Developing a Microfluidic Human Tendon-on-a-Chip (hToC) to Investigate the Role of the Vasculature in Tendon Injury

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INTRODUCTION: Tendon injuries are among the most common musculoskeletal pathologies, accounting for 16 million cases in the U.S. each year and a $240 million healthcare burden[1]. Tendon injury is often followed by a chronic inflammatory response leading to a vascularized fibrotic scar. This fibrotic healing process fails to restore pre-injury joint motion, further contributing to patient and clinical burden[2]. While monocytes from the vasculature are thought to perpetuate this excessive inflammation, their cross-talk with vascular endothelial cells in tendon healing remains unclear. Further, current *in vitro* musculoskeletal tissue models are restricted to simplified 2D platforms that lack physiological cues, including fluid flow, and thus have limited translational value. To address these obstacles, microphysiological systems (MPS), or tissue chips, have emerged as a paradigm-shifting technology to model 3D tissue organization. Previous work in our laboratory developed a human tendon-on-a-chip (hToC) that incorporates tendon and vascular compartments with patient-derived cells, a highly permeable porous membrane for cellular crosstalk, and a type I/III collagen hydrogel to mimic the extracellular matrix of healing tendon. While the hToC models a post-injury microenvironment in static culture conditions, a lack of physiological flow may affect the endothelial response to inflammatory mediators and infiltrating immune cell behavior. This necessitates the incorporation of fluid flow to provide cues for endothelial barrier maturation and enable circulation of immune cells. The objective of this research is to further develop the hToC into a plug-and-play microfluidic platform to investigate monocyte-endothelial cell interactions in models of the injured tendon and to understand the downstream effects on tissue fibrosis.

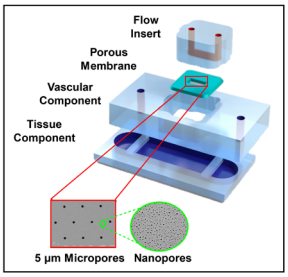

Figure 1. hToC components with flow insert. The porous membrane, which serves as a substrate for culturing the endothelial barrier, enables paracrine crosstalk between the vascular and tissue compartments as well as immune cell infiltration.

METHODS: To enable fluidic investigation of the vascular compartment of the hToC, we developed a flow insert component compatible with the geometry of the existing device (Figure 1). To facilitate high-throughput experiments, we adapted the design to be manufactured in collaboration with ALine, a microfluidic engineering company. The designed flow insert contains access ports for flow tubing integration and a channel to perfuse fluid over the endothelial barrier. As an initial test of biocompatibility, devices were assembled with the manufactured flow inserts, and human umbilical vein endothelial cells (HUVEC) were statically cultured for 24 hours. A peristaltic pump circuit was employed to confirm fluidic shear conditioning of HUVECs at physiological shear stress levels (10 dynes/cm^2). The cytoskeleton was stained for F-actin before and after flow for quantification in *Fiji* ImageJ of morphological alignment. ICAM-1 and VE-cadherin are endothelial adhesion molecules that mediate immune cell arrest and vascular integrity, respectively. The expression levels of these molecules, along with Hoechst 33342 as a nuclear counterstain, were assessed with immunocytochemistry and compared in static and fluidic culture conditions. Real-time phase microscopy, enabled by the flow insert's optical transparency, was performed to image the engagement of circulating human monocytes with the endothelium under flow. All data were analyzed using a one-way ANOVA with Tukey's post hoc test (n = 3 devices per condition) using GraphPad Prism.

RESULTS SECTION: Using COMSOL simulations, we optimized the design under the experimental flow conditions to achieve a uniform shear stress profile at the culture surface ranging from 1-10 dynes/cm^2. We adapted the flow insert design for manufacturing to improve the accessibility of the device, as well as validate the response of shear-conditioned endothelium in the hToC. Live/dead staining confirmed comparable cell viability for HUVECs cultured in the manufactured flow insert and original static hToC. We also confirmed the ease of transition from static to fluidic mode and leak-proof operation during flow (≥99%). F-actin cytoskeletal and nuclei stains demonstrated the morphological alignment of endothelial cells <30º in relation to the direction of flow, a key hallmark of endothelial morphology *in vivo* (Figure 2). ICAM-1 expression was consistent between static and flow conditions while VE-cadherin was upregulated in the presence of flow, indicating a more robust endothelial barrier. All graphical results are reported as mean ± standard error of mean from triplicate samples (*p < 0.05). Live videos demonstrated monocyte adhesion to, rolling, and transmigration through the endothelium under physiological conditions.

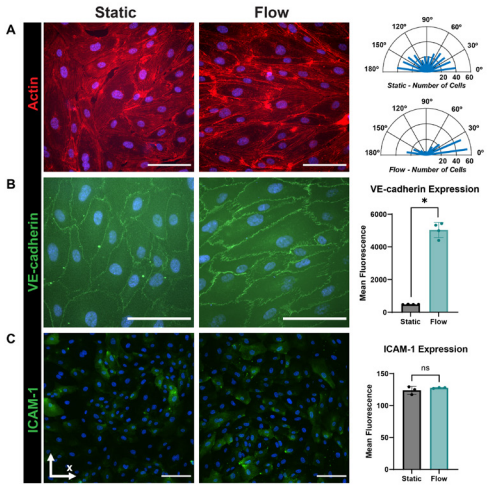

Figure 2. HUVEC cultured in hToC with flow insert for 24 hours of static culture or physiological fluidic shear stress of 10 dynes/cm^2. **A.** Cytoskeleton staining of F-actin, with radar plots quantifying alignment angle relative to the flow direction (x-axis). Expression and quantification of **B.** VE-cadherin and **C.** ICAM-1, with nuclei counterstain (scale bar = 100 µm).

DISCUSSION: Engineering physiologically relevant tissue chip models relies on recapitulating cues present *in vivo* to accurately model endothelial barrier functions in the context of tendon injury. This work demonstrates the successful development of a flow insert component that is adaptable for mass production and is used to replicate physiological cues of the vasculature. As an important step towards validating a flow response in the complete hToC injury model, this work established improved HUVEC barrier properties with flow (alignment and junctional protein expression), as well as the maintenance of ICAM-1 expression, which mediates monocyte adhesion during the initial inflammatory phase of tendon injury. Incorporating fluid flow in the hToC will facilitate the investigation of the interactions of vascular endothelium with cellular and inflammatory mediators. Future work will focus on assessing disease metrics in the hToC with fluidic conditions and investigate the mechanisms by which circulating monocytes propagate a pro-inflammatory environment as they interact with the inflamed vasculature. While limitations exist regarding a lack of complexity when compared to *in vivo* tendon injury models, employing this microphysiological system allows for a reductionist approach to better understand the driving factors that promote the progression of fibrosis.

SIGNIFICANCE/CLINICAL RELEVANCE: This research is significant because understanding the mechanisms of vascular inflammation in tendon injury is underappreciated and could be key to the development of effective therapeutic strategies, ultimately reducing the socioeconomic burden of tendon injury. The microfluidic hToC can also serve as an adaptable tool for mechanistic studies of early-stage inflammation in the context of other fibrotic diseases.

REFERENCES: **1**. Nour, S. et al *Regeneration* 2020. **2.** Nichols, A. et al. *Transl Res* 2019. **3.** de la Durantaye, M. et al. *J Orthop Res* 2014.

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