Inflammatory Signaling Modulates ECM Dynamics in Primary and iPSC-Derived Cells Using an Organ-on-Chip Model

Yasemin Sude Ergul[1], Mehmet Sertac Cekuc[1], Patrick Blankenberg[1], Monica Thukkaram[1], Roger Wise[2], Jonathon Ureel[3], Meagan Makarcyzk[4], Simon Kwoon-Ho Chow[1], Hang Lin[4], Bruce Bunnell[5], Qi Gao[1], and Stuart B. Goodman[1]
[1]Department of Orthopaedic Surgery, Stanford University School of Medicine, Stanford, *CA*; [2]Additive Manufacturing and Prototyping Facility (AMPF), Stanford University, Stanford, *CA*; [3]Department of Bioengineering, University of Washington, Seattle, *WA*; [4]Center for Cellular and Molecular Engineering, Dept. of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, *PA*; [5]Department of Microbiology, Immunology & Genetics, University of North Texas, Fort Worth, *TX*.
Email of Presenting Author: yaseminsudeergul@gmail.com

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INTRODUCTION: Human mesenchymal stromal cells (hMSCs) have attracted considerable interest for their roles in tissue repair and immunomodulation, with broad applications in regenerative cell therapy of OA [1]. Adipose-induced inflammation can accelerate the degradation of the extracellular matrix by activating destructive enzymes such as matrix metalloproteinases (MMPs), thereby reshaping both chondrogenic and osteogenic processes. The hallmark features of OA include the presence of progressive degradation of cartilage, synovial inflammation, subchondral bone remodeling, and the formation of osteophytes [2] To better understand and capture the heterogeneity of disease mechanisms, especially in complex conditions such as OA, our approach incorporates both primary hMSCs from OA patients and induced pluripotent stem cell-derived MSCs (iMSCs). The iPSC platform enables scalable production of patient-specific cell types, providing a standardized, renewable, and genetically stable source of MSC-like cells for both therapeutic exploration and mechanistic disease modeling. In our model, we use MSCs from OA patients to investigate the influence of the inflammation of the joint microenvironment on ECM remodelling. To further validate our findings, we also included iPSC-derived MSC (iMSC) samples to examine whether they exert similar effects.

METHODS: Different joint-on-chip tissue components, including synovium-like fibrous tissue and chondrogenic and osteogenic chambers, were generated by differentiating bone marrow-derived stem cells (BMSCs) for 21 days. iMSCs were generated from iPSCs (iPS12, ALSTEM) using the STEMDiff™ Mesenchymal Progenitor Kit (Cat. #05240). To compare iMSCs with primary MSCs under OA-like inflammatory conditions, each group was divided into IL-1β–induced (10 ng/mL) and healthy joint-on-chips. Then, these cells were encapsulated into GelMA and co-cultured in a single bioreactor designed for organ-on-chip applications. The three GelMAs were designed to mimic synovial, cartilage, and bone tissue, respectively—the three tissues communicate by a shared medium. The shared medium was collected from adipocyte-positive groups on day 2 and 5 for adipokine analysis using Luminex Milliplex© assay. Three joint-on-chip groups were established, including two with primary human BMSCs from different OA donors. To evaluate the effect of IL-1β-induced inflammation on cartilage tissue, matrix-degrading activity in the shared medium was quantified using the Anaspec 390 MMP FRET Substrate. Next, we examined tissue phenotypes by histological staining on the 21st day. To evaluate the effects of adipocyte paracrine communication in cartilage remodeling, a group with a fourth chamber of differentiated adipocytes was added. Frozen sections were stained with Alcian Blue to verify chondrogenic differentiation with Nuclear Fast Red counterstain. All experiments were performed in triplicate, with data presented as mean ± standard deviation.

RESULTS: Histological analysis reveals how the chondrocytes differentiated successfully, evidenced by positive glycosaminoglycan (GAG) content. Fluorescent analysis showed a significant decrease in matrix metalloproteinases (MMPs) in the shared medium in the inflammation group compared to the control for both induced and primary MSCs. The presence of adipocytes resulted in a further reduction in MMPs with statistical significance ($p < 0.01$, Figure 2). Groups with an adipocyte chamber under inflammatory conditions experienced the sharpest decrease in MMP activity. Luminex analysis reveals a significant increase in Adiponectin in the shared medium with the presence of IL-1β that was consistent across induced and primary MSCs (Figure 3).

DISCUSSION: Our findings demonstrate the significant influence adipose tissue has on cell lineage and ECM remodeling in the joint-on-chip model. We find that inflammation reduces MMP activity in our joint-on-chip model, which is potentially contradictory with current literature that suggests MMP8 plays a role in inflammatory disease. Based on our results, we hypothesize that in healthy individuals, IL-1β may be involved in a negative feedback mechanism that downregulates MMP activity. We also note that both Adiponectin, an anti-inflammatory adipokine, and MMP8 activity are elevated in inflamed conditions. We hypothesize that Adiponectin may be responsible for the reduction of MMP activity, explaining the sharp decrease in inflammation in adipocytes. We also note similar activity in iPSC-derived MSCs and primary MSCs, suggesting iMSCs have negligible differences in phenotypes when differentiated to chondrocytes and adipocytes compared to primary MSCs. Repeated trials with different primary and induced MSC sources are needed to scrutinize our results.

REFERENCES: [[1] Zhu C, Wu W, Qu X. *Am J Transl Res*. 2021;13(2):448–461. [2] Loeser RF, Goldring SR, Scanzello CR, Goldring MB. *Arthritis Rheum*. 2012;64(6):1697–707. [3] Zhu C, Wu W, Qu X. *Am J Transl Res*. 2021;13(2):448–461.

SIGNIFICANCE/CLINICAL RELEVANCE: This organ-on-chip model may serve as a physiologically relevant *in vitro* platform to study joint regeneration and disease mechanisms.

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Fig. 1.

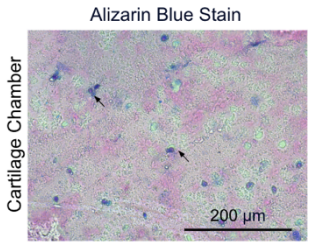

Fig. 2.

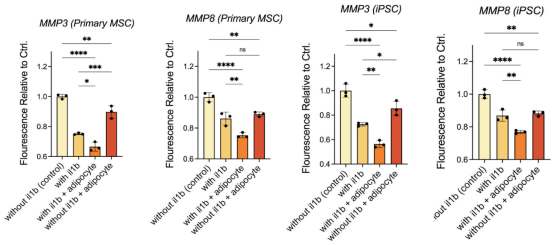

Fig. 3.

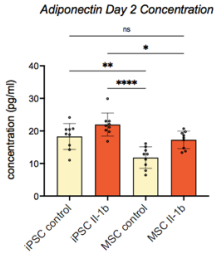

Figure 1. Histological Staining of GAG using Alcian Blue/ Nuclear Acid Fast Red **Figure 2.** Amount of MMP3 and MMP8 measured in Primary MSCs and iPSCs. **Figure 3.** Adiponectin concentration in shared medium on organ-on-chip in pg/ml.