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

Isabelle Linares M.S.[1,2], Raquel Ajalik Ph.D. [1,2], James McGrath Ph.D.[1], Hani Awad Ph.D.[2]

1. Department of Biomedical Engineering, University of Rochester, Rochester, NY. 2. Center for Musculoskeletal Research, University of Rochester Medical Center, Rochester, NY. 3. Department of Biomedical Engineering, Rochester Institute of Technology, Rochester, NY.

Listen to the poster

Leveraging Tissue Chips to Study Tendon Injury

- Tendons connect muscle to bone and facilitate joint motion
- Tendon injuries present a significant clinical burden: 16 million reported cases + $240 million in healthcare costs in the U.S. each year
- While healthy tendon is hypovascular, injured tendons are characterized by vascularized scar tissue, resulting in reduced range of motion, pain, and decreased quality of life

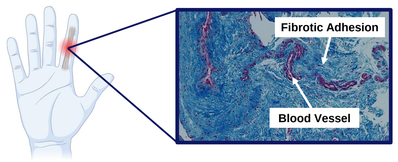


Figure 1. Flexor tendon fibrotic adhesion, characterized by disorganized matrix and infiltration of blood vessels which facilitate the arrival of immune cells to the injury site.

- Tissue chips model 3D tissue organization to investigate disease mechanisms and screen therapeutics
- Our lab developed the human tendon-on-a-chip (hToC) to model the tendon vascular inflammatory microenvironment in *static* conditions

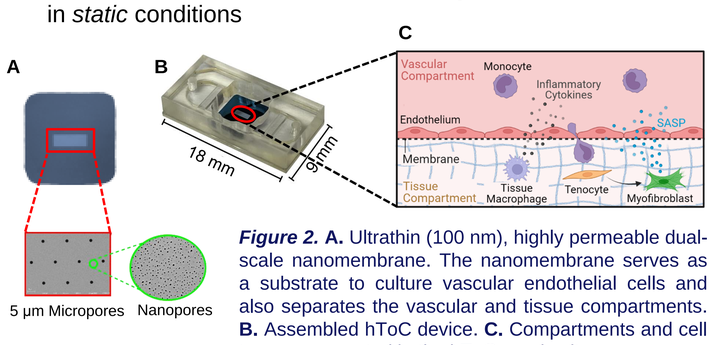


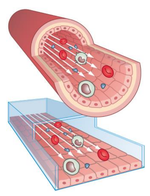
Figure 2. **A.** Ultrathin (100 nm), highly permeable dual-scale nanomembrane. The nanomembrane serves as a substrate to culture vascular endothelial cells and also separates the vascular and tissue compartments. **B.** Assembled hToC device. **C.** Compartments and cell types represented in the hToC quad culture.

Project Aim

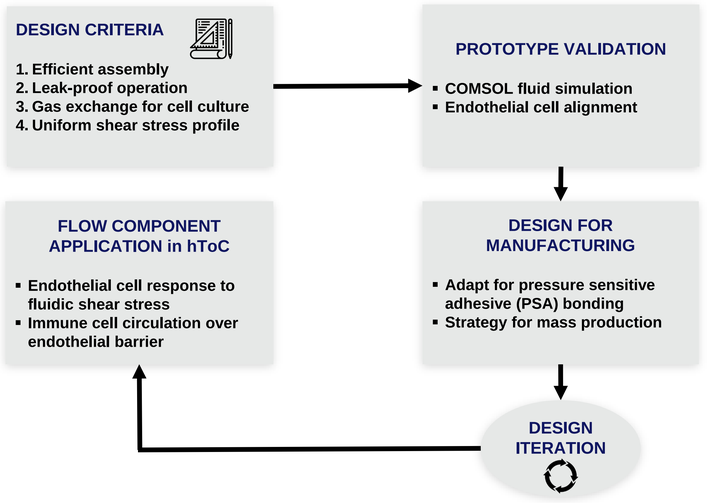
Develop a plug-and-play fluidic hToC with *physiological vascular flow*.

Hypothesis: Fluid flow will alter the fibrotic response by impacting:

- Endothelial barrier function
- Concentrations of inflammatory mediators
- Rate of immune cell infiltration



Design & Experimental Workflow



Microfluidic Component Design Process

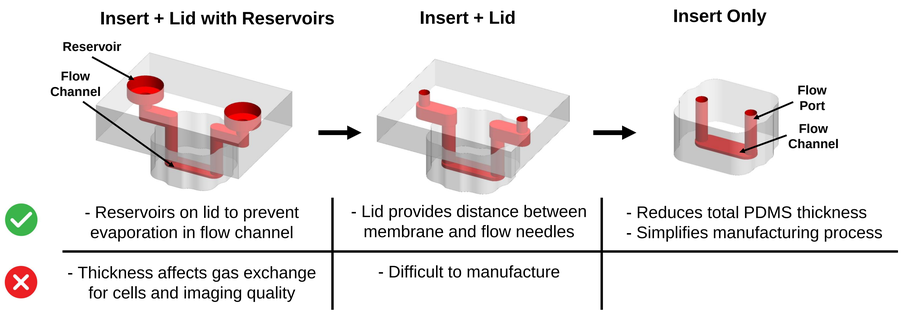


Figure 3. Design evolution of modular flow insert (red indicates flow channel geometry). Table describes advantages and disadvantages of each iteration.

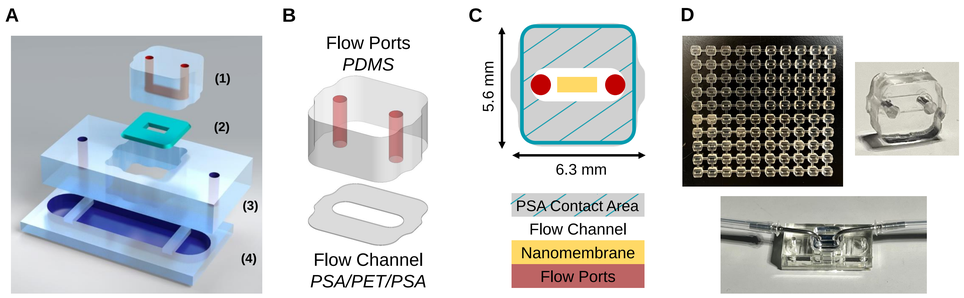


Figure 4. **A.** Exploded view of hToC components with flow insert: (1) flow insert, (2) dual-scale nanomembrane, (3) vascular component, (4) tissue component. **B.** Flow insert design for manufacturing, featuring polydimethylsiloxane flow ports (PDMS) and a pressure sensitive adhesive (PSA)/laminate (PET) flow channel. **C.** Cross section of insert. **D.** Image of mass-produced array, single flow insert, and fully assembled fluidic device with flow tubing.

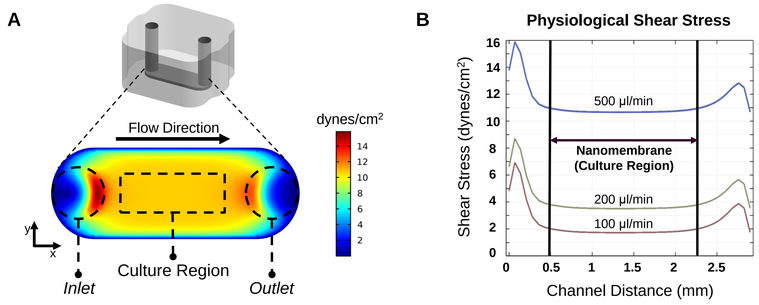


Figure 5. **A.** COMSOL fluid dynamics simulation showing a shear stress map of the flow channel. Confirms uniform shear stress at the culture surface. **B.** Physiological shear stress values across culture surface: 10.7 dynes/cm² at flow rate of 500 µl/min, 3.5 dynes/cm² at 200 µl/min and 1.5 dynes/cm² at 100 µl/min.

Endothelial Cells Elongate & Align with Fluidic Shear Stress

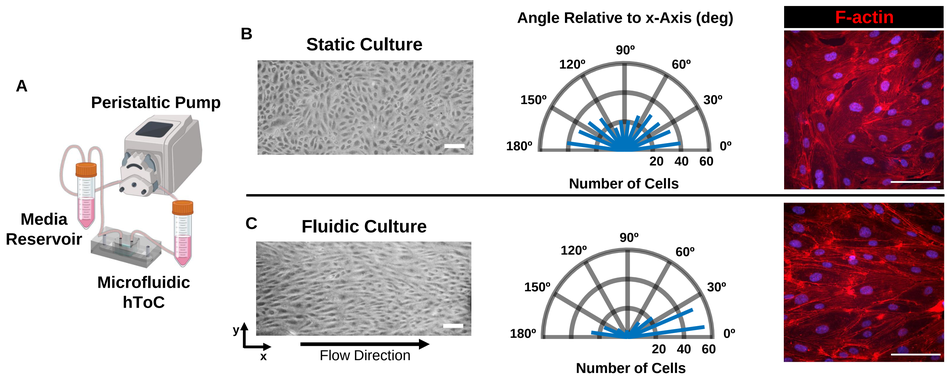


Figure 6. **A.** Peristaltic pump flow setup. Phase images of human umbilical vein endothelial cells (HUVEC) cultured in hToC with flow insert for 24 hours of **B.** static culture or **C.** physiological fluidic shear stress of 10 dynes/cm² (Phase image scale bar = 100 µm; F-actin image scale bar = 50 µm). Radar plots quantify cell alignment relative to the flow direction (along x-axis). HUVEC stained for F-actin to visualize cytoskeletal remodeling in response to flow.

Flow Influences Adhesion Molecule Expression

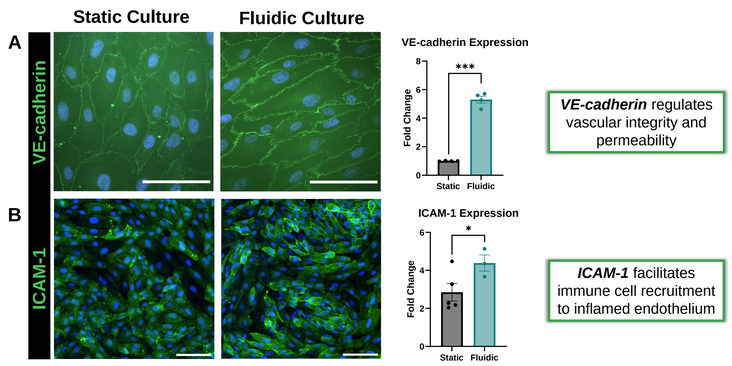


Figure 7. **A.** HUVEC cultured in hToC with flow insert for 24 hours of static culture or fluidic shear stress of 10 dynes/cm². VE-cadherin expression reported as fold change compared to static. **B.** HUVEC cultured with basal TNF-α stimulation for 24 hours of static culture or fluidic shear stress of 1.5 dynes/cm². ICAM-1 expression reported as fold change compared to unstimulated static control (Scale bar = 100 µm) *p<0.05, ***p<0.001.

Validating Immune Cell Introduction Under Flow

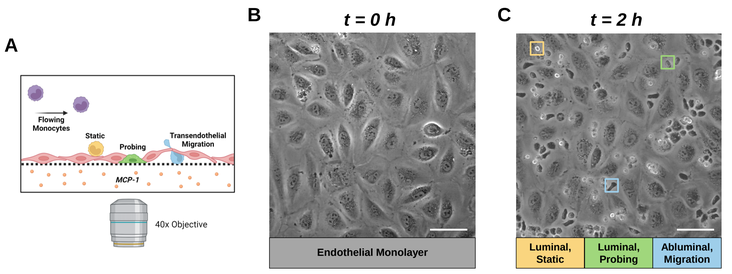


Figure 8. **A.** Circulating monocyte transmigration cascade under flow in response to 100 ng/ml MCP-1. **B.** First frame of live video showing endothelial monolayer before the arrival of monocytes. **C.** Final frame after 2 hours of monocyte circulation through the flow insert, classifying monocyte interactions with endothelium based on schematic in **A**. (Scale bar = 50 µm)

SCAN ME

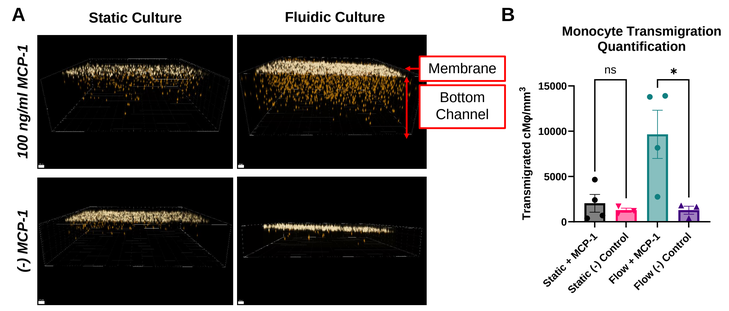


Figure 9. **A.** Confocal image stacks showing adhered (white) and transmigrated (orange) monocytes after 24 hours of either static culture or circulation (50 µl/min). 100 ng/ml MCP-1 was added to the bottom channel and compared to a (-) MCP-1 control condition. (Scale bar = 70 µm). **B.** Quantification of transmigrated monocytes (cMφ), normalized over the image stack size. *p<0.05.

Clinical Relevance & Future Directions

- Understanding the mechanisms of ***vascular inflammation in tendon injury is underappreciated*** and could be key to the development of effective therapeutic strategies
- Fluidic hToC can serve as an ***adaptable tool*** for mechanistic studies of inflammation in the context of other fibrotic diseases
- Plan to investigate the impact of circulating monocytes on the inflammatory response of the complete hToC quad culture
- Evaluate antibody inhibitors of monocyte transmigration as potential therapies

ilinares@ur.rochester.edu

linkedin.com/in/isabellelinares

References

1. Wu F. *et al.* (2017) *EFFORT*, 2(7):332-42.
2. Mehrzad R. *et al.* (2019) *Ann Plast Surg*, 83(4):419-23.
3. Brebels J. *et al.* (2022) *Polymers*, 14(5):867.
4. Mansouri M. *et al.* (2022) *Adv. Health. Mat.*, 11(21):2200802.
5. Pérez-Rodríguez S. *et al.* (2021) *Biomicrofluidics*, 15:054102.
6. Ando J. *et al.* (2011) *Antioxid. Redox Signal*, 15(5):1389-1403.

Acknowledgements

National Center for Advancing Translational Sciences
UH3TR003281