Microengineered Collagen Hydrogels with Spatial Gradations in the Fiber Alignment Landscape

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Statement of Purpose: In native tissue, cell-matrix interactions locally reorganize Type I collagen (COL1) fibers, and the degree of fiber alignment decreases away from the cell boundary, resulting in a graded landscape, as shown in **Figure 1**. Although the effects of biophysical gradients (stiffness, porosity, and ligand density) and biochemical gradients (soluble and immobilized) are well-established, cell responses to gradients in fiber alignment have not been explored in detail. This may be due to the experimental limitations of current fabrication techniques in creating well-defined alignment gradients in vitro.

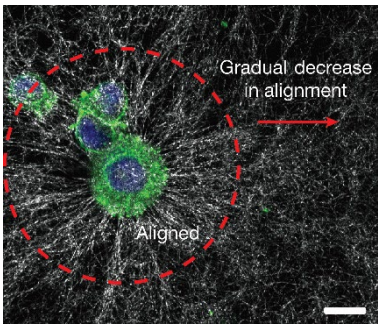

Figure 1 Confocal reflectance image of COL1 fibers remodeled by MB231 cells inducing spatial heterogeneity in alignment. Scale Bar = 25 µm.

Our previous work demonstrated that extensional flows in constricting microfluidic channels promote uniform, long-range COL1 fiber alignment in 3D hydrogels. Here, we exploit extensional flows in a constricting and expanding channel to engineer collagen hydrogels with spatial gradients in fiber alignment that can be used to quantify how different cell populations respond to heterogeneities in the collagen microarchitecture.

Methods: As shown in Figure 2A, a multi-port PDMS channel with constricting and expanding regions was fabricated using standard soft lithography and mounted to a coverslip. The microchannel was pre-filled with neutralized bovine atelocollagen solution to prime the system. Next, a COL1 solution was injected along the path from ports 1 to 3 using a syringe pump (Q = 100 µl/min or 500 µl/min) after blocking ports 2,4 and then placed in an incubator to promote COL1 gelation. Particle image velocimetry (PIV) quantified the fluid velocity along the flow path. After gelation, the fibers were imaged using confocal reflectance microscopy (CRM) and analyzed using LOCI CT-Fire to determine the coefficient of alignment (CoA), defined as the ratio of fibers within ±15° of the mode in the fiber angle histogram. Values range from 0 – 1 with CoA > 0.5 considered aligned.

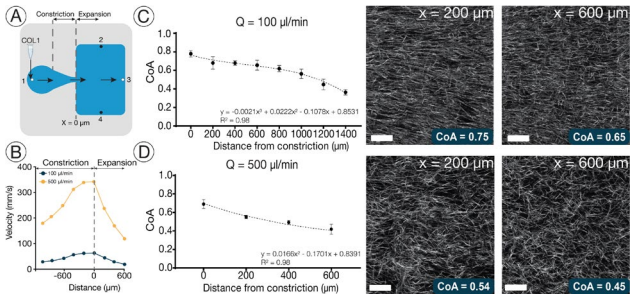

Figure 1. A) Shows the schematic of the non-uniform microfluidic chip. B) Shows the graph of COL1 solution velocity as a function of distance in constriction and expansion for the two flow rates. C) Graph of CoA vs distance from constriction at Q = 100 µl/min and CRM images of COL1 post constriction. D) Graph of CoA vs distance from constriction for flow rate of 500 µl/min and CRM images of COL1 post-constriction. Scale bar = 25 µm.

Results: The COL1 molecules in solution experience an increase in velocity along the flow direction (extensional flow) as they pass through the constricting geometry (**Figure 2B**) and experience a non-linear decrease in velocity upon entering the expansion region. The extensional flow promotes alignment, and the decelerating flow in the expansion results in a decrease in the CoA as a function of distance away from the constriction. **Figure 2, C,** and **D** show the resulting alignment profiles under flow conditions Q = 100 µl/min and Q = 500 µl/min, respectively. The CoA changed rapidly (2nd order fit) from aligned (0.6) to unaligned (0.4) over a 400 µm distance at the faster flow rate. The CoA changed gradually (3rd order fit) from 0.7 to 0.4 over a 1400 µm distance at the slower flow rate (n=3 independent experiments). The confocal reflectance images in the rightmost panels of **Figures 2, C**, **D** show the COL1 fibers at a distance of 200 µm and 600 µm into the expansion for the two flow rates. It can be seen that the transition of COL1 fibers in 500 µl/min flow rate from aligned to random occurs within 400 µm, while the fiber transition is more gradual in the 100 µl/min condition. Thus, we can control the CoA vs. distance relationship (variations in fiber alignment) simply by changing the input flow rate.

Conclusion: Here, we have demonstrated the ability to create gradients in COL1 fiber alignment using a simple microfluidic channel design. Our current work studies how endothelial and cancer cells respond to gradients in the fiber alignment landscape.