Extensional strain induces long-range alignment of collagen fibers in a microfluidic channel

Adeel Ahmed, Mehran Mansouri, Indranil M. Joshi, Ann M. Byerley, Steven W. Day, Thomas R. Gaborski, Vinay V. Abhyankar

Department of Biomedical Engineering, Rochester Institute of Technology, Rochester, NY

Statement of purpose: Collagen (COL1) fibers in the extracellular matrix are reorganized into millimeter-scale aligned domains by cell-matrix interactions. Aligned fibers guide cell motility and enable cell-cell communication by transmitting traction forces between cell populations. Given the role of aligned COL1 fibers in guiding cell behavior in vivo, replicating 3D COL1 fiber alignment across millimeter-scale domains is key to studying the cell response to ordered microenvironments, in-vitro. Microfluidic platforms have gained popularity for *in vitro* cell culture owing to favorable fluid flow properties and the ability to integrate biomaterials. Studies show that upon injecting a self-assembling COL1 solution into a microchannel of height *z* at a velocity *u*, the resulting fluid shear rate $(\dot{\gamma} = \partial u_x/\partial z)$ aligns thin (<40µm) mats of collagen fibers at the walls of the microchannels (2D surface coatings). However, microfluidic control over long-range fiber alignment of thicker 3D (>130µm) COL1 hydrogels has been challenging. This study shows that shear flow cannot align COL1 fibers in a 3D hydrogel. Instead, we show that a localized extensional component ($\dot{\varepsilon}=\partial u_x/\partial x$) in the flow promotes COL1 fiber alignment across millimeter scale. We show direct access to the 3D hydrogels using a modular microfluidic platform that enables easy cell-positioning and multi-layer hydrogel fabrication.

Methods: To generate local extensional flow, we developed a segmented microchannel (thickness = 130µm) (**Figure 1A)** with five segments of widths 10mm, 5mm, 2.5mm, 1.25mm and 0.75mm. We injected a neutralized atelocollagen solution mixed with fluorescent polystyrene beads into the channel at a flow rate Q = 50µL min^{-1} and measured the flow velocity in each segment and constriction using particle image velocimetry (PIV). The velocity measurements were used to calculate the extensional strain rates and shear rates (n=3). COL1 was allowed to self-assemble in the channels at 37˚C and imaged. The coefficient of alignment (CoA) was quantified using CT-FIRE and defined as the fraction of fibers within ±15˚ of the mode of the fiber angle histogram, CoA>0.5 was considered aligned. We decoupled the effect of extensional strain rate on fiber alignment from the effect of the shear rate by injecting COL1 into a uniform channel (no extensional component). The shear rate in the uniform channel was matched to the shear rate in a segment. We then compared the CoA of fibers that self-assembled in the segmented channel (with extensional component) to the fibers in the uniform channels (no extensional component). To directly access the collagen in each segment, we developed a two-piece channel comprising of a channel cutout and a reversibly bonded PDMS block on the cover served as a 'cover'. The channel was placed in a modular device that was fabricated using laser-cut PMMA, and magnets were press-fit into the PMMA to magnetically latch modules onto the channels to pattern cells and add ECM layers

Results: Collagen fibers that self-assembled in a uniform channel (no extensional component) did not align under any of the shear rates tested (50-1000 s^{-1}) **(Figure 1B).** In the segmented channels, he fiber CoA was 0.41±0.05 in segment a ($\dot{\varepsilon}$ = 0.3±0.14 s^{-1}) and 0.64±0.11 in segment e ($\dot{\varepsilon}$ 9.1±0.52 s^{-1}) (**Figure 1C),** suggesting that the $\dot{\varepsilon}$ directly influenced the CoA. Similar trends were observed in 250µm thick channels. To verify the role of the extensional flow in fiber alignment, we decoupled the extensional and shear component of the flow as described and found the CoA of fibers that self-assembled without a local extensional component was 0.49±0.1 and 0.64±0.11 for fibers with an extensional component of 9.1 s^{-1} at the same shear ($\dot{\gamma}$=257s^{-1}, $p < 0.05$)**(Figure 1D)**. Although the extensional flow was localized to the constriction, we found the fiber alignment to extend uniformly across each segment (5mm) with a maximum standard deviation of ±0.04 CoA **(Figure 1E)**. Using the two-piece channel and modular platform, we could directly access the collagen segments for cell patterning **(Figure 1F)** or layer-by-layer ECM fabrication.

Conclusion: This study shows that shear flows could not align 3D COL1 hydrogels and local extensional flow promotes millimeter scale COL1 fiber alignment in 3D hydrogels up to 250µm in thickness in microfluidic channels and the extensional strain rate directly influences the CoA. Using a modular platform allowed direct access to the collagen hydrogel for cell positioning and biofabrication of ordered ECM structures. Thus, COL1 fiber alignment can be easily controlled simply by changing channel geometry.

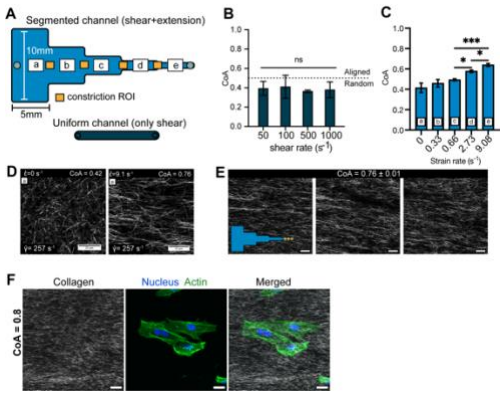

Figure 1: (A) Schematic of segmented channel and uniform channel. (B) Effect of shear rate on CoA (C) Effect of extensional strain rate on CoA (D) Fibers self-assembled with and without extension (E) Aligned collagen fibers extending across a 5mm segment (F) HUVECs align on aligned collagen segment. All data reported as mean ±SD, n=3