A Vascularized Tissue-Engineered Model of the Synovium for the *In Vitro* Study of Osteoarthritis

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INTRODUCTION. The synovium is a soft tissue that lines diarthrodial joints and contributes to joint health and homeostasis by regulating the composition of the synovial fluid, which provides anti-adhesion properties, lubrication, and nutrition to tissues within the synovial joint. Solutes like proteins, glucose, drugs, and metabolic waste products transfer into and out of the joint space across the synovium and encounter two main barriers – the synovial intima and the endothelium – which impede solute movement in a size- and molecule-dependent manner[1]. Joint solute transport is dysregulated in osteoarthritis (OA) due in large part to increased permeability of the endothelium. Molecule-specific transport properties preclude accurate *in silico* modeling based on size alone, and *in vivo* studies are difficult and costly, so *in vitro* methods are necessary to study the movement of solutes in the normal and OA joints. In this work, we describe a vascularized tissue-engineered model of the synovium with appropriate compositional, structural, and functional properties for the study of these properties and their changes in a simulated OA-like state.

METHODS. Cell sources and culture: Human synovial tissue was recovered from the joint capsule of four male individuals without known joint pathology [MTF Biologics]. Only male donor tissue was available for these studies. Fibroblast-like synoviocytes (FLS) were recovered via collagenase digestion and pooled. RFP-labeled human umbilical vein endothelial cells (HUVECs) were purchased [Angio-Proteomie] and cultured according to the supplier's recommendation. Vascularized tissue-engineered synovium: HUVECs (120k) were seeded onto the underside of 24-well Transwell inserts and allowed to attach for >2 hours. Engineered tissues were generated by embedding FLS (250k / construct) and HUVECs (120k) in Matrigel (7 mg/mL, 100uL) in the top Transwell chamber (Fig. 1A). Engineered tissues were cultured for two weeks with optional insult with interleukin-1β beginning after one week. Biochemistry: Collagen, glycosaminoglycan (GAG), and DNA content was measured by the ortho-hydroxyproline, dimethyl-methylene blue, and PicoGreen assays. Raw values were normalized against dry weight and then control. Transport assay: Diffusion through the tissues was assessed by loading the top chamber with fluorescein and fluorescently-tagged dextrans (4, 70, 150, and 500 kDa) and recovering media from the top and bottom compartments after incubation of 8-24 hours. The apparent permeability, P_{app}, was calculated as previously described[2]. In some cases, the HUVEC monolayer was removed prior to the assay. Histology and imaging: Vascular networks were visualized in live tissues without staining by imaging RFP on a confocal microscope. Tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned prior to staining with hematoxylin and eosin to visualize cells and general tissue structure. Statistics: Pairwise comparisons were made using Welch's t-test and are presented as mean ± standard deviation. Analyses were performed in GraphPad Prism.

RESULTS. Interleukin treatment caused compositional changes in engineered tissues: In response to IL treatment, collagen content decreased by 32 ± 16% ($p<0.0001$, n=11), GAG content increased by 14 ± 4.0% ($p<0.0001$, n=7), and DNA content increased by 14 ± 9.1% ($p=0.0005$, n=11) compared to untreated controls (Fig. 1B-D). Interleukin treatment caused intimal hyperplasia: H&E staining shows that the model developed the intima-subintima structure that is characteristic of the synovium (Fig. 1E). Upon treatment with IL, the intima became hyperplastic and the lining thickened from a single-cell to a multi-cell layer (Fig. 1F). Interconnecting endothelial networks were disrupted by IL treatment: Vascularized tissues formed extensive interconnecting networks of endothelial cells under normal culture conditions, but these networks were degraded in response to IL treatment (Fig. 1G-H). IL increased engineered tissue permeability: IL treatment increased tissue permeability to fluorescein and 4 kDa, 70 kDa, and 150 kDa dextrans compared to untreated controls (Fig. 1I). The relationship between permeability and hydrodynamic radius was best fit with a one-phase exponential decay model for both control and IL-treated tissues (Fig. 1J). Increases in permeability were driven by changes to the endothelium: Permeability was measured for samples with an intact HUVEC monolayer and for samples with their monolayers removed to determine the contributions of the endothelium. In untreated control tissues, removal of the endothelial barrier significantly increased total tissue permeability for all solute sizes (n=4, Fig. 1K). In IL-treated tissues, removal of the endothelial barrier caused a significant increase in permeability for only the 70 kDa dextran, and the magnitude of that change was smaller (n=4, Fig. 1L). These results indicate that the changes in permeability observed with IL treatment are largely driven by increased permeability of the vascular barrier.

DISCUSSION. In this work, we developed a functional, vascularized, tissue-engineered model of the human synovium with desirable properties that was responsive to the pro-inflammatory cytokine interleukin-1β. Engineered tissues developed interconnected vascular networks and characteristic synovial features. They responded to insult with IL-1β with relevant changes to their composition, structure, and function. The synovium is highly vascularized, and the vasculature alters tissue function by directly influencing tissue permeability and by interacting with other local cell populations. In particular, loss of endothelial barrier integrity in disease leads to increased joint permeability, which our model recapitulates. Interactions among the vasculature, the extracellular matrix, and synoviocytes have functional consequences that are clinically relevant in the context of drug clearance and the movement of other bioactive molecules, and the work presented here gives researchers a new tool to study those interactions in a controlled and reproducible *in vitro* model.

CLINICAL RELEVANCE. Transport into and out of the joint space is a critical component of joint homeostasis and affects the clearance of therapeutics after administration, but there are no suitable *in vitro* models that assess this function that recapitulate both the endothelium and synovial intima.

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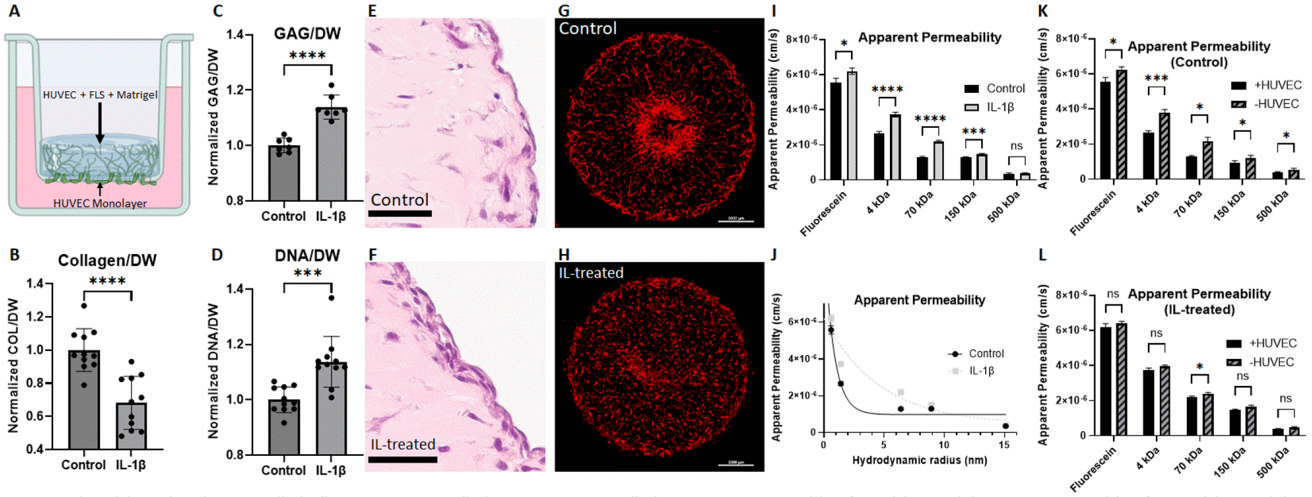

Fig. 1 A) Engineered tissue schematic. **B)** Normalized collagen content. **C)** Normalized GAG content. **D)** Normalized DNA content. **E)** H&E staining of control tissue. Scale bar: 60 μm. **F)** H&E staining of IL-treated tissue. Scale bar: 60 μm. **G)** Live-cell imaging of HUVECS (red, RFP) in control tissue. Scale bar: 1000 μm. **H)** Live-cell imaging of HUVECs in IL-treated tissue. Scale bar: 1000 μm. **I)** P_{app} for intact control and IL-treated tissues. **J)** P_{app} vs. hydrodynamic radius for control and IL-treated tissues. **K)** P_{app} for control tissues with intact and removed vascular barriers. **L)** P_{app} for IL-treated tissues with intact and removed vascular barriers. *p<0.05, ***p<0.001, ****p<0.0001.