A Novel Platform for Investigating Neuroimmune Interactions in Degenerative Disc Disease

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INTRODUCTION: Low back pain affects up to 80% of individuals over their lifetime and remains the leading cause of disability worldwide. Intervertebral disc (IVD) degeneration, particularly common in the elderly, is a major contributor. Degeneration disrupts the extracellular matrix, facilitates immune cell infiltration, and permits aberrant sensory nerve ingrowth into the normally aneural, avascular disc, driving pain. Yet, the cellular and neuronal mechanisms underlying discogenic pain remain poorly defined.

Animal models have provided critical insights into disc biology, immune involvement, and pain behaviors, but they offer limited control over cellular microenvironments and make it difficult to precisely monitor or manipulate disc-neuron crosstalk in real time. Likewise, conventional in vitro models cannot recapitulate the disc's complex, multicellular, and mechanically dynamic environment, restricting their translational relevance. Prior disc-on-a-chip systems using intact discs are also not well suited to interrogate neuroimmune crosstalk.

To address these gaps, we developed a biomimetic 3D disc model integrated with a microfluidic innervation-on-chip platform, enabling real-time analysis of how degenerative discs influence sensory neurite ingrowth and neuronal activation.

METHODS: AF and NP cells were isolated from healthy murine lumbar discs. AF cells were encapsulated in a fibrin–collagen gel (10 mg/mL fibrinogen, 1 mg/mL collagen), which contracted to induce circumferential alignment. A central cavity (3 mm) was filled with NP cells in a softer fibrin gel (5 mg/mL fibrinogen), reconstructing AF–NP anatomy. 3D discs were cultured in DMEM-F12 with 10% FBS, with degeneration induced by IL-1β (10 ng/mL, 24 h). For neuronal studies, lumbar dorsal root ganglia (DRG) were enzymatically dissociated and seeded onto poly-lysine-coated microfluidic biochips featuring a disc chamber and neuron chamber connected by 50 μm × 500 μm innervation channels. After 24 h, discs (±IL-1β) were introduced into the disc chamber. Neurite outgrowth was quantified by β-III-tubulin immunostaining; neuronal excitability was assessed by calcium imaging using Fluo-4 or GCaMP biosensors.

RESULTS: Engineered 3D discs successfully recapitulated native compartmental organization and functionality. In co-culture, degenerative discs significantly enhanced neurite outgrowth across innervation channels compared to intact discs or controls. Quantitative analysis revealed a greater number of neurite and increased directional extension, accompanied by shorter average neurite length. Calcium imaging demonstrated that DRG neurons exposed to degenerative discs or their conditioned media exhibited frequent, high-amplitude calcium transients (2.3-fold increase) and upregulation of CGRP and NGF, consistent with neuronal hyperexcitability, whereas neurons cultured with intact discs remained at baseline. Furthermore, curcumin treatment attenuated neurite extension, highlighting the model's potential for therapeutic testing.

DISCUSSION & CONCLUSIONS: This integrated 3D disc-innervation platform recapitulates both the structural hallmarks of disc degeneration and its functional impact on sensory neurons. By combining a biomimetic 3D disc with a microfluidic innervation-on-chip platform, we establish a physiologically relevant, controllable system to dissect disc–nerve crosstalk in real time. The platform bridges clinical relevance and basic science innovation, providing mechanistic insight into pain generation while serving as a versatile platform for studying disc–nerve interactions and evaluating candidate therapeutics. Together, it advances both fundamental understanding and translational strategies to alleviate chronic low back pain.

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Osteoarthritis – an evolutionary mismatch disease?

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SUMMARY: Osteoarthritis is a our most common chronic joint disease, and there is a tremendous need for new effective treatments. The last 60 years there has been a rapid change in lifestyle and diet in most modern societies. The prevalence of osteoarthritis increases rapidly due to increase in body mass index and ageing in modern societies. This talk will briefly review the concept of osteoarthritis as partly an *evolutionary mismatch disease*, i.e. our genes have not yet sufficiently adapted to the environment we live in. I will also talk about new treatment opportunities (and concerns) by GLP-1-receptor agonists.

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Temporal Proteomic Profiling of PTOA Identifies the Optimal Therapeutic Window for Anti-inflammatory Cell Therapy

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INTRODUCTION: Post-traumatic osteoarthritis (PTOA) arises after joint injury through an acute inflammatory cascade that progresses toward chronic tissue degeneration. The early molecular events that determine disease trajectory remain poorly defined, limiting development of effective disease-modifying interventions. Recognizing PTOA as a disorder of the *whole joint*, the synovium plays a significant role in cytokine and metabolic environment that drives cartilage damage. This study aimed to map the temporal proteomic response of rat synovium following destabilization of the medial meniscus (DMM) injury and to define the optimal therapeutic window for anti-inflammatory cell therapy, for example macrophage mediated.

METHODS: Synovial tissues were collected from DMM-operated and contralateral knees at 1, 3-, 7-, 10-, and 14-days post-surgery. Quantitative label-free proteomics was performed, and proteins were analyzed for differential expression, pathway enrichment, and macrophage-associated trends. Statistical and visualization analyses included PCA, hierarchical clustering, KEGG enrichment, volcano plots, and temporal trend mapping of key pathways.

RESULTS: Global proteomic clustering (PCA and hierarchical heatmap) revealed a distinct proteomic signature separating early (Days 1–3) from later (Days 7–14) post-injury time points, confirming a rapid injury-induced molecular shift (**Fig. 1A-B**). Cytokines and signaling proteins (**Fig. 1C**), including IL6, NFκB, STAT family members, and TGFB1, peaked within the first 3 days, capturing the acute inflammatory cytokine storm. Pathway enrichment analysis demonstrated strong up-regulation of acute inflammatory signaling, including chemokine, cytokine-cytokine receptor, JAK-STAT, complement, and Toll-like receptor pathways, which peaked between Days 1–3 and declined by Day 7 (**Fig. 1D**). Pathway-specific analysis demonstrated distinct temporal regulation following DMM injury: arachidonic acid metabolism and complement/coagulation cascades peaked during Days 1–3, indicating early inflammatory activation, whereas ECM–receptor interaction proteins increased after Day 7, marking the transition toward matrix remodeling and tissue repair (**Fig. 1E**). The trajectory of macrophage-associated proteins (**Fig. 1F**) revealed a clear polarization pattern, with M1-related activity dominating initially and M2-related proteins increasing from Day 7 onward, indicating the transition from inflammation toward repair. Interestingly, proteomic comparison of contralateral limbs between Day 14 and Day 1 revealed distinct molecular alterations (**Fig. 1G**). These findings suggest that DMM injury elicits a systemic or compensatory proteomic response in distant joints, likely mediated by circulating inflammatory factors and altered mechanical loading.

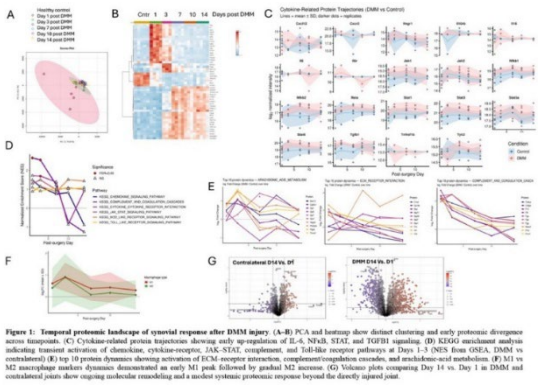

Figure 1: Temporal proteomic landscape of synovial response after DMM injury. (**A-B**) PCA and heatmap show distinct clustering and early proteomic divergence across timepoints. (**C**) Cytokine-related protein trajectories showing early up-regulation of IL-6, NFκB, STAT, and TGFB1 signaling. (**D**) KEGG enrichment analysis indicating transient activation of chemokine, cytokine-receptor, JAK–STAT, complement, and Toll-like receptor pathways at Days 1–3 (NES from GSEA; DMM vs contralateral) (**E**) top 10 protein dynamics showing activation of ECM–receptor interaction, complement/coagulation cascades, and arachidonic-acid metabolism. (**F**) M1 vs M2 macrophage markers dynamics demonstrated an early M1 peak followed by gradual M2 increase. (**G**) Volcano plots comparing Day 14 vs. Day 1 in DMM and contralateral joints show ongoing molecular remodeling and a modest systemic proteomic response beyond the directly injured joint.

CONCLUSIONS: Temporal proteomic profiling of the DMM model delineates a clear molecular chronology following injury, consisting of an acute inflammatory phase (Days 1-3), a transitional immune-metabolic phase (Day 7), and a later reparative remodeling phase (Days 10-14). The gradual reduction of inflammatory signaling and increase in M2-associated proteins suggest a shift toward resolution and tissue repair. These observations point to the period between Days 5-10 as a potentially favorable window for therapy, when modulation of the inflammatory environment may best support joint recovery. The systemic molecular changes detected in contralateral joints further indicate that joint trauma triggers both local and organism-wide responses, emphasizing the need for therapeutic approaches that address coordinated immune and metabolic regulation rather than localized inflammation alone. This study maps the molecular progression of synovial inflammation and repair following DMM injury, mirroring early PTOA. Targeting the transition between inflammation and early repair may help limit degeneration and slow PTOA progression when designing therapeutic strategies. These findings support the development of timing-optimized macrophage-based therapies such as iPSC-derive macrophages and highlight systemic effects in contralateral joints that further refine approaches for cell-based intervention

A Personalized Multi-Scale Modeling Workflow to Estimate OA-Related Cartilage Biomarkers

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INTRODUCTION: Cartilage mechanical biomarkers, such as contact pressure and strain-related measures, are increasingly used to investigate the development and progression of knee osteoarthritis (OA) within multi-scale modeling frameworks [1]. However, most implementations rely on simplified or generic representations of joint anatomy. Incorporating subject-specific articular surface geometry may improve the physiological relevance of these models. The aim of this study was to present a multi-scale modeling workflow incorporating personalized articular surface geometry and to evaluate its ability to characterize OA-specific mechanical responses in cartilage during gait.

METHODS: Seven subjects were included: two healthy controls and five with medial knee OA. For each individual, knee joint geometries were reconstructed from MRI segmentations and used to develop subject-specific musculoskeletal (MSK) [2] and FE models. MSK simulations used experimental motion data, including marker-based joint kinematics and ground reaction forces, to estimate joint loading and the contact pressure distribution at the tibiofemoral joint [3]. The resulting contact pressure maps were applied to a compartmental FE model of the medial tibial cartilage, which incorporated a fibril-reinforced biphasic material formulation to simulate tissue-level mechanical responses [4]. Distributions of contact pressure (normalized to body weight), maximum shear strain, and fibril strain were extracted at two loading peaks of the gait cycle. For each subject, these distributions were compared using Wilcoxon tests ($\alpha = 0.05$).

RESULTS: Across all evaluated biomarkers, the workflow showed a consistent tendency toward higher contact pressure, maximum shear strain, and fibril strain in OA subjects compared to healthy controls at both loading peaks (Figure 1). These differences were reflected in a shift of the distributions toward higher values in the OA group. However, statistical analysis did not reveal significant differences between groups for any of the evaluated measures.

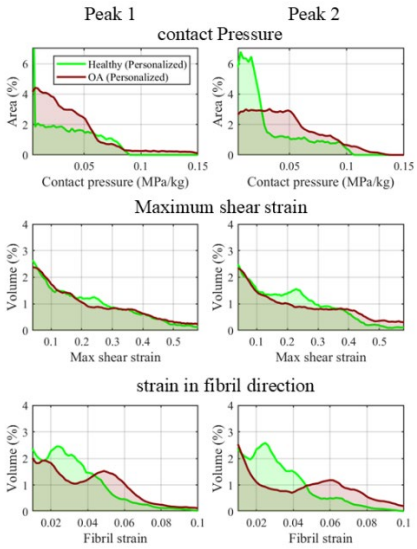

Figure 1: Histograms of outputs for healthy and knee OA subjects.

DISCUSSION & CONCLUSIONS:
This study introduced a multi-scale modeling workflow that uses subject-specific articular surface geometry to estimate cartilage mechanical biomarkers. The observed trends indicate that the method is sensitive to functional differences related to OA gait. The results are consistent with those of the previous study with scaled generic geometries [4]. Nevertheless, the lack of statistically significant differences highlights the limitations of the current sample size and the selected summary metric. Future work should focus on larger cohorts. Overall, incorporating personalized joint geometry within this framework provides a promising direction for subject-specific investigation of functional cartilage mechanics in OA.

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Biofabrication of the intervertebral disc: progress and remaining challenges

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The intervertebral disc (IVD) is a fibro-cartilaginous structure that acts as a natural shock absorber for the spine. It consists of an outer ring of collagen fibers encasing a gelatinous core. Degeneration of IVD, for which there is currently no effective treatment, is a prevalent cause of chronic back pain and significantly impacts the quality of life for millions worldwide.

Preclinical animal models of the disease have been considered; however, alternatives to animal experimentation are needed due to ethical guidelines and concerns about animal welfare.

This talk will present various biofabrication techniques (extrusion, meltelectrowriting) to design an in vitro IVD model that replicates its intricate organization. Implementing bioprinted constructs that recapitulate the IVD's complex architecture could be a crucial step toward assessing the effectiveness of cutting-edge therapeutic approaches that include cells, extracellular vesicles, mRNA, or miRNA, leading toward human and veterinary clinical trials

3D printed “Bouncy Bioglass” can regenerate articular cartilage focal defects

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INTRODUCTION: Inorganic/organic sol-gel hybrids (Bouncy Bioglasses) have potential to fulfil unmet clinical need in osteochondral regeneration. Our silica/PCL/pTHF materials consist of inorganic and organic co-networks. They have unprecedented combination of mechanical properties and control of biodegradation, including high elasticity and self-healing ability. Here, we show how they regenerate focal defects in articular cartilage in vivo.

METHODS: Our silica/PCL/pTHF materials are made via sol-gel chemistry and in situ polymerisation[1]. The hybrid inks were 3D printed in log-pile-like architectures with pore channel sizes of 250 μm and 500 μm[1]. In vitro studies were carried out with human bone marrow derived stem cells (hBMSCs)[2]. A sheep model was used with 6 mm focal defects. Microfracture was carried out before implantation of the scaffolds.

RESULTS: We found the scaffolds can guide bone marrow stem cells differentiation down a chondrogenic route and produce articular cartilage-like matrix, but only when the pore channel size was 250 μm. This was shown in vitro[2] and confirmed in vivo (3 months), with the scaffolds that had 250 μm pores promoting high quality cartilage regeneration (Fig 1) with high collagen II and glucosaminoglycan (GAG) content, while defects remained when scaffolds with 500 μm pores, or control, were used.

For the bone regeneration, the challenge is introducing osteostimulation by incorporating calcium, while sharing load with host bone. Our traditional calcium salts that we use in sol-gel do not work well, so we had to move to use of calcium alkoxide precursors, but this actually increased printability and mechanical properties[3]. In vitro cell studies show improved osteostimulation compared to osteogenic media[4].

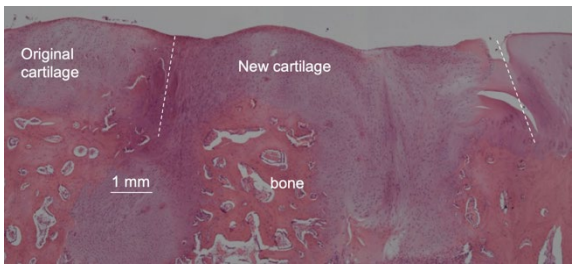

Fig. 1: Cartilage regeneration 3 months after scaffold implantation in 6 mm cartilage defects in an ovine model.

ACKNOWLEDGEMENTS: EPSRC and National Institute for Health Global Health Research.

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A nasal chondrocyte-based inflammation resistant therapy for osteoarthritis

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INTRODUCTION: Nasal chondrocytes (NCs) have emerged as a promising cell source for focal knee cartilage repair [1], however, the synovium-driven chronic inflammatory environment of osteoarthritis (OA) may impair effective regeneration. This study aimed to: (i) identify anti-inflammatory cytokines that can mitigate synovial inflammation, including IL-1Ra [2]; (ii) engineer NC-based tissues expressing the most effective candidate; and (iii) evaluate their resilience and therapeutic potential in vitro using different OA mimicking models.

METHODS: Synovia (N=16) and synovial fluid (OASF, N=54) were collected from knee OA patients undergoing arthroplasty (i) Various anti-inflammatory cytokines (IL1Ra, IL1R2, IL10, IL37) were screened to assess their impact on mitigating inflammation in a synovial explant culture. (ii) NCs were lentivirally engineered to stably express IL-1Ra (NC-IL-1Ra-mCh) and mCherry (NC-mCh), and subsequently cultured as chondrogenic spheroids, while IL-1Ra's release kinetic was assessed over time. (iii) Engineered NCs expressing mcherry alone or with IL-1Ra were cultured in presence of OASF or inflammatory cytokines (INFL: IL-1β/IL-6/TNFα), and OA cartilage organoids [3].

RESULTS: (i) Among the cytokines tested, IL-1Ra was the most promising anti-inflammatory cytokine, satisfactorily mitigating OA-synovium-derived inflammation, significantly downregulating key pro-inflammatory markers like IL-8, IL-6 and PTGS2 at the mRNA level and CCL2, CCL22, NGfb at the protein level. (ii) Engineered NCs secreted IL-1Ra persistently throughout 8 weeks of culture and formed tissue with glycosaminoglycans levels akin to untransduced NCs. (iii) When exposed to OASF or INFL, NC-IL-1Ra-mCh exhibited reduced production of inflammatory cytokines and less pronounced fibrotic responses compared to NC-mCh, with (Fig.1A). Furthermore, NC-IL-1Ra-mCh integrated well with OA cartilage organoids, reverting their degenerative traits (Fig.1B).

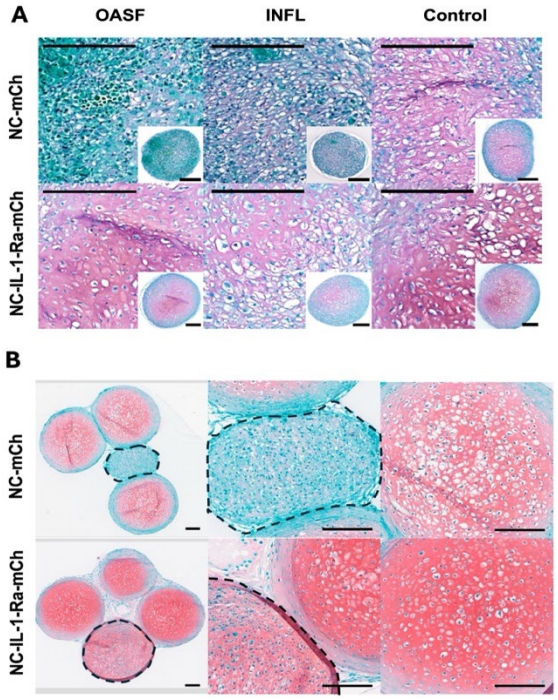

Fig.1. Safranin-O/Fast Green stainings of NC-mCh and NC-IL-1Ra-mCh chondrogenic constructs exposed to OA synovial fluid (OASF) or inflammatory stimulus (INFL) (**A**), and their effect on proteoglycan content of OA cartilage organoids following co-culture (**B**).

DISCUSSION & CONCLUSIONS IL-1Ra emerged as the most effective candidate for counteracting OA synovial inflammation. Its sustained secretion by engineered NCs supports cell-mediated delivery as a more robust alternative to recombinant protein administration. The ability of NC-IL-1Ra-mCh constructs to resist OASF-induced degradation and fibrosis, while and reversing OA-like traits in cartilage organoids positions them as active modulators of the joint microenvironment. Ongoing work aims to elucidate the paracrine and cellular mechanisms driving these regenerative effects.

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Extracellular Matrix Stiffness Drives Proliferative and Metabolic Dysregulation in Nucleus Pulposus Cells during Intervertebral Disc Degeneration

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INTRODUCTION: Extracellular matrix (ECM) stiffness is a critical regulator of intervertebral disc degeneration (IDD), remodeling the biomechanical microenvironment of nucleus pulposus cells (NPCs). As the main cells maintaining disc homeostasis, NPCs respond to stiffness changes with alterations in proliferation, metabolism and differentiation. However, the precise mechanisms by which ECM stiffness coordinates these processes remain incompletely understood.

METHODS: Hydrogel-based stiffness models were employed to investigate phenotypic changes of NPCs cultured on soft or rigid matrices; puncture-induced IDD models were established to validate in vivo relevance; single-cell RNA sequencing was performed to characterize alterations in specific NPC subpopulations; molecular assays were applied to further verify underlying mechanisms.

RESULTS: ECM stiffness regulates NPCs fate through coordinated mechanotransduction pathways linking cytoskeletal tension to transcriptional and metabolic regulation. Increased stiffness enhances actin polymerization and RhoA-dependent cytoskeletal tension, promoting nuclear translocation of Yes-associated protein (YAP) and Myocardin-related transcription factor-A (MRTF-A).

Nuclear YAP forms a complex with TEAD1 to directly activate transcription of Cyclin B1, thereby accelerating G2/M transition and driving cell cycle progression. Single-cell RNA sequencing further revealed that proliferating nucleus pulposus cells predominantly exhibit a fibrotic phenotype. This establishes a stiffness-dependent YAP/TEAD1–Cyclin B1 axis that sustains proliferative signaling.

In parallel, MRTF-A mediates stiffness-induced metabolic reprogramming. Following nuclear translocation, MRTF-A transcriptionally represses Kidins220, an upstream activator of AMP-activated protein kinase (AMPK). Downregulation of Kidins220 reduces AMPK phosphorylation and activity, leading to decreased expression of key glycolytic enzymes, including PFKM and PFKFB3, and suppression of glycolytic flux and ATP production.

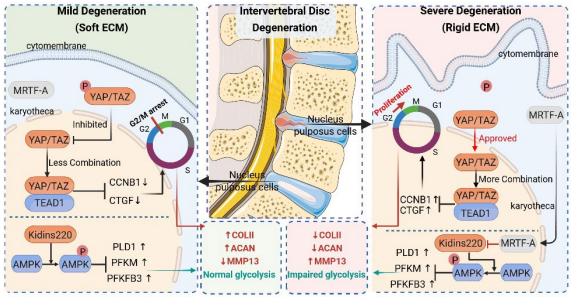

Fig 1: Schematic illustration showing the possible mechanisms by which YAP induces NPCs proliferation and degenerative phenotypes, and by which the rigid matrix regulates cell glycolysis in degenerated NPCs.

DISCUSSION & CONCLUSIONS: These signaling pathways establish a cellular state, characterized by the coexistence of YAP-driven cell cycle activation and MRTF-A–mediated metabolic suppression. Single-cell RNA sequencing analysis reveals that proliferating NPCs are enriched for a fibrotic phenotype. Concurrently, impaired glycolysis downstream of the MRTF-A signaling pathway reduces energy production, further exacerbating the imbalance between cellular demand and metabolic capacity. Targeting mechanotransduction pathways may help restore cellular homeostasis and represents a promising therapeutic strategy.

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Joint injury: new insights from animal models on injury risk and long-term consequences

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INTRODUCTION

Joint injury is a major cause of post-traumatic Osteoarthritis (ptOA) and long-term disability. The knee joint is particularly susceptible, with injury to the meniscus and anterior cruciate ligament (ACL) amongst the most common. Australia has the highest incidence of ACL rupture (ACLr) in the world (91/100k pop^{n}). ACLr numbers have escalated rapidly in the past 20 years. Young people aged 5-14 yrs are especially impacted - increasing by 10.4%/yr in females, 7.3%/yr in males. Approximately 50% of ACLr result in ptOA within 15 years, irrespective of intervention. The reason individuals with apparently similar injuries have different ptOA risk and trajectory is unclear.

Prior joint loading history and mild injury are known predisposing factors and potential intervention points for preventing ACLr and ptOA. However, identifying individuals with prior mild injury is problematic as they are not tracked (even in elite athletes), there are no methods/measures to define mild-injury severity, and potential impacts are long-term limiting clear association. To address this problem, we have developed a controlled knee-joint injury model in mice mimicking a mild resolving ACL-sprain (ACLs) [1]. Together with surgical and non-surgical critical joint injury models, we have investigated the molecular mechanisms that predispose to ACLr and/or ptOA, and whether these can be targeted to modify long-term outcomes.

METHODS

In different studies, male 10-12wk old C57Bl6 mice had either no injury (naïve), mild single impact knee injury to 75% of ACL rupture load (ACLS), single impact to ACL failure (ACLr), surgical ACL transection (ACLt), surgical destabilization of the medial meniscal (DMM), or sham surgery (Sham). Cohorts of animals received peri-injury treatment with NSAIDs or specific anti-IL12/IL23 agents. Outcome measures included: pain behaviours (tactile allodynia, knee hyper-algesia, hind-limb weight bearing), flow-cytometry and mRNA expression (RT-PCR) of knee synovial tissue, biomechanics (ACL strength), and knee joint histopathology, as previously described [1-3] All experiments were conducted according to DEPART and ARRIVE guidelines [4], with data analysed using standard parametric and non-parametric tests, Pearson's correlation, weighted-kappa, and ordinal or linear regression as appropriate.

RESULTS AND DISCUSSION

ACLs did not induce any structural joint injury or progressive long-term ptOA pathology or pain behaviour – mimicking a resolved mild joint injury in people. However, ACLs resulted in progressive weakening of the ACL and long-term neural sensitization (tactile allodynia). This was accompanied by evidence of delayed (14 wks post injury) onset of synovitis with increased macrophage activation, CD4 T-cells and accompanying up-regulation of cytokines (IL1, IL6) and metalloproteinases (Adamts4, Adamts5, Mmp13). These changes along with increased subchondral osteocyte loss may explain the increased cartilage proteoglycan loss in joints with DMM preceded by ACLs. Importantly, ACLs-induced longer-term ACL weakening, neural sensitization, synovitis and associated inflammatory and catabolic molecular changes could be abrogated by a 1-week course of NDAIDs immediately post ACLs.

Joint injuries that induced ptOA (DMM, ACLt, ACLr) had a distinct spatiotemporal Th1 and Th 17 response in synovial tissue and draining lymph node with little systemic change. The Th1 response subsided once the cartilage damage reached its maximum, whereas Th17 cells continuously accumulated in the synovial tissue. Importantly, model-specific temporal differences in both Th1/Th2 and Th17/Treg cells were observed. Targeting the Th1/Th17 polarization of CD4 T-cells by inhibiting IL12/IL23 for just 5 days peri-ACLr injury, markedly inhibited long-term synovitis, synovial fibrosis and cartilage chondrocyte apoptosis, and slowed cartilage erosion and subchondral bone remodelling.

CONCLUSIONS

These studies have for the first time defined molecular mechanisms underlying mild-injury induced ACLr and ptOA risk, and critically that these risks are modifiable.

ACKNOWLEDGEMENTS

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Cartilage regeneration and joint preservation: A challenging translational journey to the clinical practice

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Cartilage regeneration remains an open challenge. After 30 years from the earliest studies showing the potential to regenerate cartilage, most of the promising preclinical findings did not translate into clinical practice. Also, while some procedures are available in some health care systems, there is currently no agreement on their real potential, both from the clinical perspective in terms of symptom improvement, as well as disease-modifying properties. This created a situation where there is no gold standard, and indications are often controversial at best. To further complicate this scenario, problems and limitations with methodological approaches to evaluate treatment results, pressures from reimbursement systems, and market strategies not always supported by preclinical and clinical evidence led to a rather heterogeneous field. Moreover, cartilage treatments are often applied in joints affected by early osteoarthritis processes, which brings the complexity of an abnormal joint environment into the equation. Addressing the joint environment actually became the main focus of the most used treatment approaches in clinical practice, being less invasive and less expensive than regenerative treatments focused on the articular surface.

Many challenges also affect the development and study of injectable approaches to improve joint homeostasis. Even when a treatment has a clear mechanism of action and disease-modifying effects proven in preclinical studies, demonstrating its clinical benefit remains difficult. Clinical research faces multiple biases—such as confirmation, present, selection, attrition, and detection biases—that complicate the interpretation of study findings. Designing studies that minimize these biases is essential to clarify treatment potential.

Placebo effects are significant and complex. A meta-analysis of knee osteoarthritis trials showed that saline injections provide substantial pain relief and improvements in function and stiffness, with effects lasting up to six months [1]. These findings underline the importance of accounting for placebo when designing studies to evaluate treatment efficacy. Assessing treatment success mainly relies on patient-reported outcomes, which are subject to symptom fluctuations and recall bias [2]. Pain peaks prior to follow-up assessments can distort patient self-evaluations, which supports the need for more reliable evaluation tools. Moreover, improvements must be interpreted considering their clinical relevance, which adds another layer of complexity. The minimal clinically important difference (MCID) [3] is used to identify improvements meaningful for the patient, yet there is no consensus on how to calculate it. A recent study applying 17 methods found wide variability, raising doubts about MCID's role in clinical research.

In summary, developing and validating new treatments involves multifaceted challenges, from targeting the disease effectively to designing robust studies and interpreting outcomes. To do this, collaborative efforts [4] are key to improving innovation and its translation in clinical practice.

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The immediate postoperative joint milieu is predominantly alkaline under normal and staphylococcal septic conditions

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INTRODUCTION: During periprosthetic joint infections (PJI), the joint environment is thought to be acidic, which has led to the development of various therapies and devices that respond to this presumed acidity. This study employs a continuous indwelling wireless pH capsule alongside J-P drains comprising 4 experimental cohorts to characterize pH dynamics under aseptic and septic conditions, with and without local antibiotics. We then compared these results with human wound/synovial fluid collected from arthroplasty patients. Our findings strongly support an alkaline milieu in the postoperative joint replacement wound fluid. **METHODS:** First, a single hip from four adult female mixed-breed sheep was operated on and evaluated. An AlphaONE Wireless pH Capsule Reflux Monitoring System (LABORIE MEDICAL TECHNOLOGIES CORP.) and a J-P drain were sutured in place next to the open coxofemoral joint for continuous pH monitoring and fluid collection. AlphaONE began recording pH data at time zero. The J-P drain was sampled at wound closure (time zero) and every 6-12 hours for 96 hours, as fluid output allowed, then immediately analyzed for pH using a calibrated digital pH meter. Venous blood pH was also recorded. Then, six sheep were enrolled, with a 4-week washout period between surgeries on the left and right hips, respectively. In the 1st hip surgery, sheep received 250 mg Ceftiofur Sodium locally via the J-P drain every 24 hours for 96 hours, followed by a 4-week medication washout period. Then the procedure was repeated on the opposite hip, which was inoculated with 3 ml of *Staphylococcus aureus* ATCC 25923 ($1 \times 10^{\wedge 5}$ CFU/mL in PBS) via the JP drain after closure. Three of the six sheep received 250 mg Ceftiofur Sodium locally via the J-P drain every 24 hours for 96 hours, 24 hours after inoculation. Human samples: 99 human synovial fluid samples were collected during outpatient office visits and at the time of surgery for both index and revision arthroplasty and were analyzed for pH using pH paper. Infection status was unknown for this sampling. Normality tests and homogeneity of variance tests were performed on the data. A simple linear regression model was used to evaluate the relationships among pH, time, infection status, and antimicrobial treatment. Comparisons across time points for parameters such as infection status, antimicrobial treatment, and wound/synovial fluid pH were conducted using repeated-measures analysis of variance, with a significance level of $P < 0.05$. **RESULTS:** All animals completed the study. The AlphaONE System produced continuous pH readings for 96 hours. The starting pH ranged from pH 6.3 to pH 7.8. Across all cohorts, postoperative wound fluid pH was consistently alkaline (≥7.7 after 24h). The venous pH was stable at ~7.5. Infection effect: *In situ* pH closely converged with J-P drain pH in septic cohorts but less so in aseptic cohorts, suggesting infection reduces wound fluid compartment gradients. In human wound fluid/synovial samples, the mean pH, independent of underlying pathology, was alkaline, centered at pH 7.9. The correlation between changes in pH and wound fluid status over time and across treatment groups (infected, non-infected, +/-antimicrobial treatment) was not statistically significant. **DISCUSSION & CONCLUSIONS:** The environment created in the postoperative period differs from that of the native joint, posing a unique set of environmental stressors for opportunistic pathogens. One reason septic joints and/or PJI are notoriously hard to treat may be the decreased effectiveness of antimicrobials in the alkaline local environment, suggesting that pH should be considered when developing a clinical diagnostic or treatment plan for septic joints and/or infected arthroplasty patients. Our findings may guide the exploration of mechanistic, diagnostic, and therapeutic questions addressing septic joints, PJI, and joint cross-talk.

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Chronic stress-induced spatiotemporal alterations in corticosterone converting enzymes and adrenergic receptors in experimental osteoarthritis

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INTRODUCTION: Chronic stress activates both the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS) and is increasingly recognized as a risk factor for osteoarthritis (OA) [1, 2, 3]. However, the role of local glucocorticoid and adrenergic signaling remains unclear. Therefore, we investigated the changes in adrenergic receptor (AR) and corticosterone (CORT)-converting enzyme expression linked to degeneration and inflammation in a murine model of OA.

METHODS: OA was induced in twelve-week-old male C57BL/6J mice by destabilization of the medial meniscus (DMM), with sham controls. A subset was exposed to chronic mild stress (CMS) for 4 or 12 weeks. Stress response was evaluated by measuring serum CORT and splenic noradrenaline (NE). Cartilage degeneration and synovitis were quantified using histological scoring. ARs (α2A and β2) and CORT-converting enzyme (11β-hydroxysteroid dehydrogenase-1/-2 (11β-HSD1/2)) expression in cartilage and synovium was analyzed using immunohistochemistry (IHC) and quantified as the ratio of positive cells to total cells. Ongoing analyses include glucocorticoid receptor (GR) and tyrosine hydroxylase (TH) expression. Data were analyzed using Kruskal-Wallis/Dunn's test and Spearman's rank correlation. A p-value <0.05 was considered statistically significant.

RESULTS: Serum CORT and splenic NE increased in all CMS groups at both time points (CORT: both CMS-Sham and CMS-DMM $p < 0.05$ at 4 and 12 weeks, NE: both CMS-Sham and CMS-DMM $p < 0.01$ at 4 weeks, $p < 0.001$ at 12 weeks). CMS exacerbated DMM-associated cartilage degeneration and synovitis at both 4 (cartilage: $p < 0.05$, synovium: $p < 0.05$) and 12 weeks (cartilage: $p < 0.01$, synovium: $p < 0.01$). No change in 11β-HSD1 expression was observed, while 11β-HSD2 decreased in CMS-Sham and DMM cartilage (both: $p < 0.05$) and increased in DMM synovium ($p < 0.05$) at 4 weeks, with both returning to baseline by 12 weeks. α2A-AR expression increased in CMS-Sham and CMS DMM cartilage at both 4 (both: $p < 0.001$) and 12 weeks (both: $p < 0.01$) and increased only in CMS DMM synovium after 12 weeks ($p < 0.05$). β2-AR was elevated in CMS-Sham and DMM cartilage (both: $p < 0.05$ at 4 weeks, both: $p < 0.01$ at 12 weeks) at all-time points and in the synovium of CMS-DMM mice at 12 weeks ($p < 0.001$). Moreover, synovial β2-AR expression positively correlated with splenic NE ($r = 0.650$, $p < 0.01$), cartilage degeneration ($r = 0.518$, $p < 0.01$), and synovitis ($r = 0.506$, $p<0.01$).

DISCUSSION & CONCLUSIONS: CMS seems to accelerate local OA-associated processes primarily via SNS/AR-mediated mechanisms rather than HPA/glucocorticoid signaling indicated by an early loss of 11β-HSD2 (converts CORT in its inactive form) in the cartilage as well as general β2-AR dominance. Our findings underscore the potential of the β2-AR as therapeutic target in OA.

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Identification of novel skeletal stem/progenitor cells in the perichondrium

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The adult skeleton is maintained by region-specific skeletal stem/progenitor cells (SSPCs) in the bone marrow, growth plate, periosteum, cranial suture, and vertebral endplates. However, whether SSPCs exist in the perichondrium remains poorly understood. We have recently discovered Procr+ chondroprogenitors in the superficial region of the articular cartilage and meniscus, which can be activated by mechanical stimuli to maintain articular cartilage homeostasis, or promote the regeneration of articular chondrocytes after injury. Prospective isolation of Procr+CD200+CD105+ superficial cells followed by orthotopic transplantation could robustly regenerate hyaline cartilage to repair articular defects. Our recent study further reveals novel SSPCs in the perichondrium that initiate endochondral ossification of embryonic long bones, and give rise to distinct postnatal SSPCs. These cells remain in the perichondrium encircling the growth plate in postnatal mice, which not only generate osteoprogenitors in the bone marrow and periosteum, but also differentiate into chondrocytes in the articular surface and resting zone of the growth plate. Importantly, these cells are tightly regulated by mechanical stimulation and Wnt signaling, which could be harnessed to repair articular and growth plate injuries. Taken together, we have identified novel SSPCs in the perichondrium that critically regulate osteochondral lineage specification and regeneration of the adult skeleton.

CENTRAL ROLE OF PYROPHOSPHATE METABOLISM IN SPINE HEALTH

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INTRODUCTION: Intervertebral disc degeneration is increasingly recognized not as a uniform process, but as a spectrum of distinct, mechanistically driven phenotypes, each representing a unique pathological axis with specific molecular determinants and clinical consequences (1). Disc degeneration primarily contributes to chronic low back and neck pain, underscoring an urgent need to delineate these phenotypes, including fibrosis, ectopic calcification, herniation, and mixed states. Among these, disc calcification remains the least studied despite its potentially profound biological and clinical implications (1). Ectopic calcification, defined as the pathological mineralization of soft tissues, has been extensively investigated in vascular, dermal, and cartilaginous systems; however, its occurrence within the intervertebral disc is poorly understood and conceptually underappreciated.

METHODS: Recent studies employing genetically engineered and injury-induced mouse models will be discussed.

RESULTS: Clinically, disc calcification is associated with poor surgical outcomes and pain that is often refractory to conservative management. At the molecular level, this phenotype is marked by hypertrophic chondrocyte-like differentiation, elevated expression of tissue-nonspecific alkaline phosphatase (TNAP), ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), and ANK, along with increased cell death, inflammation, and disruption of inorganic phosphate (Pi) and pyrophosphate (PPi) homeostasis (2). Notably, mineralization patterns differ across AF, NP, and EP compartments, indicating strong spatial regulation (2). Emerging evidence demonstrates that local control of PPi homeostasis—mediated by the ANK–ENPP1 axis—dominates over systemic mineral metabolism in driving calcification (3). Furthermore, genetic predisposition, aging, and biomechanical stressors such as trauma collectively shape susceptibility to this phenotype.

DISCUSSION & CONCLUSIONS:

Disc calcification represents a distinct and underrecognized degenerative axis within the broader landscape of intervertebral disc disease. Its pathogenesis is governed by tightly regulated local molecular events, including dysregulated phosphate metabolism, altered cellular differentiation states, and cell death. Recognizing and mechanistically defining this phenotype is essential for reshaping our conceptual framework of disc degeneration. Importantly, identifying the pathways that drive local mineralization offers a path toward developing disease-modifying strategies. Advancing our understanding of intervertebral disc calcification will not only fill a major gap in spine biology but also enable more precise and effective therapeutic interventions for chronic, degeneration-associated spinal disorders.

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Single-cell transcriptomics informs the development of bone marrow lesions in early osteoarthritis

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INTRODUCTION: Recognizing and treating early-stage osteoarthritis (OA) represents a critical therapeutic imperative. Subchondral bone marrow lesions (BMLs) constitute a pathognomonic imaging feature of both incipient and progressive OA [1-3]. However, owing to limited access to human early-stage OA specimens and the absence of suitable animal models [4], the pathological characteristics and molecular mechanisms driving BMLs remain poorly defined, thereby impeding early therapeutic development.

METHODS: We established a standardized mouse model recapitulating BMLs by combining anterior cruciate ligament transection (ACLT) with forced wheel running. Using single-cell RNA sequencing (scRNA-seq) during BML development alongside genetic lineage tracing, we investigated BML pathogenesis and evaluated therapeutic effects via pharmacological targeting. Mendelian randomization was used to analyze the gene expression versus OA risk.

RESULTS: BMLs in the model correlated significantly with cartilage degeneration and exhibited aberrant bone matrix accumulation. Misfolded collagen within BMLs disrupted extracellular matrix homeostasis by impairing mechanical adaptation and collagen fiber alignment, culminating in localized subchondral bone lesions. scRNA-seq revealed that proteasome dysfunction and abnormal secretion of misfolded collagen, driven by dysregulated heat shock protein 70 (HSP70) and deubiquitinase 19 (USP19) expression in osteoarthritic subchondral osteoblasts, constitute a key mechanism of BML formation. Furthermore, WNT5A secreted by these osteoblasts mediated crosstalk with hypertrophic chondrocytes (HTCs), accelerating HTC hypertrophy and death. Pharmacological HSP70 activation by TRC051384 suppressed collagen misfolding/secretion and prevented BML formation. Mendelian randomization demonstrated a significant association between proteasome gene expression and human OA risk.

DISCUSSION & CONCLUSIONS: Collectively, our study provides a standardized BML mouse model and delineates cellular and molecular mechanisms driving BML formation, identifying promising therapeutic targets for early OA intervention.

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UNLOCKING CD44+ INTERVERTEBRAL DISC CELLS IN HOMEOSTASIS AND DEGENERATION FOR THERAPEUTIC TARGETS IN DISCOGENIC PAIN

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INTRODUCTION: Discogenic pain (DP) is characterized by a strong inflammatory response associated to intervertebral disc degeneration (IVDD) and absence of spine deformation [1]. However, the underlying mechanisms of DP are not completely elucidated, namely at early stages of IVDD.

Previously, IL-1β+needle puncture-stimulated nucleus pulposus (NP) organ cultures from bovine origin were established as standard models of early-stage IVDD [2]. In this context, our team has identified CD44, a cell surface glycoprotein involved in cell-extracellular matrix (ECM) interactions and the main ligand for hyaluronic acid (HA), as an up-regulated marker in NP cells [3]. Therefore, the aim of this work was to further characterize CD44-based myeloid cell subsets, important regulators of inflammation, in IVDD, that could reveal new therapeutic targets for DP.

METHODS: Bovine IVD (~1 year old) were collected as described [3]. NP and annulus fibrosus (AF) were separated and enzymatically digested overnight to obtain NP/AF cells. The fresh NP/AF cell phenotype was first characterized by flow cytometry and immunohistochemistry (IHC) for the expression of CD44, CD45 (pan-hematopoietic cell marker), CD14 (monocyte/macrophage lineage marker) and MHC-II (cell activation marker). Then, NP/AF cells were maintained in culture for 7 days under both basal and pro-inflammatory conditions (IL-1β, 10ng/ml, 48h), with diclofenac treatment applied 3h after stimulation. In parallel, NP/AF explants were cultured in 3D organ culture under basal and degenerative conditions (IL-1β+21G-needle puncture). Cell viability was evaluated by a fixable viability dye and cell phenotype was assessed by flow cytometry. In the end, proteomics data of CD44+ versus CD44- bovine NP cells were analysed to identify cell-surface proteins that could distinguish the CD44 NP cell subset.

RESULTS: Both freshly isolated NP and AF cells revealed absence of CD45 expression (no hematopoietic/myeloid cells) and similar expression levels of CD44 (15%), being CD14 and MHC-II more highly expressed in AF than in NP cells (maximum 45% for CD14), which was confirmed by IHC. Co-expression analysis revealed that the most expressed subset was CD45-CD44-CD14+ cells in AF (35%).

In 2D, both NP/AF cells maintained adherent and suspension cell fractions, with lower viability for AF cells. CD44 was highly expressed in both NP adherent/suspension cells (>95%), and barely expressed in AF cells (<10%). CD14 was expressed at significantly higher levels in NP and AF suspension cells (55%), compared to adherent fraction, while MHC-II expression was absent.

When stimulated with IL-1β in 2D culture, only CD44+CD14- cells and CD44 fluorescence intensity in adherent NP cells significantly increased. The 2D cultures were then treated with Diclofenac, an anti-inflammatory drug, and no reduction of these markers was observed. On the other hand, CD44 increase with IL-1β was once again verified.

When looking into proteomics data of surface-membrane proteins, we have identified integrin subunit alpha 6 (CD49d) with >1.5-fold change in CD44+ NP cells, that could be further explored as potential therapeutic target associated to CD44 in the context of IVDD.

DISCUSSION & CONCLUSIONS: The results obtained exclude the presence of myeloid-derived cells in the IVD. Also, the results show that the 3D environment is essential to study CD44 in IVDD and point for the relevance of a non-adherent NP cell fraction. Moreover, the results confirmed the importance of CD44 in IVDD, suggesting additional surface markers that could be exploited in the future to better understand the significance of CD44 as therapeutic target in IVDD.

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Mechanically Preconditioned Synovial Mesenchymal Stem Cells Organoid Exosomes for Osteoarthritis Repair

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INTRODUCTION: Osteoarthritis (OA) involves cartilage degeneration, inflammation and cell senescence with limited treatments. Synovial mesenchymal stem cells (SMSCs) show strong chondrogenic and paracrine potential. We hypothesized that exosomes from mechanically preconditioned SMSC organoids can reduce chondrocyte senescence and mitigate OA, and an injectable ROS-responsive hydrogel can improve local delivery and efficacy.

METHODS: Human SMSCs were isolated and mechanically stimulated before organoid culture. Exosomes from preconditioned and control groups were extracted and characterized.In vitro: Protective effects on H_2O_2-injured chondrocytes were evaluated by viability, senescence, inflammation, ROS and mitochondrial function. Key miRNAs and signaling pathways were analyzed.In vivo: Exosome-loaded hydrogel was injected into OA joints; therapeutic effects were assessed by imaging, histology and senescence markers.

RESULTS: Human SMSCs were isolated, characterized, and mechanically preconditioned before organoid formation. Effects on proliferation and chondrogenic differentiation were assessed using CCK-8, EdU, qPCR, and immunofluorescence. Exosomes from preconditioned and control groups were extracted and verified by TEM, nanoparticle tracking, western blotting, and uptake assays. In vitro, H_2O_2-induced chondrocyte senescence models were used to evaluate viability, migration, chondrogenic markers, β-galactosidase activity, inflammation, ROS, mitochondrial respiration, and mtDNA integrity. miRNA sequencing and validation identified key exosomal cargos and signaling pathways. For translation, exosomes were encapsulated in an injectable ROS-responsive hydrogel and evaluated in vivo using fluorescence imaging, micro-CT, histology, and senescence analyses.

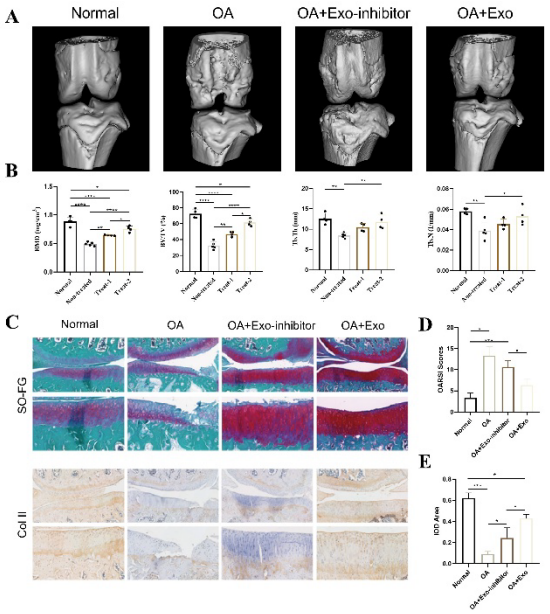

Figure 1 SMSCs Organoid-derived exosomes improve cartilage regeneration in OA.

DISCUSSION & CONCLUSIONS: Mechanical preconditioning boosts the therapeutic potency of SMSC organoid-derived exosomes. These exosomes protect against OA by suppressing chondrocyte senescence, inflammation and mitochondrial dysfunction. Combined with ROS-responsive hydrogel, this strategy achieves stable local delivery and strong translational potential.This study provides a promising exosome therapy for OA based on mechanically optimized stem cell organoids.

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Mesenchymal stromal cell spheroid-loaded microscaffolds for intervertebral disc repair

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INTRODUCTION: Mesenchymal stromal cell (MSC) injection is a promising strategy to mitigate intervertebral disc (IVD) degeneration, but the harsh IVD environment limits MSC survival and therapeutic efficacy. MSC spheroids have gained interest as a potential solution, yet their mechanical stability is often insufficient and survivability after injection remains a key challenge. We recently demonstrated that scaffolded spheroids (S-SPH) can be differentiated to enhance mechanical stability and express a nucleus pulposus (NP)-like phenotype [1]. Here, we investigated (i) the differentiation towards an NP-like phenotype in S-SPH in multiple MSC donors and (ii) its effect on survivability in an *ex vivo* organ culture model.

METHODS: Porous microscaffolds (⌀200µm) were fabricated from PCL-based resin (DEGRAD INX, BIO INX, Belgium) using a custom-built two-photon polymerization 3D printer. S-SPH were generated by seeding human bone marrow MSCs onto microscaffolds (2k cells/scaffold) and cultured for 14 days under NP-differentiating conditions (+GDF5, low-glucose DMEM, 2% oxygen), negative conditions (-GDF5, high-glucose DMEM, normoxia), or non-differentiating expansion medium (n=9 MSC donors). S-SPH area was quantified (n=15 per donor). Gene expression was evaluated using RT-qPCR and some S-SPHs were stained with SafraninO/ FastGreen. Survivability post injection was assessed using differentiated and non-differentiated PKH26-labelled S-SPHs. For this, 250 S-SPHs in 200 µL PBS were injected into papain-degenerated bovine caudal IVDs (~0.16 U, applied 2 days prior to injection, 3 daily physiological loadings). IVDs were harvested after one night of free swelling post-injection, and S-SPH viability was evaluated using CalceinAM and DAPI staining.

RESULTS: S-SPH were formed successfully with all 9 donors (Fig.1A). Differentiated S-SPH had a 37.2 % and 32.3 % larger area than negative and non-differentiated S-SPH respectively and formed a denser cell–matrix construct. ACAN (Ø 44.5 -fold) and COL2 (Ø 474.9-fold), as well to a lesser extent COL1 (Ø 2.7-fold) were upregulated in differentiated S-SPH in 8/9 donors. COL10 was not upregulated. Survivability after ex vivo injection was substantially improved following differentiation: while only 31.0±5.9 % of S-SPH remained viable in the non-differentiated group, 82.2±18.2 % were viable in the differentiated S-SPH group.

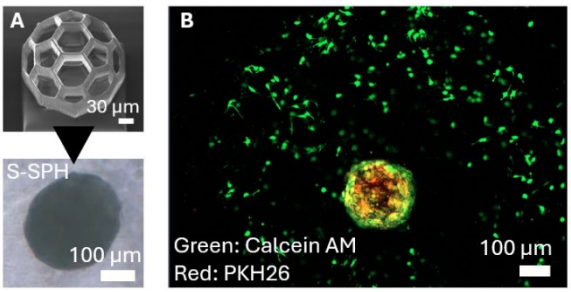

Figure 1: A) S-SPH Formation B) Viable differentiated S-SPH surrounded by NP tissue.

DISCUSSION & CONCLUSIONS: Differentiation was consistently achieved across multiple MSC donors, confirming reproducibility of this approach. Increased S-SPH area indicates more matrix production in the differentiated group, while the predominance of ACAN and COL2 imply an NP-like phenotype rather than a fibrocartilaginous shift. S-SPH were successfully delivered into bovine IVDs and NP-like S-SPH showed markedly improved survivability, indicating greater robustness than non-differentiated constructs. Future studies will assess long-term survivability and evaluate anabolic and anti-inflammatory potential of differentiated S-SPH.

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Organ-on-a-chip models of inflammatory joint disease

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INTRODUCTION:

The Queen Mary Centre for Predictive in vitro Models (CPM) provides a world-leading centre for the development and use of a wide range of in vitro models. This presentation describes the use of a Translational Readiness Framework (TRF) to increase adoption of organ-chip models within a defined context of use. As exemplars, the presentation will describe a range of different models of inflammatory joint disease, developed by the Knight group.

METHODS:

Vascularised synovium model with immune cells: Using the Emulate Chip-S1, patient-derived human fibroblast-like synoviocytes (hFLS) were seeded in the top channel, separated by a porous membrane from the bottom channel which was seeded with HUVECs (Fig. 1b). Media in the synovial channel l was supplemented with ± IL-1β (1ng/ml), and THP-1 monocytes added to the endothelial channel, to induce an inflamed synovitis model[1]. The entire chip was stimulated with physiological cyclic tensile strain (0.2Hz, 12%).

Vascularised osteochondral model: BMP2 was tethered within a within a 3D hydrogel of heparin methacryloyl (HepMA) + gelatin methacryloyl (GelMA). Human bone marrow mesenchymal stem cells (hBM-MSCs) were seeded into the gel and a differential buoyancy method used to create a BMP2 gradient to drive osteochondral differentiation within an open-well format such as the Emulate Chip-A1 or a Transwell[2].

RESULTS:

The synovium chip showed maintenance of hFLS and endothelial phenotype based on assessment of morphology, gene and protein expression[1]. Inclusion of IL-1β drove an inflammatory disease state with increased THP-1 adhesion and migration (Fig. 1c). Ongoing studies are incorporating resident macrophages and using autologous hFLS for patient stratification and personalized medicine with validation against known therapeutics (Fig. 1d). Within the osteochondral model, a BMP2 buoyancy gradient was successfully achieved. Tethered BMP2 drove osteochondral differentiation over a 21 day period[2]. Further studies are developing an inflamed joint disease model by incorporating inflammatory cytokines, immune cells and conditioned media from the synovium chip.

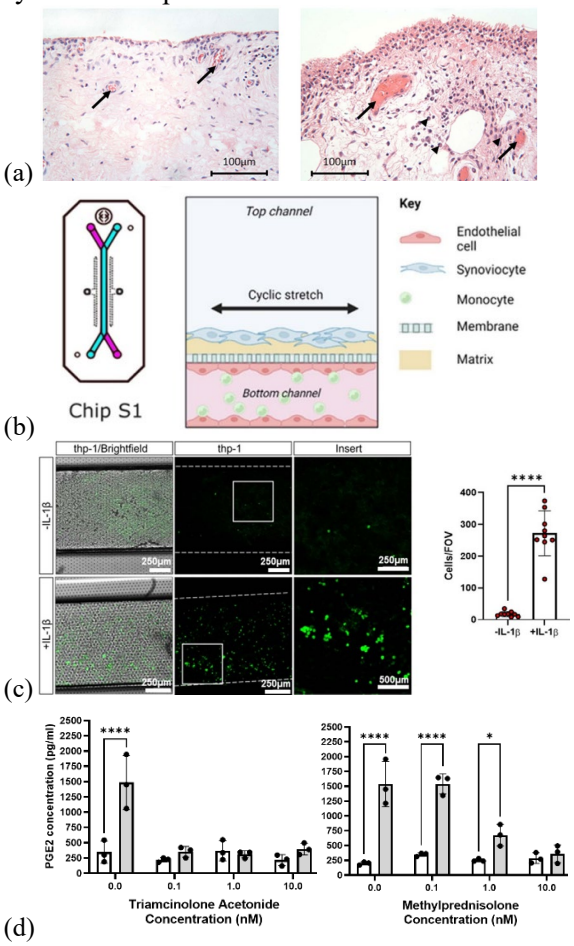

Fig. 1 The vascularised synovium model. *(a) Normal and inflamed synovium. (b) Chip configuration. (c) Increased adhesion of FITC-labelled THP-1s to endothelium with IL-1β. (d) Validation showing suppression of inflammatory PGE2 release with known therapeutics.*

DISCUSSION & CONCLUSIONS:

Development of in vitro models for widespread adoption for fundamental research or therapeutic testing is supported by early consideration of the TRF. We have successfully developed a range of industry-ready inflammatory join disease models covering synovium, cartilage and bone.

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(1) DOI:8/1748-605x/acf976
(2) DOI: 10.1177/20417314251326256

FROM PEOPLE TO MOLECULES: IN SILICO INSIGHTS FOR PRECISION HEALTH IN SPINE AND SYNOVIAL JOINTS

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INTRODUCTION: **A**ccording to the Global Burden of Disease 2021, intervertebral disc degeneration (IDD) and knee osteoarthritis (KOA) affect 255 M and 364 M people worldwide, with clinical care largely limited to symptom relief. KOA peaks at age 75–80, whereas IDD peaks earlier (55–60 years) with poor patient stratification. To enable preventive and reversible precision therapies, scalable computer models integrate morphometric, biomechanical, metabolic, and inflammatory data to identify early-stage disease endotypes.

METHODS: The Biomechanics and Mechanobiology lab at UPF integrates medical imaging into personalized 3D organ finite element (FE) models of the IVD [1]. FE formulations couple biomechanical responses with reactive metabolic transport for nucleus pulposus (NP) cell glycolysis, evaluated using Northern Finland Birth Cohort (NFBC) data [2]. Local mechanical fields, glucose, and lactate concentrations are translated into NP cell activity via a novel Parallel Network (PN) methodology for precision endotyping [3]. These are complemented by fuzzy logic-like regulatory network models (RNM) [4] mapping pathways from mechanoreceptors to transcription factors. In KOA, RNM capture articular chondrocyte regulation in Kellgren-Lawrence 2-3 patients to weight mechanical versus inflammatory stimuli on matrix regulation, nociception, and oxidative stress. Synovial fluid proteomics (Luminex) and synthetic data are mined alongside clinical outcomes [5], then extended to model macrophage polarization.

RESULTS & DISCUSSION: Multiscale FE simulations in a patient cohort (My Spine) showed that lactate accumulation associates with anterior IVD height due to a trade-off between diffusion distances and volumetric deformations. In the NFBC, this morphometrics-based metabolic risk explained early IDD better than standard MRI features, while systemic plasminogen activation uniquely explained nearly 30% of early IDD. PN modelling suggested that metabolic risk is relevant primarily when NP cells are TNF-positive [6], and RNMs captured path orchestrations (C-JUN, P65, FOXO) under pro-inflammatory stress. Mechanosensitive RNMs revealed a topological hierarchy where metabolic/stress routers (ROS, mTORC1) gate upstream mechanosensor signals flowing to master regulators like NF-κB and SOX9. Reversing this required pairing stress-driver attenuation (e.g., FAK, PIEZO1) with cytoprotective/anabolic activation (e.g., SOX9, NRF2). In KOA, high-throughput RNM simulations suggested that mechanical stress plays a minor role in idiopathic OA compared to synovitis. Anti-inflammatory treatments might manage nociception but not restore tissue alone. Synovial MCP-1 differentiated WOMAC pain, which personalized RNM simulations strongly linked to chondrocyte oxidative stress management via FOXO, indicating oxidation targets may be more clinically relevant than pro-inflammatory ones [5]. Finally, macrophage modelling identified TNF as a major synovitis marker and IL-10 as a key immunosuppressing molecule.

CONCLUSIONS: Multiscale, personalized computer modelling integrations mark a significant paradigm shift in understanding IDD and KOA. IVD simulations reveal that mechano-metabolic trade-off where specific anatomical dimensions dictate harmful lactate accumulation in early IDD. KOA changes in our explored cohort seemed primarily driven by synovitis and inflammatory profiles, with patient pain tightly linked to innate immune cell activation and how chondrocytes manage oxidative stress.

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ACKNOWLEDGEMENTS:

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Pericellular Matrix Restoration Enhances Resistance of Chondrocytes to Inflammatory and Mechanical Stress

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INTRODUCTION:

In native cartilage, chondrocytes are enclosed within a specialized pericellular matrix (PCM), forming a functional unit known as the chondron, which buffers mechanical and biochemical stress. However, current cartilage repair strategies such as autologous chondrocyte implantation (ACI) rely on enzymatically isolated chondrocytes, resulting in loss of the PCM and disruption of this protective microenvironment. The aim of this study was to determine whether restoring collagen VI (COL-VI)-enriched PCM around isolated chondrocytes can reconstruct a chondron-like structure and improve cellular resistance to inflammatory and mechanical stress.

METHODS:

Isolated human chondrocytes were cultured in alginate beads for 2 weeks in chondrogenic media to support pericellular matrix formation. Reconstituted chondrons were subsequently harvested by dissolving the alginate.

To evaluate responses to inflammatory and mechanical stimulation in a 3D environment, chondrons were then embedded in Gelatin methacryloyl (GelMA) constructs and exposed to interleukin-1β (IL-1β, 1 ng/mL) and multiaxial mechanical loading (10% static and 10–20% dynamic compressive strain, 0–12° shear, 1 Hz, 1 h/day) for 7 days, modeling the early osteoarthritic microenvironment. Isolated chondrocytes without PCM were embedded in GelMA as controls. Structural and phenotypic changes were assessed by immunofluorescence and histological staining, quantitative gene expression analysis (qPCR), and protein-level assays (ELISA).

RESULTS:

Pericellular COL-VI enrichment was detected in >95% of reconstituted chondrons following 2 weeks of alginate culture (Figure 1A). After 7 days in GelMA, reconstituted chondrons retained a chondron-like pericellular structure, characterized by a distinct collagen-rich pericellular compartment by Safranin O /Fast Green staining (Figure 1B).

Compared to isolated chondrocytes, reconstituted chondrons exhibited enhanced matrix synthesis, with significantly higher gene expression of COL2 and COMP. Compared with chondrocytes, IL-1β stimulation of chondrons showed attenuated inflammatory (COX2, NOS2, IL6 (Figure 1C), IL8), catabolic (MMP3, ADAMTS4), angiogenic (VEGF), and neurogenic (NGF, Figure 1D) gene expression. Reduced IL6 and IL8 secretion in chondrons was confirmed by ELISA.

Notably, multiaxial loading amplified IL-1β-induced inflammatory and neurogenic responses in chondrocytes, including increased IL6 (Figure 1C), IL8, COX2, and NGF expression (Figure 1D). In contrast, this mechano-inflammatory response was markedly reduced in chondrons.

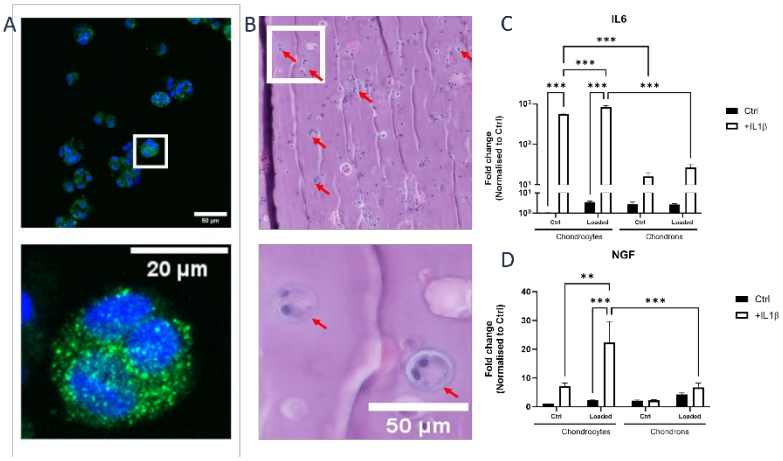

Figure 1 Pericellular Matrix Restoration Enhances Resistance of Chondrocytes to Inflammatory and Mechanical Stress
*Immunostaining of COL-VI (A, green) and Safranin O/Fast Green staining (B, red arrows showing chondrocytes with PCM), and inflammatory (IL6, C) and neurogenic (NGF, D) gene expression of chondrons vs chondrocytes, in response to IL1β and multiaxial mechanical loading. N=3 Donors and n=9 replicates. Bars represent mean values with error bars indicating SEM. Statistically significant differences indicated **P < 0.01, ***P < 0.001.*

DISCUSSION & CONCLUSIONS:

Restoring a COL-VI-enriched PCM enables reconstruction of a chondron-like micro-environment and enhances resistance to inflammatory and mechanical perturbations. These findings highlight the pericellular matrix as a critical functional component in cartilage biology and suggest that preserving or restoring the chondron may improve the robustness of cell-based cartilage repair strategies.

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Exploring TRPV4 Mechanosensitivity to Substrate Stiffness and Mechanical Loading in Annulus Fibrosus

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INTRODUCTION: Low back pain is the leading cause of disability worldwide and is strongly linked to intervertebral disc (IVD) degeneration. The annulus fibrosus (AF) is highly sensitive to mechanical cues; excessive loading disrupts matrix homeostasis, increases inflammation, and promotes progressive stiffening and structural failure. Mechanosensitive transient receptor potential (TRP) channels, particularly TRPV4, have been implicated in inflammatory and pain pathways[1,2], yet their contribution to AF mechanobiology remains insufficiently defined. We hypothesized that TRPV4 regulates key AF cell responses to substrate stiffness and cyclic mechanical loading via Ca^{2+} signaling and downstream degenerative gene programs.

METHODS: To model degenerative stiffening, we fabricated polydimethylsiloxane (PDMS) substrates spanning low, mid, and high stiffness (9, 63, and 240 kPa) and integrated them into custom stretching chambers. Substrate modulus was confirmed by tensile testing, and strain transfer during cyclic loading was validated by digital image correlation. Using bovine AF cells, we assessed pharmacologic TRPV4 activation (GSK101) across stiffness conditions by (i) intracellular Ca^{2+} flux (Fura-2 assay) and (ii) transcriptomic profiling (RNA-seq after 18 h stimulation). In parallel, we evaluated mechanically induced responses using cyclic stretch (8-12%, 1 Hz, 14 h) and quantified candidate mechanosensitive gene regulation by RT-qPCR.

RESULTS: PDMS chambers reliably reproduced the target stiffness range and transmitted strain effectively. TRPV4 activation induced robust transcriptional changes (637 differentially expressed genes), including candidates associated with inflammation and matrix regulation. TRPV4-mediated Ca^{2+} flux was stiffness-dependent, with the lowest responses on soft (9 kPa) substrates compared with stiffer conditions. While stiffness alone produced modest global transcriptional differences, cyclic stretch regulated multiple TRPV4-related genes; ongoing inhibitor studies test TRPV4 specificity during mechanical stimulation.

DISCUSSION & CONCLUSIONS: This platform enables controlled investigation of the combined effects of extracellular matrix stiffening and mechanical loading on TRPV4-dependent AF mechanotransduction, providing mechanistic insight relevant to annular degeneration and disc herniation-associated pain.

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Human Disc-on-a-chip: Modelling Human Intervertebral Disc Degeneration via Microfluidic and Hydrogel-Based System

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INTRODUCTION: Discogenic back pain mainly arises from intervertebral disc (IVD) degeneration, which is driven by disruption of extracellular matrix (ECM) homeostasis, inflammation processes, and heightened sensory hyperinnervation (1). Conventional animal and two-dimensional *in vitro* models have provided valuable insights but fail to capture the complex human IVD phenotype within a physiologically relevant three-dimensional context. In this study, we aimed to develop an IVD model integrating a microfluidic gradient device with ECM-based hydrogels, mimicking the heterogeneous IVD consisting of nucleus pulposus (NP), annulus fibrosus (AF), and sensory neurons.

METHODS: Disc-on-a-chip was fabricated using stereolithography with PDMS. incorporating variations in microfluidic channel and chamber geometries, with hydrogel concentrations and compositions, to mimic the NP, AF, and sensory regions integrated with a microelectrode array. Computational simulations were performed to analyse flow rate and velocity within the microfluidic system. The microfluidic slab was evaluated for surface hydrophilicity and roughness. Hydrogels were characterized for their mechanical properties. The inner core hydrogel consisted of HA/COLII for NP, surrounded by COLI hydrogel for AF, with iPSC-derived sensory neurons positioned peripherally. Cell-encapsulated hydrogels were assessed for viability, metabolic activity, phenotypic marker expression, and local field potentials of the iPSC-derived neurons were recorded.

RESULTS: Computational simulations showed that higher pressure increased shear stress and velocity, while curved-microchannel designs produced uniform flow and reduced pressure. Both hydrogels at 3 mg/mL exhibited greater stiffness, viscosity, and shear stress, while 2 mg/mL of hydrogel provided optimal viscosity resistance. NP cells demonstrated a rounded shape morphologically, and both AF and NP cells were evenly distributed throughout the hydrogel. NP cells exhibited the expression of COL2A1, KRT19, ACAN, and Brachyury, and AF cells expressed COL1A1 and FN, confirming the maintenance of region-specific phenotypes. Expression of peripherin and PGP9.5 confirmed the presence of sensory neurites, while expression of Nav1.7 further validated the nociceptive phenotype of the iPSC-derived sensory neurons within the co-culture system.

DISCUSSION & CONCLUSIONS: This research developed a human disc-on-a-chip model that mimics the structural and cellular diversity of the intervertebral disc. The use of ECM-based hydrogels and microfluidic gradients facilitated the establishment of spatially defined populations of NP, AF, and sensory neurons, promoting region-specific phenotypes. Computational simulations showed the impact of microchannel design on shear stress and flow homogeneity, whereas hydrogel optimization preserved cell viability and tissue-specific phenotype. The detection of peripherin and PGP9.5 validated the effective integration of sensory neurons. This approach provides a human-relevant substitute for animal and 2D models, facilitating mechanistic investigations of inflammation. It offers a prospective preclinical instrument for evaluating anti-inflammatory treatments for discogenic back pain.

ACKNOWLEDGEMENTS: This study is ethically approved with C.A. 3235 Molecular profiling of intervertebral disc degeneration underlying inflammatory pain in humans and funded by the Health Research Board, Ireland, under the Emerging Investigator Award for Health (EIA-2022-010).

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The "village-in-a-dish" approach as an innovative cellular model for investigating variability in response to loading in chondrogenic cells

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INTRODUCTION: The use of induced pluripotent stem cells (iPSCs) for *in vitro* population genetics has gained popularity, enabling the modelling of complex (non-monogenetic) diseases. However, traditional culturing approaches are often time-consuming, expensive, and influenced by high technical variability. To overcome these limitations, the village-in-a-dish approach has emerged [1].
This approach implies the culturing and, when applicable, the differentiation of iPSCs from multiple donors at the same time in a single dish. This method increases throughput, reduces variability, and lowers costs, making it particularly useful for population genetic studies that require a high number of donors. Recently, our goal has been to implement the village-in-a-dish method to investigate osteoarthritis heterogeneity. We started by examining a fundamental cellular process connected to osteoarthritis that we hypothesized could be influenced by genetic variations present in the general population, such as response to loading.

METHODS: We first obtained 80 human iPSC lines from skin fibroblasts of donors 25 to 75 years old. The lines were reprogrammed using the non-integrative Sendai virus method and all expanded in vitronectin-coated plates and mTeSR+. In order to characterise cell behaviour and cluster composition in the "village" setting compared to the individual mono-cultures, a mini-village of four iPSC lines was established, using iPSC lines stained with different cytoplasmatic dyes. For the cluster composition analysis, proliferation assay, time-lapse analysis, Functional Single Cell (FUN)-seq pipeline [2] and variance estimation analysis of the study covariates was performed. After, an 11-day chondrogenic differentiation protocol was applied [3] and, at day 8, cells were transferred to FlexerCell FX-6000 compatible plates to allowed exposure to mechanical loading (cyclic tensile strain). Census-seq and RT-qPCR was used to characterise the cells after differentiation, followed by single cell RNA-sequencing to characterize them after loading.

RESULTS: By comparing iPSC lines cultured individually or in a "village", we confirmed that proliferation rate was not significantly influenced by culture conditions. Additionally, we imaged the composition of iPSC colonies within a village setting, demonstrating that cluster composition arises largely by random chance. Using FUN-seq, we demonstrate that growth in clusters containing cells from multiple donors or from a single donor does not significantly affect transcriptional profiles, supporting the validity of the village model. As proof of principle, a village of five iPSC lines was established and successfully chondrogenically differentiated as confirmed by the expression of *SOX9*, *COL2A1* and *AGCN*. We are currently performing experiments using chondrogenic maxi-villages of 25 iPSC lines each under loading, where a Vireo-based demultiplexing strategy will be applied for single-cell genotype-phenotype associations.

DISCUSSION & CONCLUSIONS: For the first time, we (1) performed an in-depth analysis to confirm the validity of the "village" setting for *in vitro* population studies, (2) chondrogenically differentiated a village of human iPSCs, and (3) successfully adapted the chondrogenic protocol for compatibility with the FlexCell device. Ultimately, this experimental setup will be used to identify genetic variations (e.g., SNPs) that confer differential responses to mechanical loading in specific donors. We believe this approach can establish new foundational knowledge about variability in response to loading in the context osteoarthritis.

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Imaging Neuroplasticity in Knee Osteoarthritis

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INTRODUCTION: Joint pain is one of the most prevalent symptoms in osteoarthritis. A critical gap in understanding mechanisms underlying joint pain is a lack of comprehensive anatomical and functional descriptions of pain-sensing nerves around and in the knee joint. Developing novel methodologies to enable visualization and quantification of these nerves is expected to lead to identification of novel targets for future treatment of joint pain. In this talk, lightsheet imaging and *in vivo* calcium imaging will be discussed as two methods to improve our understanding of anatomical and functional changes in joint innervation.

METHODS:
All animal procedures were approved by the Rush University Medical Center IACUC.

Lightsheet imaging: Following fixation, the samples were cleared using a modified DISCO protocol and immunolabeled with RFP and PGP9.5. Secondary antibodies conjugated with AF568 or AF647 were used. Following refractive index matching, samples were imaged by light sheet microscope (Zeiss Lightsheet 7).

In vivo calcium imaging: $Na_V1.8$cre;GCaMP6sloxp or Pirt-GCaMP3 mice were used for experiments. The L4 dorsal root ganglion (DRG) was exposed for two photon microscopy. Mice were kept under isoflurane anesthesia during the entire procedure. Neurons were recorded while stimuli were applied to the knee joint. The numbers of neurons responding before and after injection were calculated using Fiji.

RESULTS:
Through lightsheet imaging, we observed robust sensory innervation in specific regions of the healthy intact mouse knee joint including the infrapatellar fat pad, anterior meniscal horn and cruciate ligament insertion sites. In models of joint pain, we could detect increases in innervation in parts of the joint that were associated with structural changes in pathology[1] such as the medial synovium and medial subchondral bone.

We also showed that the i*n vivo* calcium imaging method was sensitive to detect changes in the numbers of responding neurons to mechanical stimuli applied to the knee joint caused by osteoarthritis[2] and was sensitive to detect changes caused by genetically[3] or pharmacologically[4] targeting specific molecules.

DISCUSSION & CONCLUSIONS: We demonstrate clearing-enabled light sheet microscopy and an accompanying analysis workflow for the intact mouse knee joint. The workflow can also examine multiple targets and can accommodate tissues from multiple species. Through *in vivo* calcium imaging of the DRG we show that increased numbers of small to medium-sized DRG neurons respond to mechanical stimuli in models of osteoarthritis, suggesting that nociceptors have become sensitized by lowering the response threshold. Targeting mechanosensitive ion channels intra-articularly can modulate these responses. Collectively these methods allow for more comprehensive analyses of *in situ* changes in the sensory nervous system in and around the knee joint.

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The potential of MSCs and MSC-EVs as new therapeutics for osteoarthritis

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INTRODUCTION:

Mesenchymal stem cells (MSCs), as well as extracellular vesicles (EVs) derived from MSCs are being increasingly studied for their therapeutic effects in a range of chronic diseases, including musculoskeletal diseases. However, their potential for conversion into clinical therapy is limited by shortcomings such as suboptimal efficacy and limited survival (MSCs) or low yield (MSC-EVs). At least part of the problem may be solved by modulating the culture environment of MSCs to improve the therapeutic effects of both the parent MSCs and of MSC-EVs in the treatment of specific musculoskeletal diseases, such as osteoarthritis. Moreover, MSC-EVs may be used as an augmentative factor to improve the bioactivity of scaffold implants for treating musculoskeletal injuries, such as in tendon-bone healing. This talk will introduce some of our recent work on improving the therapeutic efficacy of MSCs and MSC-EVs through a range of preconditioning methods, and applying them as novel therapeutics in preclinical models of musculoskeletal diseases such as osteoarthritis.

A Neurovascular Disc System to Study the Role of Endothelial Cells in Nociceptive Axonal Growth

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INTRODUCTION:

Low back pain is associated with sensory nerve ingrowth in intervertebral disc (IVD). Endothelial cells (ECs) are often found alongside these nerves, [1] while their role in nerve growth is not well understood.

In our previous work, we applied hydrodynamic forces to spatially organize bovine dorsal root ganglion (DRG) neurons around annulus fibrosus (AF) explants, the outer region of the IVD, to investigate their crosstalk. [2]

The presented study examines whether incorporating ECs into the DRG compartment affects nerve growth within a degenerative IVD context.

METHODS:

A multi-compartment system was developed by hydrodynamically assembling DRG cells around bovine AF explants. Within this model, AF explants pre-treated with inflammatory cytokines (IL-1β and TNF-α, 10 ng/mL each) were compared to untreated controls to determine whether inflammation enhances nociceptive axonal growth. (Figure 1)

To further investigate the role of endothelial cells, the DRG compartment surrounding cytokine-treated AF explants was co-cultured with human umbilical vein endothelial cells (HUVECs) and compared to conditions without HUVECs, allowing assessment of whether ECs promote nociceptive axon extension. (Figure 1)

After 48 hours of co-culture, axons were labelled using the nociceptive neuronal marker calcitonin gene-related peptide (CGRP). Axonal branching was quantified using an automated Sholl analysis algorithm. Concentric rings of increasing diameter were generated from the neuronal soma, and the number of axon-ring intersections was measured as an indicator of axonal density and complexity.

RESULTS: A total of 759 CGRP-positive neurons were analyzed. The intersection number, representing axonal branching density, increased by 34.1% in the cytokine-primed AF group compared to the non-primed control ($P = 0.0033$). This value was further elevated by 23.7% with the addition of ECs to the DRG compartment ($P = 0.033$). The combined effect of cytokine treatment and EC addition resulted in a 65.9% increase in the intersection number ($P < 0.001$). (Figure 2)

DISCUSSION & CONCLUSIONS:

Sound-based hydrodynamic forces are efficient to assemble neurovascular compartment when studying multi-tissue crosstalk. Results from the multi-compartment system show that ECs may play a critical role in IVD nerve ingrowth and represent a potential therapeutic target for treating discogenic pain due to innervation.

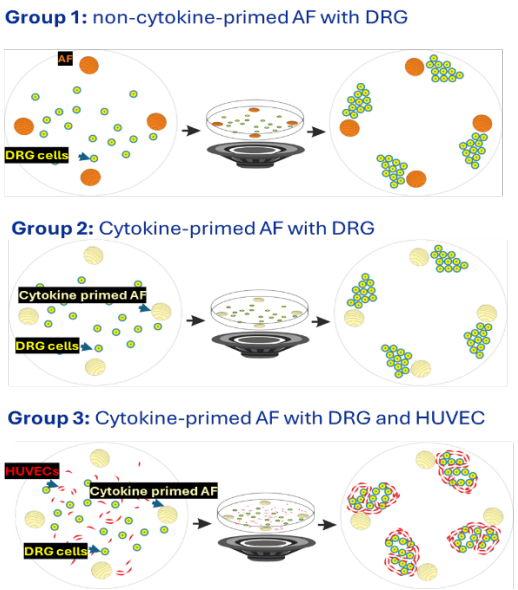

Figure 1: Study group design.

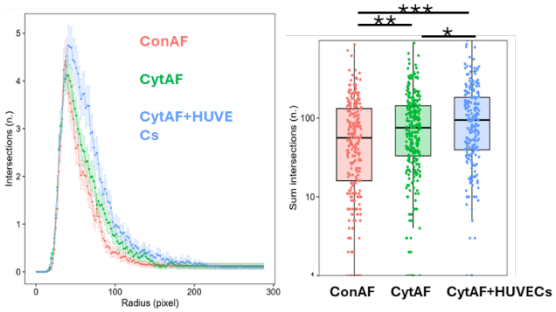

Figure 2: Sholl analysis of CGRP+ axons.

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Intradiscal propionic acid levels in chronic low back pain patients (CLBP) with Modic type 1 changes (MC1) are associated with systemic immune cell activation

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INTRODUCTION: Disc infection with *Cutibacterium acnes* (*C. acnes*) can trigger MC1 in vivo, and MC1 bone marrow immune response depends on the amount of *C. acnes* in the adjacent disc. Inconsistent response of MC1 patients to antibiotics may reflect differences in *C. acnes* related MC1 pathophysiology. Magnetic resonance spectroscopy (MRS) of discs is a non-invasive tool to measure intradiscal propionic acid (PA), a main *C. acnes* metabolite. Using disc MRS, we tested whether CLBP patients with high disc PA have a distinct blood immune profile, enabling non-invasive stratification of MC1 subtypes.

METHODS: CLBP patients (n=200) from the UCSF comeBACK cohort underwent lumbar MRI for Modic change grading (MC1, MC2, MC0), and lumbar MRS for intradiscal PA quantification. Patients were stratified into tertiles of maximum disc PA. Systemic immune signatures were compared between highPA and lowPA within each MC type. Analyses included whole-blood transcriptomics, multiparametric flow-cytometry immunophenotyping, and serum cytokine profiling (40 analytes). Group differences were tested by ANOVA or DESeq2, and gene-set enrichment analysis. FlowSom algorithm was used for unsupervised clustering of flow cytometry data.

RESULTS: HighPA was associated with systemic immune responses exclusively in MC1. Transcriptomics: MC1 highPA patients had enrichment of innate and adaptive-immune pathways, MC2 and MC0 patients didn't (Table 1). Flow cytometry: Among >1000 analysed immune cell populations, 12 of 13 enriched subsets in MC1 highPA were B cells (Fig.1a), and B cell subsets correlated with intradiscal PA in MC1 but not in MC2 or MC0. FlowSom clustering revealed that the $CD73^{+}IgD^{+}$ B cells were significantly enriched in highPA patients. Cytokines: In MC1, hierarchical clustering revealed that PA–cytokine correlation profiles aligned closest with those of IgD^{+} B cells.

Table 1. *Blood transcirptomics. Normalized enrichment scores (NES) in "PA-high" vs. "PA-low" by MC type.*

Description	MC1	MC2	MC0
Defense response to bacterium	2.00	ns	1.87
Phagocytosis, recognition	2.09	ns	ns
Phagocytosis, engulfment	1.94	ns	ns
Adaptive immune response	1.85	1.90	ns
Pos. regulation of B cell active.	2.04	ns	ns
B cell receptor signaling pathway	1.89	ns	ns

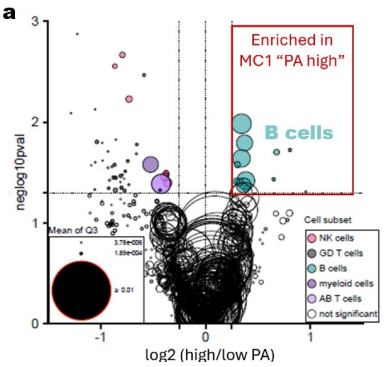

Fig.1. *Comparison of proportions of 1021 immune cell populations between highPA and lowPA in MC1 patients. Generalized linear mixed effect model; symbol size corresponds to the estimated population size in PAhigh.*

DISCUSSION & CONCLUSIONS: The amount of intradiscal PA, a *C. acnes* metabolite, stratifies MC1 patients by a systemic B-cell activation phenotype. This suggests that MC1 comprises distinct immunological subtypes that may reflect adaptive immune responses to bacterial antigens. This extends the concept of MC1 as not purely local but involving a systemic immune component, providing a novel mechanistic bridge between disc metabolism and microbial activity. MRS measurement of intradiscal PA may offer a promising biomarker for differentiating MC1 subtypes and stratifying patients for therapies.

Notochordal cells lead the way to regeneration

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Notochordal cells originate from the notochord, a transient midline structure essential for early vertebrate development. In developmental biology, the notochord is pivotal in patterning of the tissues and in neural tube formation. Furthermore, the notochord is the structural precursor of the vertebral column. Ultimately, the notochord regresses with remnants of notochordal cells residing within the nucleus pulposus of the intervertebral disc (IVD).

Notochordal cells residing in the core of the developing IVD, secret and deposit a glycosaminoglycan-rich matrix and their presence is associated with preservation of healthy NP. Species that suffer from the consequences of IVD-related low back pain – humans and dogs – loose these large vacuolated notochordal cells. They become gradually replaced by the smaller NP cells, which - as degeneration sets on - loose their ability to deposit healthy matrix and contribute to the low grade inflammatory and pro-catabolic IVD environment. The dog was employed as a model to capture, at the single-cell level, the heterogeneity of the resident nucleus pulposus cells (NPCs) and their transition during different life stages.

Notochordal cells represent a powerful biology avatar; one that played a central orchestrating role in embryonic development and can be harnessed to achieve NP regeneration. Their biologic effects are mediated through the secretome, which contains soluble factors, extracellular vesicles as well as a wealth of extracellular matrix molecules[1].

Over the course of several collaborative projects we have come to better understand their differentiation and transition to nucleus pulposus cells, as well as appreciate even more their regenerative potential. Through single cell sequencing at the transcriptomic and epigenetic level, focusing on H3K27 methylation, along the different life stages of notochordal cells, we mapped notochordal cell heterogeneity from healthy to degenerate and clinically painful discs, and identified governing mechanisms at the cellular level (unpublished data).

During this keynote lecture Tryfonidou will synthesize the insights of several collaborative efforts in which iPS technology, notochordal cells and the secreted specialized matrix and extracellular vesicles are the protagonists.

Where do we stand with iPS-based approaches to generate notochordal-like cells? How does the notochordal cell matrix contribute to this? In what way are extracellular vesicles involved in this? By providing the newest insights to these questions, the lecture will sketch the notochordal cell-avatar in the context of NP regeneration and new avenues towards exploiting their biology cues to therapeutic approaches for IVD-related low back pain.

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Filth or Fuel? Lactate's Role in Disc Metabolism and Epigenetic Regulation

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INTRODUCTION: The intervertebral disc lacks significant blood supply and is structured with a central hypoxic nucleus pulposus (NP) encased by the more oxygenated annulus fibrosus (AF). Traditionally, lactic acid—a major glycolytic byproduct of the NP—has been considered a detrimental metabolic waste that lowers tissue pH and compromises disc cell survival and performance[1]. Given that lactic acid converts readily to lactate within disc tissue, this study aimed to explore whether AF cells could metabolize lactate as an energy substrate rather than requiring its removal as a cellular waste product. Additionally, this research examined lactate's potential role as an epigenetic metabolic regulator by evaluating its capacity to trigger histone lactylation[2] in disc cells to regulate matrix homeostasis and transcriptional reprogramming.

METHODS: To test AF cell's capacity of utilizing lactate as a biofuel, import and conversion of lactate to tricarboxylic acid (TCA) cycle intermediates and amino acids in rabbit AF cells were measured by heavy-isotope (^{13}C-lactate) tracing experiments using mass spectrometry[3]. To study the epigenetic role of lactate, histone lactylation was quantified in intervertebral disc tissues and primary AF and NP cells. Using disc cell culture models, exogenous lactate treatment, transcriptomic profiling (RNA-seq), and chromatin mapping for histone lactylation marks (CUT&Tag) were employed to assess lactate's effects on histone modifications, gene expression, and cell viability.

RESULTS: Heavy-isotope tracing experiments revealed that AF cells imported and converted lactate into TCA cycle intermediates and amino acids using in vitro cell culture and in vivo models, indicating utilization of lactate as a carbon source by AF cells. Intervertebral disc tissues displayed elevated histone lactylation levels. Treatment with exogenous lactate enhanced histone lactylation in both AF and NP cells in a dose- and time-dependent manner. Furthermore, lactate improved disc cell viability across basal, inflammatory, and oxidative stress conditions. Transcriptomic profiling identified lactate as a mediator of metabolic adaptation, matrix remodeling, and inflammatory dampening. CUT&Tag analysis revealed location-specific histone lactylation signatures corresponding to observed transcriptional alterations, indicating epigenetic regulation mediated by lactate.

DISCUSSION & CONCLUSIONS: These findings support a lactate-dependent metabolic symbiosis within the disc, wherein lactate generated by hypoxic, glycolytic nucleus pulposus cells is consumed by the more oxygen-rich annulus fibrosus cells through oxidative phosphorylation to fuel energy production and matrix synthesis. This work fundamentally reframes the current understanding of disc lactate from a metabolic waste product to a critical biofuel source. Furthermore, this study establishes lactate as an epigenetic signaling molecule within the disc microenvironment, demonstrating that histone lactylation serves as a mechanistic link connecting lactate metabolism, chromatin remodeling, and disc cell adaptation—thus revealing the metabolic–epigenetic regulatory networks underlying intervertebral disc biology.

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High-resolution spatial transcriptomic and proteomic mapping of the developing human intervertebral disc

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INTRODUCTION: The intervertebral disc (IVD) is a fibrocartilaginous tissue made up of a central nucleus pulposus (NP) surrounding by an outer annulus fibrosus (AF). It acts as a shock absorber and enables flexibility of the spine. During development, the NP is populated by notochordal cells, which are gradually replaced by small chondrocyte-like cells during adolescence. This transition coincides with the onset of the gradual deterioration of the IVD structure, indicating that notochordal cells are vital for maintaining IVD homeostasis. However, the function of human notochordal cells and their influence on the surrounding cellular and extracellular microenvironment during development is poorly understood. To address this, we applied a high-resolution spatial multi-omics approach combining spatial transcriptomics and proteomics to define gene and protein distribution patterns governing human IVD development.

METHODS: To define the spatial transcriptomic landscape of cells in the developing IVD, histological sections were prepared from formalin-fixed paraffin-embedded human fetal spine tissues at 6 developmental stages: 5 and 7 weeks post-conception (WPC) representing pre-notochordal segmentation, and 9, 12, and 14WPC representative of post-notochordal segmentation. The sections were stained, imaged, and processed using the 10X Genomics Visium high-definition (Visium HD) protocol with a 2µm resolution. Data were analysed using SpatialData and Seurat software packages. To characterise the protein composition of the developing IVD, MALDI imaging mass spectrometry was applied at the same ages. Data were analysed using SpaceRanger and SCiLsLab. This study was approved by the UK NHS Research Ethics Committee and full written informed consent was obtained from all donors.

RESULTS: High-resolution spatial transcriptomics revealed significant differences in the cellular distribution and gene expression profiles of spine cell populations, particularly notochordal cells, as development progressed. Differential expression analyses showed that 5WPC and 7WPC notochordal cells had higher expression of genes regulating embryonic skeletal system development, most notably *SHH.* By contrast, these genes were downregulated following notochordal segmentation and cells at 9, 12 and 14WPC expressed higher levels of genes involved in structural maintenance, cell growth and extracellular matrix synthesis. Interestingly, we observed two regionally distinct notochordal populations (Notochord 1 and 2) present only during segmentation at 7WPC. Notochord 2 had high expression of cell migration genes including *CRIP1 and TPPP3,* suggesting that this cell population regulates notochordal segmentation and spine patterning. Through spatial proteomics, we observed early regional specialisation in the IVD, characterised by matrisomal patterning, including an inverse distribution of collagen Type I and Type II in the AF and NP, respectively.

DISCUSSION & CONCLUSIONS: In summary, our data provides a high-resolution spatial map of all cell types in the developing spine, offering novel insights into their behaviour within their natural microenvironment. It further demonstrates the complex tissue heterogeneity in the developing spine and offers novel insights into how IVD cells regulate their extracellular microenvironment. Together this data enhances our understanding of notochordal cell behaviour during development and provides insight into key molecular pathways which could potentially inform cell reprogramming strategies aimed at repairing impaired cell function during disc degeneration.

Direct In Vivo Capture of the Nucleus Pulposus Secretome

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INTRODUCTION: Low back pain is a major global health burden and is closely linked to intervertebral disc (IVD) degeneration. The nucleus pulposus (NP), a gelatinous core tissue, is essential for disc hydration, mechanical function, and overall integrity. Although NP health is central to IVD homeostasis, how NP cells communicate molecularly with surrounding tissues—including the annulus fibrosus (AF) and endplate (EP)—remains poorly defined. Also, much remains to be learned about the crosstalk between IVD and other tissues. Secreted proteins are critical mediators of tissue crosstalk. Identifying factors released by NP cells, as well as circulating proteins that regulate disc microenvironment, is essential for understanding factors that regulate disc homeostasis. To address this, we generated a novel genetic mouse model with an *in vivo* proximity-labeling strategy to directly capture the NP secretome under physiological conditions. This approach enables cell-specific identification of NP-derived secretory proteins, and non-native circulating proteins present within the disc.

METHODS: Male and female mature (1-4 months of age) mice were used in accordance with NIH guidelines and IACUC approval. *ShhCre* mice[1] (NP-specific in IVD) were crossed with a conditional *BirA**G3-ER secretome reporter allele[2] to generate NP-specific-secretome mice. *BirA**G3-ER littermates lacking Cre served as controls. All mice received biotin daily for about 10 days via drinking water and intraperitoneal injection.
Serum, lumbar and caudal spine were collected. The L5-S1 IVDs were fixed for histological analysis, and remaining IVDs were processed for protein analysis. Biotinylated proteins were pulled-down using streptavidin beads and subject to label-free LC-MS/MS in DIA mode. Proteins identified at 1% FDR against Uniprot mouse protein database were used for further analysis.
Protein localization and functional enrichment were assessed using UniProt, PantherDB, and Gene Ontology. Immunostaining, reporter expression, and Western blotting were used for validation. RNAsesq was performed on the NP cells isolated from the lumbar IVDs of age-matched mice to generate transcriptome signature of NP cells.

RESULTS: The *BirA**G3-ER reporter enables permanent *ShhCre*-dependent activation of fluorescent reporter and ER-localized biotin ligase in NP cells, providing precise control over in vivo secretome labeling. Immunostaining confirmed biotinylated proteins exclusively in NP tissue from *ShhCre; BirA**G3-ER mice. Western blotting confirmed expression of BirA ligase protein expression and biotinylation of proteins. Proteomic analysis of NP pull-downs identified diverse secreted proteins, including extracellular matrix components, signaling molecules, and growth factors. Integration of proteomics data with NP transcriptomic data confirmed NP-expressed proteins while also revealing additional proteins not expressed by NP cells, suggesting tissue crosstalk and contributions from other *Shh*-expressing cells outside of the IVD. Proteomics of serum identified NP-secreted proteins present in circulation-indicating long-distance crosstalk.

DISCUSSION & CONCLUSIONS: This study establishes cell-specific *in vivo* biotinylation as a robust and selective method for mapping secretome. Comprehensive proteomic profiling reveals NP cell secretory network that likely coordinates molecular signaling within the IVD, and other tissues via circulation. These findings also provide molecular insight into the influence of circulating factors on the disc microenvironment. By defining NP-associated secreted proteins with potential regulatory and reparative functions, this work establishes a foundation for identifying early biomarkers of disc degeneration and developing targeted strategies to prevent or treat low back pain and IVD disease.

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The Relevance of Skeletal Innervation: From Basic Research to Advanced In Vitro Modelling

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The skeleton is increasingly recognized as a highly innervated and biologically dynamic organ rather than a merely structural tissue. Neural networks within skeletal tissues are spatially organized and functionally integrated with bone, cartilage, synovium, vasculature, and bone marrow niches, where they contribute to tissue homeostasis, remodelling, regeneration, inflammation, and pain perception. Growing evidence indicates that neuro-skeletal interactions constitute a critical regulatory axis in skeletal development, maintenance, repair, and disease progression.

Alterations in skeletal innervation and neuronal signalling have been reported in several musculoskeletal pathologies, including fractures, bone cancer, and arthritic diseases. These neurobiological changes are increasingly associated with inflammatory processes, pathological tissue remodelling, and chronic pain. In osteoarthritis (OA), for example, alterations in joint innervation are closely linked to synovial inflammation, subchondral bone remodelling, cartilage degeneration, and persistent nociceptive signalling. Despite pain being the predominant clinical symptom and major cause of disability in OA patients, current therapeutic approaches remain insufficient, largely because they fail to address the neurobiological and inflammatory mechanisms underlying disease progression and chronic pain.

The presentation will discuss current advances in the understanding of neuro-skeletal interactions, with particular focus on the bidirectional communication between peripheral nervous system and skeletal tissues in health and disease. The presentation will further introduce the scientific rationale and conceptual framework underlying the SINPAIN project, which aims to develop innovative therapeutic strategies targeting both inflammation and pathological innervation in osteoarthritis. Emphasis will be placed on the growing role of advanced human micro-physiological systems and in vitro models as powerful tools to investigate neuro-skeletal crosstalk and support the development of next-generation therapies for musculoskeletal disorders.

Exploring the bioactivity of synovial fluid in osteoarthritis as a vector for intra-joint biomolecular cross-talk

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INTRODUCTION: In the early days, osteoarthritis was mainly attributed to mechanical wear and tear mechanisms of the joint, evidenced by degenerative pathological changes in the cartilage compartment and inflammatory responses of the synovium. In the past decades osteoarthritis has been increasingly recognized as a "whole joint disease", with cellular and molecular crosstalk mechanisms as important drivers of an active disease process. However, the medium or vector via which these molecular crosstalk events occur remains largely underexplored. Synovial fluid is a serum ultra-filtrate with a composition that is largely influenced by the local joint tissues (1). Osteoarthritic synovial fluid contains a plethora of biomolecular factors including cells, extracellular vesicles, proteins, crystals, metabolites and RNAs. While synovial fluid is the main vector via which intra-articular tissue or cellular crosstalk occurs, it is mainly considered as a depot for diagnostic osteoarthritis biomarkers. In contrast, our investigations highlight that there is critical pathological bioactivity contained within osteoarthritic synovial fluid, which acts as a vector for disease activity and progression. This makes us approaching synovial fluid as a critical joint compartment in its own right (2).

METHODS: Primary OA patient material was used under medical ethical permission and informed consent. A variety of molecular cartilage biological techniques were used using standard operating procedures, including RT-qPCR, luciferase reporter assays, immunoblotting, ELISA, cell culture, lentiviral transduction, protein mass-spectrometry and antibody array methodology. Accompanying statistical testing was applied, with specific statistical tests depending on the specific experimental outline.

RESULTS: Our recent cell signaling studies have demonstrated that exposure of chondrocytes to OA synovial fluid leads to activation of MAPK, RhoGTPase, AKT and NFkB signaling events. These are signaling pathways that are well-known to be involved in pathological OA-related processes. Downstream, the activation of these cell signaling cascades activate pathological transcription factor activity, such as SRF, p65 and AP1. The cellular consequences for the chondrocyte following exposure to OA synovial fluid are dominated by fibrotic differentiation accompanied by increased cell proliferation. Also ribosome synthesis is being altered by OA synovial fluid, with impact on preferential translation of proteins involved in fibrosis and inflammation. While it is expected that OA synovial fluid bioactivity is the result of the combined biomolecular composition of synovial fluid, reductionistic investigations have highlighted pathological responses of many of the individual synovial fluid constituents. Extracellular vesicles in synovial fluid induced NFkB, CRE, SFR and SRE transcriptional activity as a function of OA progression. The protein composition of OA synovial as compared to non-OA synovial fluid is drastically different. Dominant proteins that we found higher expressed in OA synovial fluid are cytokines (i.e. IL16/17B/17F/23A/24/25/26 and TNF), chemokines (i.e. CCL11/24/26, CXCL1/10/12/13 and CX3CL1), growth factors (i.e. VEGFB/D, FGF8/11/20, IGF2, EGF and EREG) and DAMPs (i.e. SAA1 and S100A10/12). Many of these signaling proteins are established upstream initiators of pathobiological responses in the OA joint. For a good number of proteins that are higher expressed in OA synovial fluid, we could identify a tissue-dependent source, with IGF2, AHSG, FN1, CFB, KNG and C8 as validated examples. A major pathological crystal species in OA synovial fluid is basic calcium phosphate (BCP). Besides the established role of BCP crystals in inducing IL6 and MMP expression in chondrocytes, we found that exposure of chondrocytes to BCP crystals leads to activation of a TGFbeta signaling axis that aggravates inflammatory responses. We regard the biomolecular composition and associated cumulative bioactivity of OA synovial fluid as a patient-specific fingerprint of the disease status of the intra-articular tissues involved in OA. Using a cell-based screening approach to biologically interpret the variety of biomolecular signals present in end-stage knee OA synovial, we identified two major OA synovial fluid clusters. These could be separated by AP1 and SIE transcriptional activity. Ribosome biosynthesis is one of the major cellular pathways that is differentially impacted by these two OA synovial fluid subgroups.

DISCUSSION & CONCLUSIONS: The field is increasingly appreciating the bioactive role of OA synovial fluid as a vector for intra-joint cross-talk in OA. It is expected that studying the intrinsic bioactivity of OA synovial fluid will open new avenues for developing OA disease-modifying therapies and provide a unique and agnostic basis for using emerging technologies to discover bioactive pathways towards better targeted and biomolecular stratification approaches for the treatment of OA.

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Dynamic compression strain drives chondrogenic transcriptional fate through actin cytoskeleton dynamics, TGF-β/SMAD signalling and YAP1 translocation

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INTRODUCTION: Mesenchymal stromal cells (MSC) mobilization, including microfracture, is often used for cartilage repair. However, it typically produces repaired tissue with inferior properties compared to hyaline cartilage, as MSCs tend towards fibrocartilaginous or hypertrophic differentiation. While MSCs are known to sense extracellular mechanical cues, such as those provided by biomaterials, most research has focused on their impact on chondrogenic phenotype and cytoskeletal organization. Consequently, the effects of externally applied mechanical forces on MSC-mediated regenerative responses remain poorly understood. Here we aimed to assess whether distinct dynamic compression (DC) strain could modulate chondrogenic transcriptional fates, actin cytoskeleton arrangement and YAP1 localization in human bone marrow derived MSC (hBMSCs) embedded within gelatin methacrylate (GelMA) hydrogels.

METHODS: A custom multiaxial bioreactor was used to apply 10% or 20% DC (with a 10% static offset) combined with 1 Hz shear. In the acellular study, 10% or 15% (w/v) GelMA hydrogels with latent Transforming Growth Factor Beta 1 (LTGF-β1) were subjected to a single loading cycle (1, 4, or 6 h) or maintained under free-swelling (FS) conditions. The cellular study was divided into two parts. In the first part, 1 million hBMSCs (4 donors) in 10% (w/v) GelMA were mechanically stimulated 4 h/day for 10 days or FS-cultured (active TGF-β1–free); in the second, 5 million hBMSCs (5 donors) in 15% (w/v) GelMA were mechanically stimulated 1 h/day, 5 days/week for 2 weeks or under the same FS conditions. Samples were collected and analyzed by TGF-β1 ELISA, qRT-PCR, RNAscope in situ hybridization, and fluorescent and immunostaining.

RESULTS: In 10% (w/v) GelMA hydrogels, a 4 hours 10% DC loading cycle increased exogenous LTGF-β1 activation in cell-free hydrogels and promoted a fibrocartilaginous/hypertrophic gene expression profile compared to 20% DC strain. Although both DC conditions enhanced SMAD2/3 reporter gene expression, indicating pathway activation, this was not accompanied by an increase in the activation of endogenous LTGF-β1, and *COL2A1* expression was not detected. Following optimization of the hydrogel formulation and loading regime, 15% (w/v) GelMA hydrogels exhibited increased LTGF-β1 activation under 20% DC strain, exogenously compared to 10% (w/v) GelMA hydrogels and endogenously to FS control. Additionally, 20% DC strain supported a more stable chondrogenic transcriptional profile within hydrogels. Spatial differences (**Figure 1**) were observed at the hydrogel's uppermost part, where 10% DC strain promoted a spread morphology, stress fibre formation, and nuclear YAP1 localization hydrogels, while 20% DC strain induced a rounded morphology, cortical F-actin, cytoplasmic YAP1 localization, and increased *COL2A1* transcripts hydrogels.

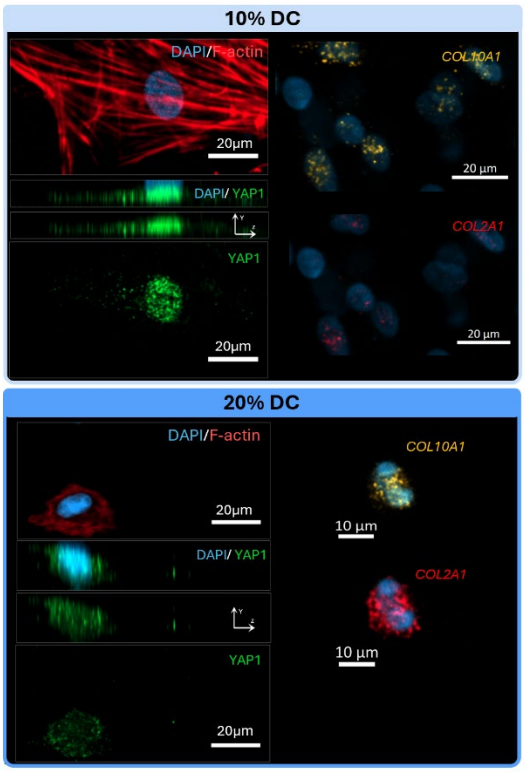

Figure 1: *Spatial DC strain–dependent early hBMSC chondrogenic transcriptional fate via cytoskeleton organization and YAP1 translocation in uppermost part of GelMA hydrogels.*

DISCUSSION & CONCLUSIONS: In this study, we demonstrate a customizable hydrogel–bioreactor system in which controlling LTGF-β1 activation dictates *COL2A1* expression and determines whether hBMSC adopt a hypertrophic or stable chondrogenic gene expression profile across the hydrogel. Additionally, DC strains spatially regulate chondrogenic fate via mechanotransduction, with effects observed at the hydrogel's uppermost part. These findings could guide rehabilitation protocols and mechanosensitive scaffold design to enhance MSC-mediated repair after microfracture.

Objective Imaging Biomarkers for Knee OA: A Data-Driven View of Cartilage Degeneration

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INTRODUCTION: Knee osteoarthritis (KOA) is a progressive joint disease marked by degeneration of articular cartilage and altered joint biomechanics[1]. Despite the clinical demand for early diagnosis, conventional radiographic grading remains subjective and insensitive to subtle structural changes[2]. Quantitative cartilage features extracted from medical imaging (MRI and CT) might serve as objective imaging biomarkers of joint status. This work presents a data-driven framework for characterizing cartilage degeneration using multimodal imaging features and analytical methods.

METHODS: A total of 133 knees (53 diagnosed with KOA, 80 controls) recruited within the SINPAIN EU project underwent MRI and CT scans. Cartilages were segmented from MRI and reported to CT through a registration process, allowing for the study of radiodensity (HU) and morphological features[3]. From the four major cartilage regions (femoral, medial tibial, lateral tibial, patellar), two categories of quantitative biomarkers were extracted:

1. Density-Morphology features (16 total): Cartilage mean and standard deviation of CT-based HU values, volume, and surface.
2. Radiomic features: 1272 MRI-based and 958 CT-based texture, shape, and wavelet-derived descriptors.

Group differences were assessed using permutation tests (10000 iterations). Some machine learning (ML) algorithms (SVM, Logistic Regression, and Random Forest) were also used to classify the disease outcome. Together, statistical analysis and ML were used to identify the most discriminative biomarkers.

RESULTS: On the Density-Morphology dataset, statistical significance ($p < 0.001$) was retrieved by density heterogeneity across all cartilage regions, volume for medial tibial and patellar cartilages, and femoral cartilage surface. ML analysis also proved the capability of these features to consistently distinguish KOA samples from healthy ones. The SVM algorithm achieved the highest performance with an F1 score of 0.86 ± 0.07. The SVM feature importance indicated that all cartilage volumes were the most predictive features.

Regarding MRI- and CT-based radiomics, ML algorithms achieved accuracies above 90% across both datasets and different algorithms. From these, the most predictive biomarkers included textural features (also from the wavelet transform) and shape descriptors, capturing microstructural changes not visible at naked eye.

DISCUSSION & CONCLUSIONS: These findings highlight the clinical value of quantitative imaging for detecting cartilage changes. Density heterogeneity reflects adjacent regions of abnormally high and low density, showing that degeneration progresses unevenly. Similarly, reductions in cartilage volume and an increase in surface area provide direct evidence of typical morphological changes associated with KOA. Texture and shape descriptors of MRI- and CT-based radiomics confirmed it: although less interpretable, microstructural alterations are strongly associated with knee status.

The consistent significance of medial compartment features across analyses reflects the well-established pattern of increased mechanical loading in this region, supporting its role as the earliest and most sensitive site for detecting degenerative changes.

Altogether, the identified biomarkers provide more sensitive and reproducible indicators of cartilage degeneration, improving diagnostic confidence. This lays the foundation for a more interpretable and clinically precise assessment.

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Charge-driven Gene Delivery to Cartilage

Prof. Ambika Bajpayee

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Osteoarthritis (OA) remains a major unmet clinical challenge because cartilage presents multiple barriers to therapeutic delivery: it is avascular, densely charged, and populated by sparsely distributed, largely quiescent chondrocytes. These features severely limit the penetration, retention, and intracellular trafficking of conventional drug and gene delivery systems. This talk will discuss how charge-guided biomaterial design can transform these physiological barriers into transport advantages for targeted gene delivery to cartilage. Prof. Bajpayee will present the principles underlying electrostatically driven transport systems that exploit the high fixed negative charge density of cartilage to achieve rapid tissue penetration, enhanced uptake, and prolonged retention of therapeutic cargos. Emphasis will be placed on the development of a new class of charge-engineered extracellular vesicles and hybrid non-viral carriers capable of traversing the full thickness of cartilage and delivering nucleic acids to resident chondrocytes. The talk will further address one of the central challenges in cartilage gene therapy: enabling nuclear access in quiescent, non-dividing cells where nuclear entry is intrinsically inefficient. Emerging strategies to overcome these intracellular barriers—including modulation of vesicle surface charge, membrane fusion properties, endosomal escape, and nuclear targeting mechanisms—will be discussed in the context of mRNA and plasmid delivery. Together, these advances illustrate how integrating biophysics, biomaterials engineering, and cell biology can enable disease-modifying gene therapies for OA and other degenerative musculoskeletal diseases.

Protective Effects of IL-1Ra Gene Therapy on Intervertebral Disc Degeneration: *In Vitro* and *In Vivo* Findings in Beagle Dogs

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INTRODUCTION: The pro-inflammatory cytokine IL-1β drives catabolic processes in intervertebral disc (IVD) degeneration, one of the main causes of low back pain. Inhibiting IL-1β signaling with local gene delivery of IL-1 receptor antagonist (IL-1Ra) offers a promising therapeutic approach. IL-1Ra gene delivery using a high-capacity adenovirus vector (HCAd) inhibits IL-1β–induced catabolic pathways in human nucleus pulposus cells (NPCs) and matrix degradation in human NP explants. This study evaluates the translational potential of IL-1Ra gene therapy by integrating mechanistic *in vitro* analyses with validation in a clinically relevant large animal model of IVD degeneration.

METHODS: NPC pellet cultures from five dogs, transduced with HCAd-IL-1Ra, were challenged with IL-1β (1ng/mL) for seven days. Matrix composition, histological features, and gene expression were assessed to determine the protective effects of IL-1Ra gene therapy. The therapeutic potential of HCAd-IL-1Ra was evaluated in a dog model of moderate IVD degeneration induced via partial nuclectomy. Six weeks post-induction, affected discs were treated with intradiscal injections of HCAd-IL-1Ra (40 µL, 2.5×10^{10} vector particles; N=6) and monitored longitudinally using MRI (T2 mapping). At six months, systemic safety was assessed post-mortem and disc tissues were halved along the sagittal plane, one for histology and the other one for biochemical analysis including GAG content and multiplex ELISA.

RESULTS: IL-1β triggered a catabolic shift in dog NPC pellets, characterized by reduced GAG deposition and content, accompanied by increasing IL-1β expression (Fig1), confirming the culture model. HCAd-IL-1Ra treatment effectively maintained an anabolic NPC phenotype. Gross pathology and histopathology confirmed systemic safety of HCAd-IL-1Ra *in vivo*. Additional assessment revealed moderate IVD degeneration in the untreated discs at T=6 months (reduced T2 relaxation; increased PGE2, CCL2, IFNγ, and NGF). Although effect on inflammatory mediators was not observed with HCAd-IL-1Ra treatment, it resulted in reduced histopathological scores (Fig. 1) and halted the progressive decline in T2 relaxation. On histology, IL-1Ra treated discs had 4.5-fold higher % $SOX9^+$ (P=0.015) and 1.6-fold higher % $TBXT^+$ (P=0.072) NPCs, compared to degenerative control, suggesting that it supports a healthy NPC phenotype.

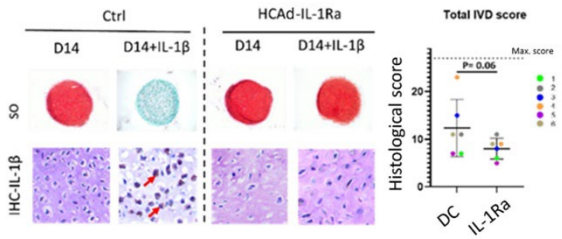

Figure 1. Safranin O/Fast Green stain (SO) and IL-1β immunohistochemistry on NPC pellets (N=5), and histological score in degenerative control (DC) vs. IL-1Ra treatment in a dog model (N=6). Red arrows: IL-1β positive NPCs.

DISCUSSION & CONCLUSIONS: Together, these results indicate that IL-1Ra gene therapy not only halts IL-1β-mediated matrix degradation but may also create a microenvironment that promotes SOX9 and TBXT expression in NPCs, highlighting IL-1Ra's translational potential as a disease-modifying therapy for IVD degeneration.

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Ex Vivo Exploration of PCRX-201 in Human OA Joint Tissues

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INTRODUCTION:
Osteoarthritis (OA) is a degenerative joint disease affecting over 595 million people worldwide, characterised by progressive cartilage breakdown, subchondral bone remodelling and synovial inflammation, contributing to joint dysfunction. Central to OA pathogenesis is an imbalance between catabolic and anabolic processes driven by inflammatory cytokines, particularly interleukin-1 beta (IL-1β). PCRX-201, a high-capacity adenoviral vector encoding interleukin-1 receptor antagonist (IL-1Ra), is designed to inhibit this IL-1-driven inflammation. This study aimed to leverage an *ex vivo* human osteochondral and synovial explant co-culture system to evaluate PCRX-201's therapeutic effects in a physiologically relevant setting. Although PCRX-201 is already being tested in clinical trials, the *ex vivo* model allows investigation of multiple variables including minimally effective dose, molecular pathways, and drug combinations within samples from individual patients, providing mechanistic insight and personalised therapeutic screening. Supporting the evaluation of PCRX-201 as a gene therapy to suppress the catabolic environment driven by IL-1 in OA, enhancing understanding of its mode of action and optimizing treatment strategies.

METHODS: An *ex vivo* OA culture model was developed using human osteochondral and synovium explants harvested from patients undergoing joint replacement surgery (Ethical approval: Sheffield Research Ethics Committee 20/SC/0144, 12182). Both high-grade and low-grade osteochondral explants were co-cultured with patient-matched synovium in a compartmentalised system enabling cellular crosstalk via secretome signalling, closely mimicking *in vivo* joint interactions. Explants were pre-cultured for 7 days under physiologically relevant conditions: hypoxia and low-glucose, serum-free media, prior to addition of PCRX-201 at three doses to investigate minimal infective dose. Cultures were maintained for a further 14 days. Conditioned media collected throughout the 21-day culture period were analysed using Luminex technology for 42 proteins to characterise inflammatory and catabolic responses to determine the minimally effective dose. Lactate dehydrogenase (LDH) assays monitored cytotoxicity across the 21-day culture period. Infectivity, tissue viability, and protein expression changes were assessed through histology, immunohistochemistry (IHC), and ELISA.

RESULTS Cell viability across all cultures was confirmed by the absence of apoptotic IHC staining and supported by lactate dehydrogenase (LDH) assays, which indicated no cytotoxicity throughout the culture period and across PCRX-201 dosages. ELISA quantification confirmed a dose-dependent increase in IL-1Ra protein in the conditioned media, while IHC demonstrated increased cellular immunopositivity within both cartilage and synovial tissues (Figure 1). These results highlight both the presence and sustained localisation of the therapeutic protein, with IHC showing immunopositivity trends in target tissues and ELISA supporting robust delivery and distribution to the OA joint microenvironment.

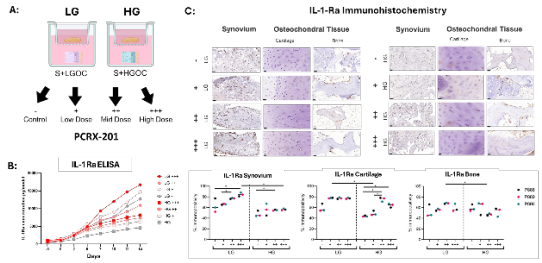

***Figure 1: Ex vivo* co-culture model of human high-grade (high- grade osteochondral and synovium) and low-grade (low-grade osteochondral and synovium) explants**. A: Experimental design. B: IL-1Ra ELISA. C: IL-1Ra Immunohistochemistry.

DISCUSSION & CONCLUSIONS: This comprehensive evaluation of PCRX-201 within a physiologically relevant human *ex vivo* OA joint model supports its potential to modulate IL-1 driven inflammation and catabolic signalling. Future work will expand on this by integrating inflammatory challenges to facilitate assessment of tissue-specific reactivity. The model will also be utilised to determine influence of potential interactive effects of common OA pain medications to mirror the multifaceted treatments patients receive in clinic, optimising treatment strategies of PCRX-201 in a physiologically relevant setting to inform clinical importance. The exploration of PCRX-201 within this *ex vivo* OA model highlights it capabilities for use in advancing gene therapy as a targeted disease-modifying approach for OA with the aim of advancing patient stratification and precision medicine techniques.