Developing an *in vitro* Osteochondral Micro-Physiological System for Modelling and Testing Therapeutics for Osteoarthritis

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INTRODUCTION: Musculoskeletal diseases are the leading cause of disability, and often affect a combination of tissues including bone, muscle, and cartilage **[1]**. Currently, animal models remain popular for musculoskeletal research, even though they may not accurately represent human biology **[2]**. However, poorly planned and inconsistent animal research wastes up to $24 billion per year **[3]**. *In vitro* models could be used to replace certain animal studies, but to be biologically relevant they must capture the three-dimensional nature of musculoskeletal tissues as well as their crosstalk. In this study, we develop a micro-physiological *in vitro* system that uses a biphasic bioreactor and multiple cell types and scaffolds to mimic native biology and crosstalk of the osteochondral unit of articular joints. We validate the efficacy of the model to emulate osteoarthritis (OA) by treating with inflammatory factors, and we then test relevant OA therapeutics. We show how our *in vitro* model can recapitulate cross talk across tissue types, enabling the testing of candidate therapeutics.

METHODS: *Seeding the Osteochondral Construct:* For the cartilage region, human bone-marrow derived mesenchymal stem cells (BM-hMSCs) are seeded in 5 %methacrylated gelatin (GelMA), 2.5 %methacrylated hyaluronic acid (HAMA), and 0.15% lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) for UV photo-crosslinking. Cartilage constructs are pre-differentiated for 28 days in chondrogenic medium containing 10ng/mL TGF-β3. For the bone region, BM-hMSCs are seeded onto a 3D printed 10 % GelMA, 50 % NanoHydroxyapatite, 0.2% genipin-crosslinked scaffold **[4]**. After osteogenic differentiation for 14 days with 10mM β-glycerophosphate and 10nM 1,25-dihydroxy vitamin D3, human umbilical vein endothelial cells (HUVECS) and BM-hMCSs (4:1 ratio) are seeded into the pores of the 3D printed scaffold in a 5% GelMA, 0.0025% fibrinogen, 0.00004% thrombin suspension crosslinked *in situ*, and cultured in 50:50 osteogenic and vasculogenic medium for another 14 days. The chondral and osseous components can be combined in the biphasic bioreactor prior to experiments. *Generating an Osteoarthritic Environment:* To cause inflammation in the differentiated construct, the cartilage constructs are treated with inflammatory cytokines for 7 days either alone, or while cultured in the bioreactor with the osseous constructs, using chondrogenic maintenance medium containing only 2ng/mL TGF-β3 and either: control (no additives), interleukin 1β (IL-1β) (20ng/mL), interleukin 6 (IL-6) (100ng/mL), tumor necrosis factor α (TNF-α) (1000ng/mL), combination of all three cytokines, or M1 macrophage conditioned medium (M1CM). *Analysis of Chondrogenesis and Cartilage Inflammation:* Chondrogenesis is assessed by RT-qPCR (*SOX9, ACAN, COL2*), histology, immunofluorescence (collagen II and aggrecan), and glycosaminoglycan (GAG) assay. The effect of pro-inflammatory treatments is assessed similarly with the addition of RT-qPCR for *MMP1, MMP3, MMP13, COL1, COL10*, and immunofluorescence for collagen I, and matrix metalloproteinase (MMP) assay. Osteogenic differentiation is assessed by Alizarin Red staining, RT-qPCR (*COL1, COL10, BSP2, RUNX2, ALP*), and immunofluorescence (collagen I, Runx2, ALP). Using GFP-HUVECs, vessel formation can be confirmed by microscopy.

RESULTS SECTION: *Cartilage Gels:* Chondrogenesis of the gels is shown in Figure 1. There is strong collagen II deposition in the matrix of the gel, and only cellular expression of collagen I. *Vascularized bone component:* There is strong calcium deposition in the osteogenic region of the scaffold, seen by Alizarin Red staining. Collagen I staining allows visualization of the vasculature formed by the HUVECS (Figure 1, white arrow) as well as matrix deposited by the osteogenic BM-hMSCs (Figure 1, yellow arrow). *Structure of Complete Osteochondral Construct:* The combined osteochondral construct shows region specific expression of bone and cartilage markers respectively (Figure 2). Masson's trichrome staining shows strong collagen deposition in both cartilaginous and osseous regions, with calcification visible in the osseous region. This is confirmed by collagen I and collagen II staining, the latter only in the cartilaginous region. Alcian blue staining shows GAG deposition in the cartilaginous region. This confirms the biphasic bioreactor supports osteochondral constructs and a separate cartilaginous and vascularized osseous region reminiscent of the osteochondral junction in articular joints.

DISCUSSION: Our preliminary data show that we have successfully developed a multi-tissue micro-physiological system simulating OA. Moving forward, we will probe the role of the osseous region in regulating cartilage response to pro-inflammatory stresses by comparing responses with and without the osseous region. Additionally, we aim to use this system to test OA therapeutics like Rapamycin and TNF inhibitors. A limitation of the current study is that the inflammatory conditions are not fully validated to represent the articular environment. To further validate our model in this sense, we plan to either perfuse the system with synovial fluid from OA patients.

SIGNIFICANCE/CLINICAL RELEVANCE: Using a micro-physiological system to test therapeutics for OA prior to animal studies can both minimize the number of animals required for preclinical testing and save on animal costs. These advance *in vitro* systems will help advance knowledge of musculoskeletal diseases development as well as allow rapid testing of potential therapeutics for treatment.

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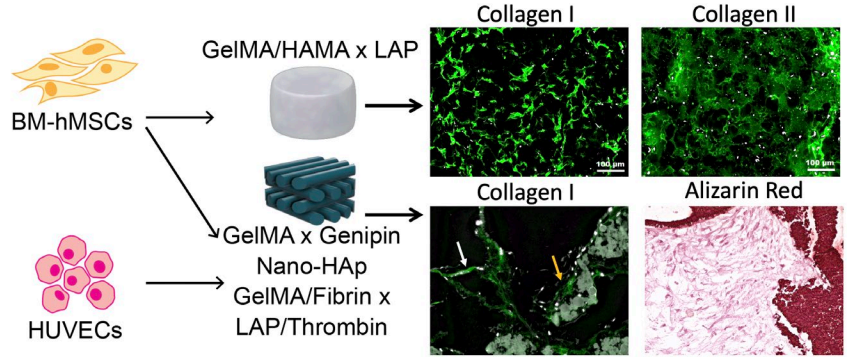

Figure 1: *The separate components accurately represent cartilage and bone respectively.*

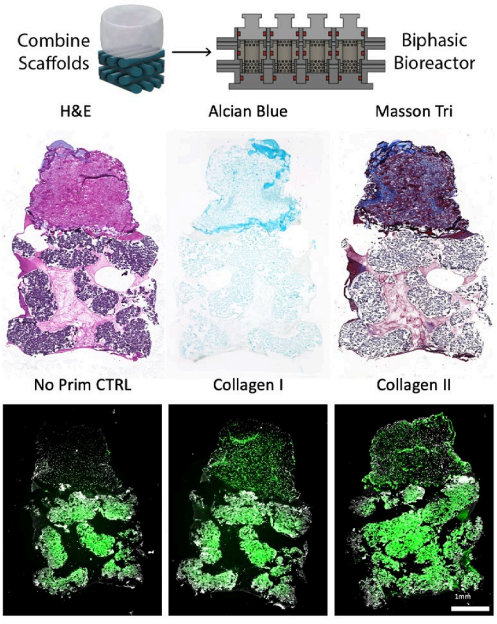

Figure 2: *Tissue specific differentiation in the bioreactor.*