as the inflammatory milieu (e.g. IL-1 $\beta$ ) that can be used to understand efficacy of therapy in a physiological environment. The development of these models can enable therapies to undergo clinical trials in patients without the necessity for long-term animal studies. This presentation will describe the state of the art in this field and will discuss whether there is potential to speed up translation from bench to bedside in the future.

# EORS 2023

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**27-29 SEPTEMBER | PORTO, PORTUGAL**

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