# DEVELOPMENT OF AN *EX VIVO* OSTEOARTHRITIC KNEE MODEL

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## INTRODUCTION

**Osteoarthritis** (OA) is a degenerative joint disease affecting 595 million people worldwide. Pathological changes during OA are characterised by **cartilage breakdown, subchondral bone remodelling, and synovial inflammation** *(Figure 1)*, leading to **pain and disability.**

Investigating these pathways in an ***ex vivo*** **synovial joint model** that replicates the **complex interactions** between cartilage, bone, and synovium is crucial for **understanding disease mechanisms** and evaluating potential **therapeutic strategies.**

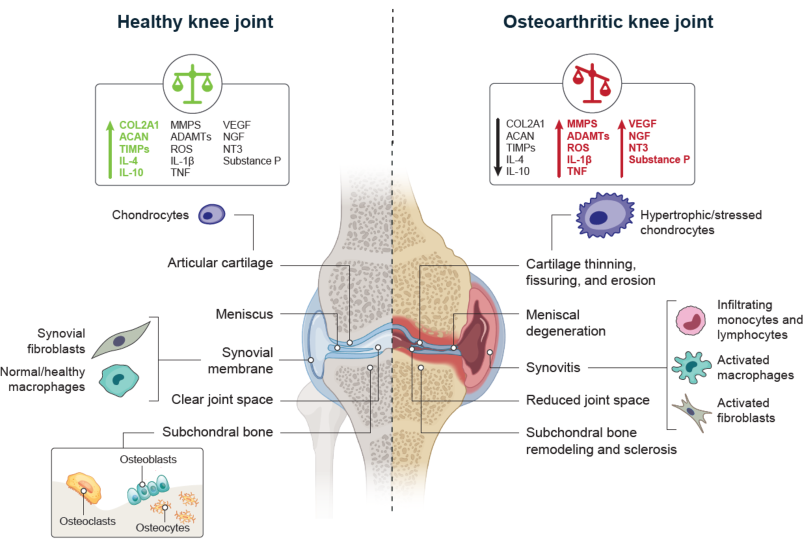

**Figure 1**: Schematic representation of a healthy and osteoarthritic knee joint. The LHS highlights normal physiology and factors involved in homeostasis of a healthy knee joint, the RHS illustrates key features of osteoarthritis pathophysiology.

## EXPERIMENTAL DESIGN

This work focused on the initial development of an *ex vivo* model of knee OA. Whereby human low-grade osteochondral (LGOC), high-grade osteochondral (HGOC) and synovium (S) explants were harvested from patients undergoing knee joint replacement surgery and cultured either alone or in co-culture for up to 21 days (*Figure 2*). Low- and high-grade tissues were defined by Kellgren–Lawrence grades 1–2 and 3–4, respectively.

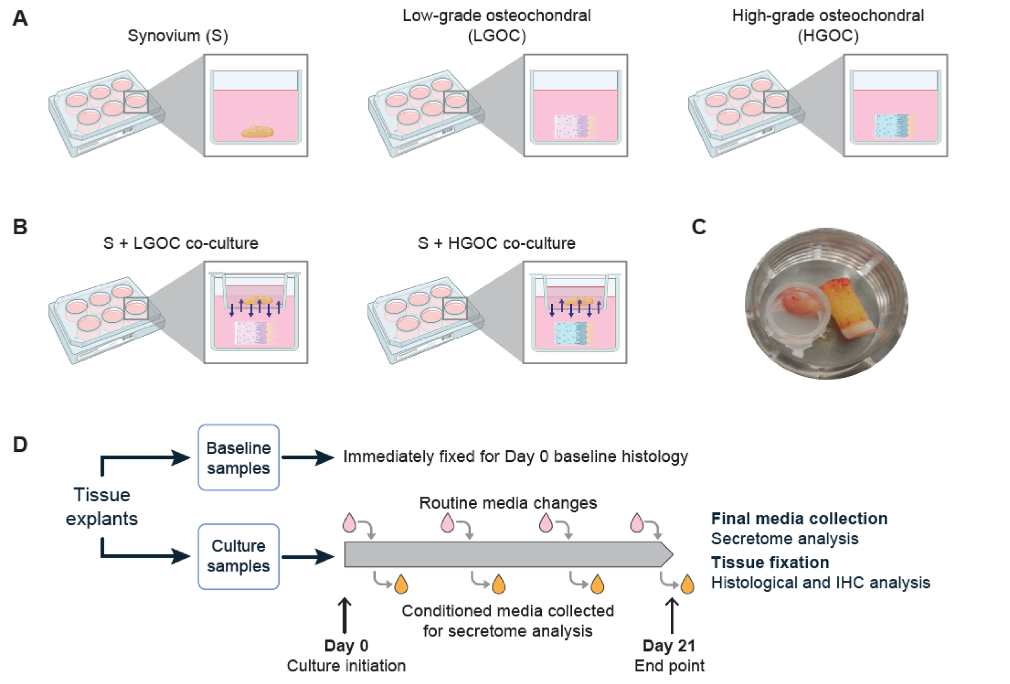

**Figure 2: A** Schematic of singular cultures. **B:** Schematic of co-cultures. Culture conditions: Physioxia: 5% $O_2$, Temperature: 37°C. Media: Dulbecco's Modified Eagle Medium (low glucose, 1 g/L), +Penicillin–Streptomycin, +Insulin–Transferrin–Selenium supplement, +L-Proline, +Albumax, +L-Glutamine, +Amphotericin B, +Ascorbic Acid. **C:** Image of *ex vivo* knee OA tissues. **D**: Experimental timeline and analysis.

## RESULTS

### 1) Histology confirm the culture of specific tissue explants with cell viability maintained throughout long-term culture

Histology confirmed retention of tissue architecture (H&E), proteoglycans (Safranin-O), and collagen (Masson's Trichrome) characteristic of low- and high-grade osteochondral tissues (*Figure 3*). Synovium tissues following 21-day culture retained inflamed and fibrotic areas (Picrosirius Red) (*Figure 4*).

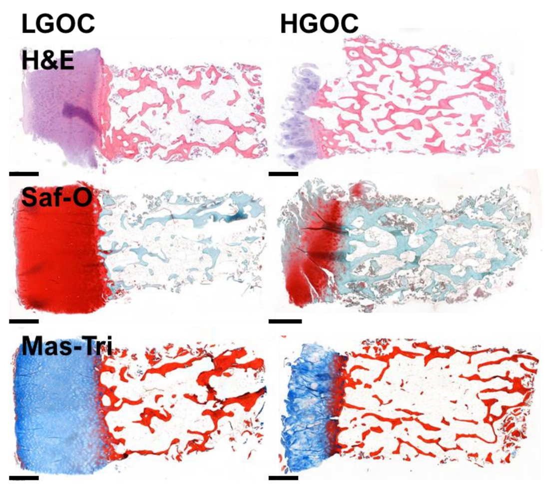

**Figure 3:** Histological staining of low-grade and high-grade osteochondral explants following 21days culture. Haematoxylin & Eosin (H&E), Safranin-O (Saf-O) and Masson's Trichrome (Mas-Tri).

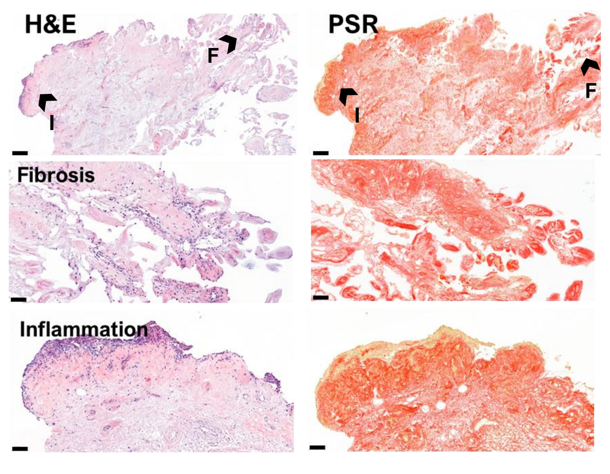

**Figure 4:** Histological staining of synovium explants following 21 days in culture. (H&E) and Picrosirius Red (PSR). Arrows indicate regions of fibrosis (F) and inflammation (I), shown in greater detail in the images below.

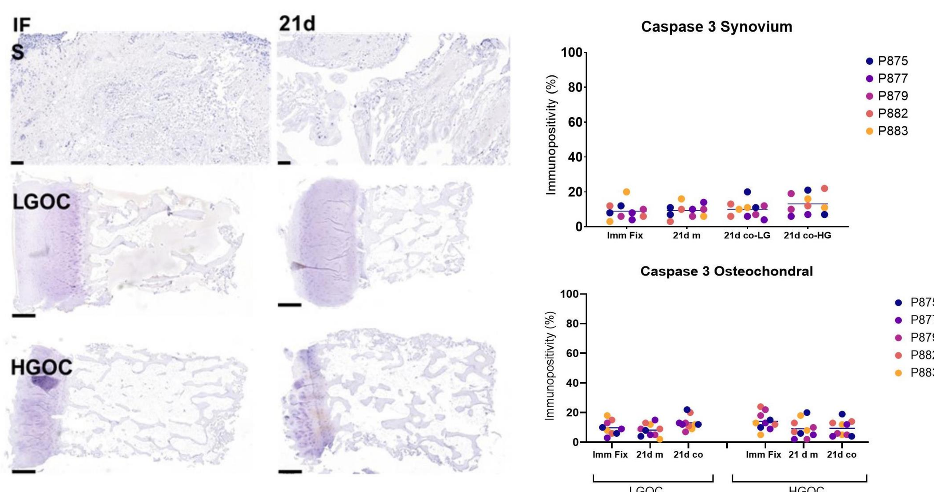

**Figure 5:** IHC staining of Caspase-3 in explant cultures, immediately fixed (IF) and following 21 days in culture (21d) and semi-quantification of immunopositivity. N=5 patients: P875, P877, P879, P882 and P883. * P<0.05.

Minimal Caspase-3 staining and no significant change in immunopositivity pre- vs post-culture (*Figure 5*) indicated sustained cell viability throughout the extended culture period.

### 2) Extracellular matrix and cellular populations were maintained in long-term culture

Immunohistochemistry (IHC) confirmed collagen type II preservation in cartilage and bone during prolonged osteochondral explant culture (*Figure 6*). Synovium explants maintain macrophage (CD68) and fibroblast (Vimentin) populations in extended culture *(Figure 7).*

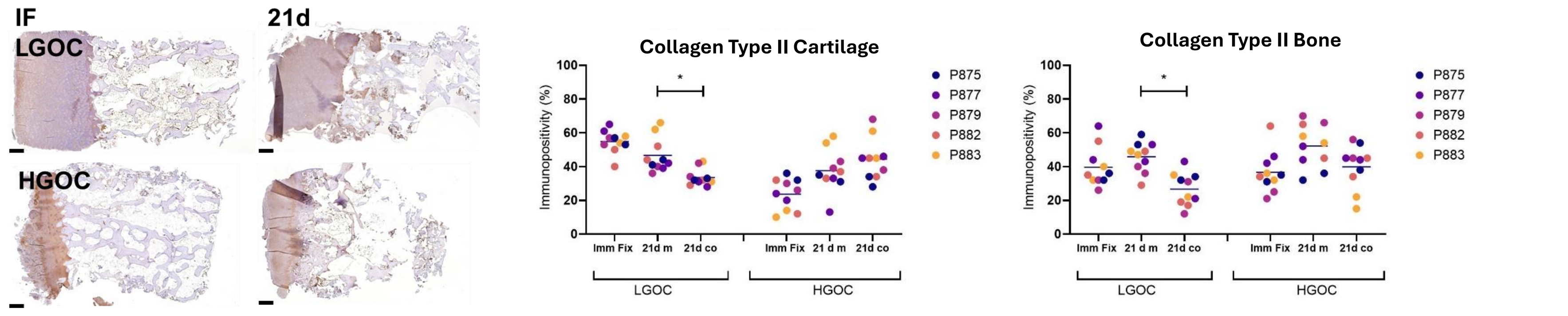

**Figure 6:** IHC staining of collagen type 2 in low-, high-grade, immediately fixed (IF) and post-21 days of culture (21d) and semi-quantification of immunopositivity. * P<0.05.

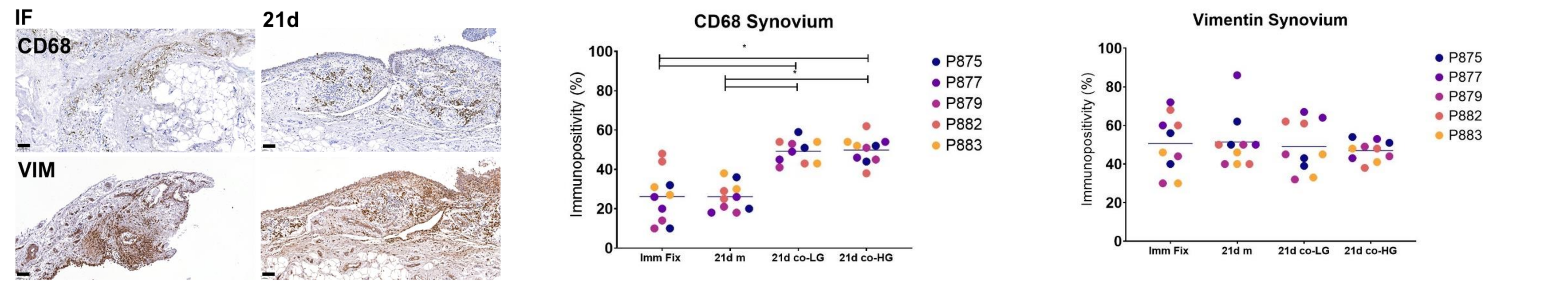

**Figure 7:** IHC staining of CD68 and vimentin (VIM) in synovium immediately fixed (IF) and post-21 days of culture (21d) and semi-quantification of immunopositivity. * P<0.05.

### 3) Secretome analysis revealed distinct differences in the secretome through prolonged culture and donor variability

Volcano plots illustrated differential secretome protein expression over time in single and co-culture conditions *(Figure 8)*. Volcano plots showed that synovium addition significantly altered the secretome in low- and high-grade osteochondral cultures, comparing conditions with and without synovium at day 2 and day 21 (*Figure 9*). Scatter plots of MMP-3 and MMP-13 highlight donor variability (*Figure 10*); MMP-3 showed a significant increase in synovium cultures, while MMP-13 increased from day 2 to day 21.

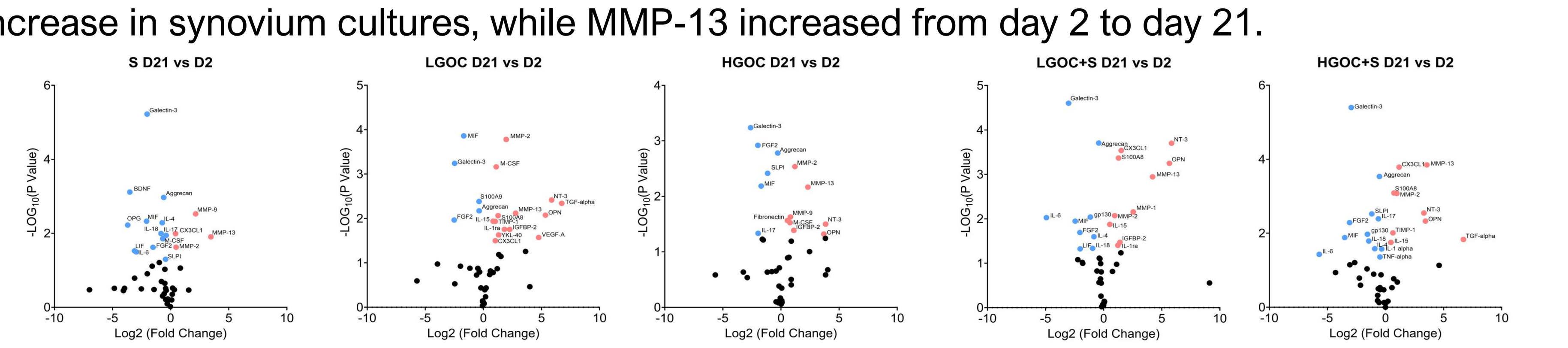

**Figure 8**: Volcano plots showing differential protein expression at day 21 vs day 2 for Synovium (S), Low-grade osteochondral (LGOC), High-grade osteochondral (HGOC), LGOC+S, and HGOC+S. Red indicates upregulation, blue downregulation.

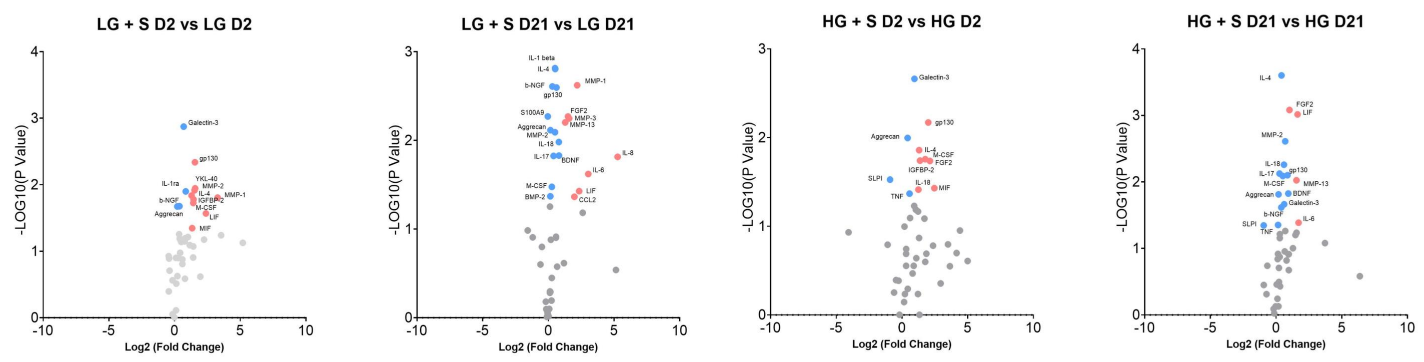

**Figure 9**: Volcano plots showing the impact of synovium addition, with log2 fold-change and significance comparing Low-grade (LG) and high-grade (HG) osteochondral cultures with or without synovium (S) at day 2 and day 21,Red indicates upregulation, blue downregulation.

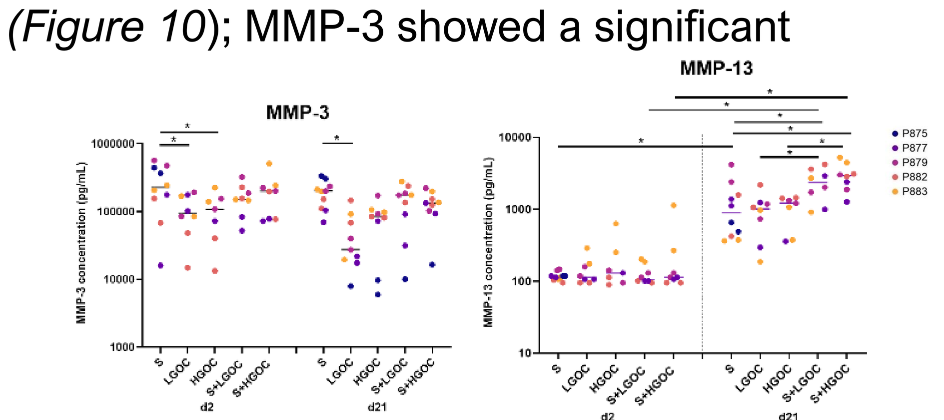

**Figure 10**: scatter plot of MMP-13 protein secreted at day 2 and day 21 across single-culture (S, LGOC, and HGOC) and co-culture (S+LGOC and S+HGOC). * P<0.05.

### 4) Functional biomarkers of OA are maintained in *ex vivo* culture

IHC staining (*Figure 11*), confirmed IL-1β expression in all tissues, with highest positivity seen in synovium and an increased expression in high-grade osteochondral samples. IL-1R1 persisted for 21 days in both cultures indicating sustained responsiveness to inflammatory cytokines, crucial for studying and therapeutically targeting OA inflammatory pathways.

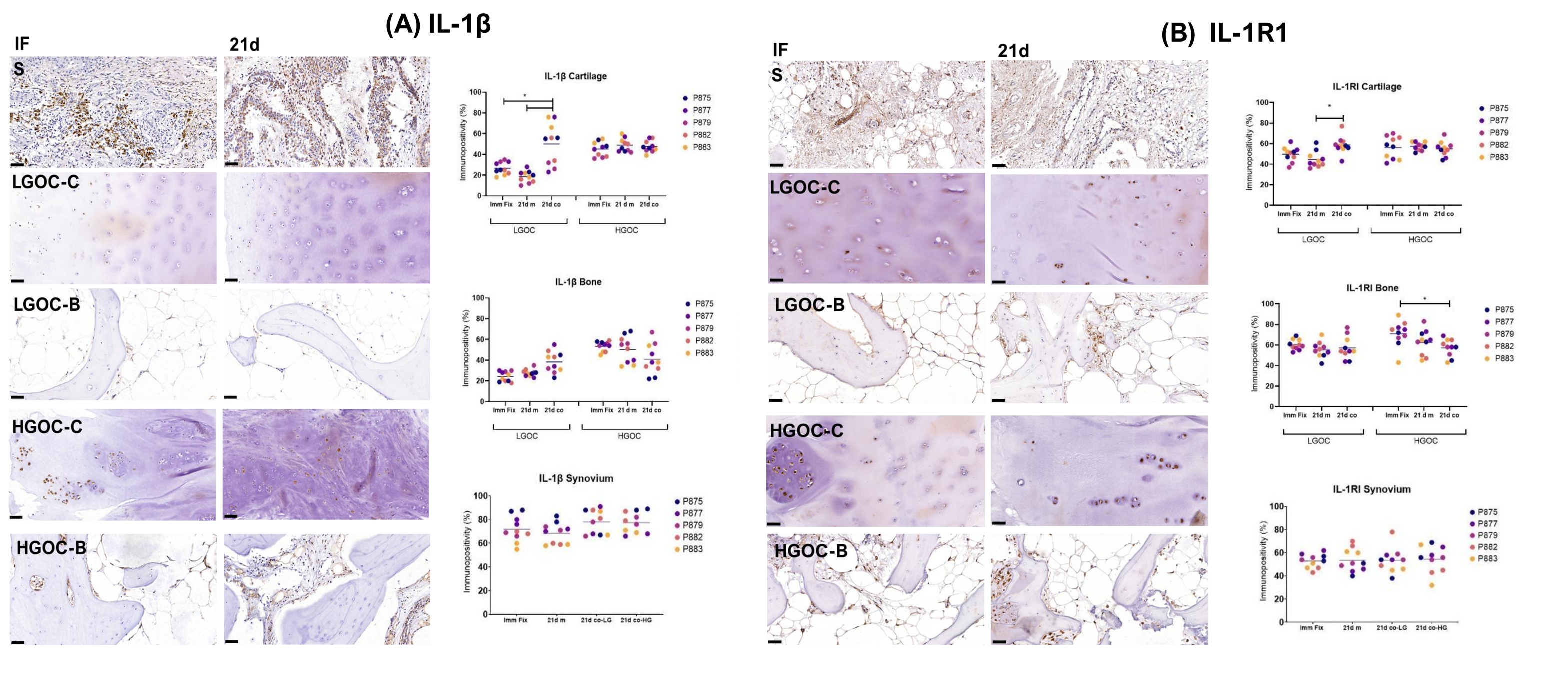

**Figure 11**: IHC staining of IL-1β(A) and IL-1R1(B) immediately fixed (IF) and post-21 days of culture (21d) in synovium (S), low-grade (LG) and high-grade (HG) osteochondral (cartilage: C, bone: B) explants and semi-quantification of immunopositivity. * P<0.05.

## CONCLUSIONS

- **Successfully developed a co-culture system that highlights complex interactions** between cartilage, bone and synovium.
- Within the model **synovium tissue played a central role in driving secretion of inflammatory cytokines and catabolic enzyme.**
- Future work will involve **further interrogation into the cross-talk between intra-articular tissues**; synovium, cartilage and bone, to analyse the interactions within the model and develop understanding into OA pathophysiology.
- This model lays the foundation for **future work aimed at testing therapeutics** within the system and advancing the development of targeted treatments for OA.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST STATEMENT

This work was funded by Pacira Biosciences Inc. and Versus Arthritis. DJ and MK are employees of Pacira Biosciences Inc.