Microfluidic Patterning of Aligned Type I Collagen Fibers on Compliant Polyacrylamide Gels

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Abstract

The microstructure of the corneal stroma is organized into lamella of highly aligned type I collagen fibrils. The diameter and spacing of these fibrils allows light in the visible spectrum to pass through and endows the cornea with its transparent optical properties. Keratocytes reside in the corneal stroma and maintain this highly organized microstructure. Upon injury, however, these cells differentiate into myofibroblasts and exert increased mechanical forces, which help close the wound to restore tissue integrity but also distort the aligned collagen lamellae and can cause corneal hazing. Earlier work in our lab has shown that decreases in ECM stiffness can inhibit the myofibroblastic differentiation of cultured primary keratocytes. These experiments involved the use of polyacrylamide hydrogels, which were functionalized for cell culture using unpolymerized type I collagen. Here, we used microfluidic devices to create patterns of aligned type I collagen fibers on polyacrylamide hydrogels to more closely mimic the microstructure of the collagen lamellae in the corneal stroma. These substrates will be used for subsequent cell culture studies investigating the role of ECM stiffness in behavior of cultured corneal keratocytes.

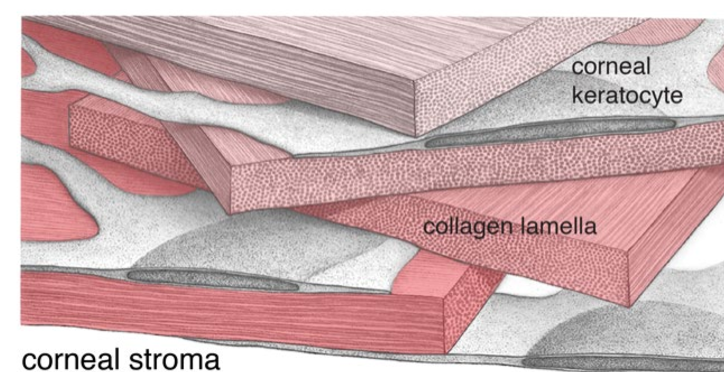


Figure 1: Microstructure of corneal stroma. Adapted from Hogan et al., *Histology of the human eye*, 1971.

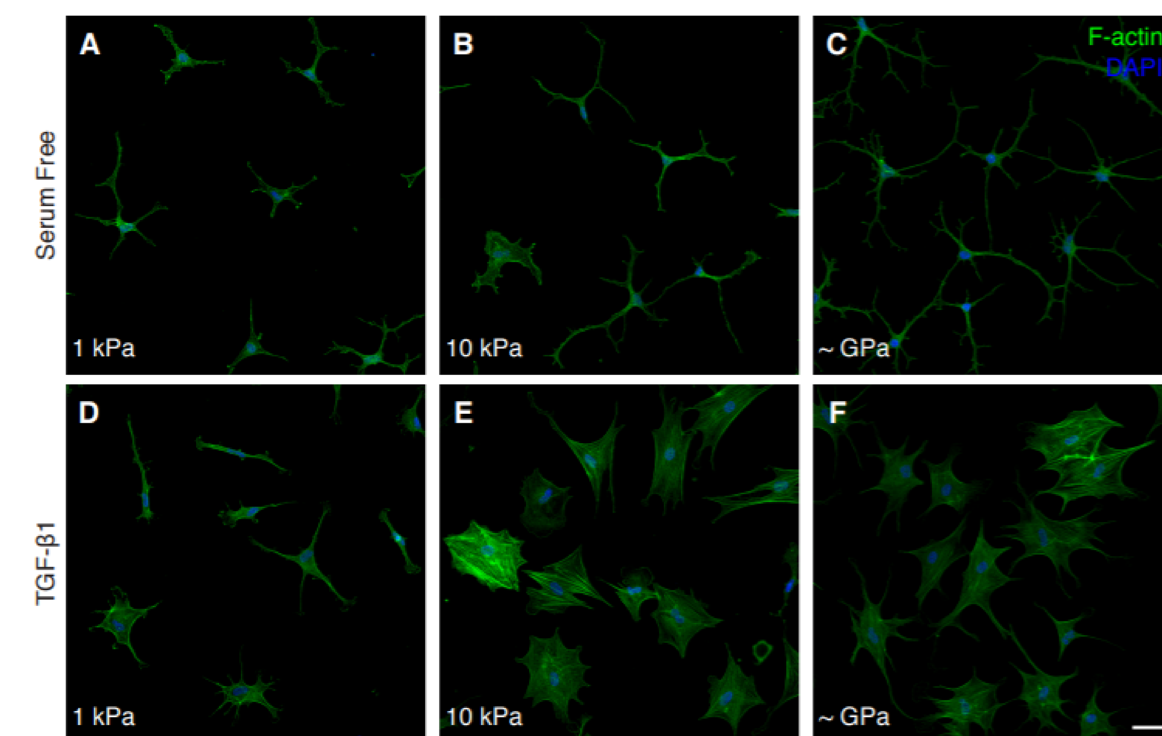


Figure 2: Substratum stiffness modulates the myofibroblastic differentiation of cultured primary keratocytes in the presence of TGF-β1. Adapted from Maruri et al. (in preparation).

Materials and Methods

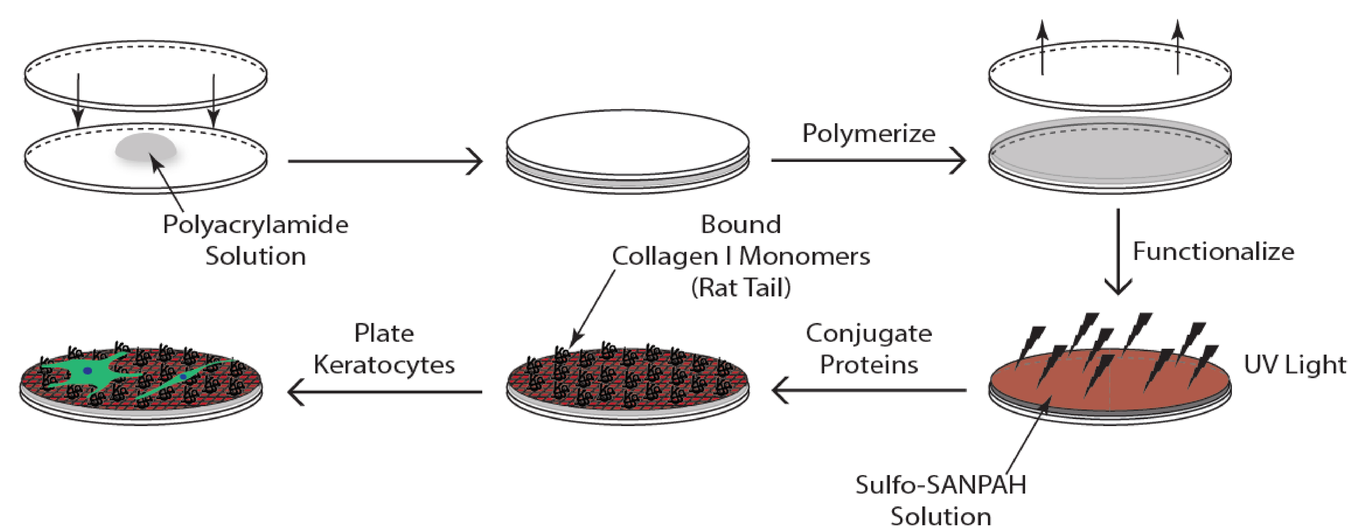


Figure 3: Polyacrylamide (PAAM) gels were fabricated using the above protocol. PAAM solution, whose stiffness can be tuned by altering the acrylamide to bisacrylamide ratio, is sandwiched between a hydrophobic top slide and a hydrophilic bottom slide. After polymerization, the top slide is popped off, exposing the gel for functionalization.

Materials and Methods

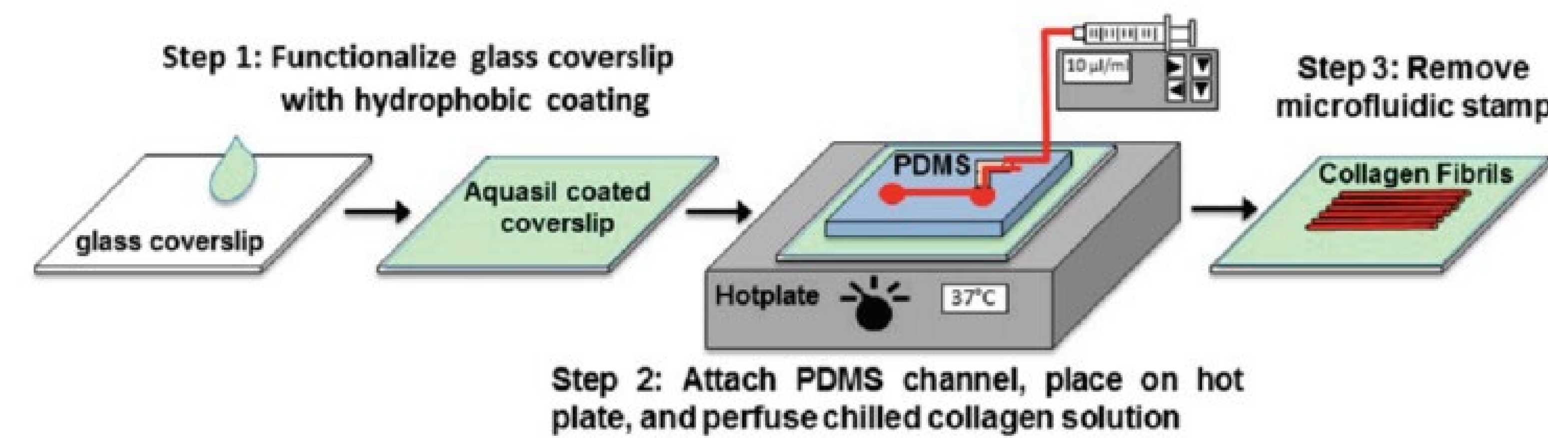


Figure 4: Microfluidic patterning of aligned type I collagen fibers on glass coverslips. Adapted from Kivanany et al., *J. Funct.* Biomater., 2018.

Microfluidic devices were created using polydimethylsiloxane (PDMS), which were then plasma treated and temporarily sealed onto either aquasil treated glass coverslips (~GPa) or stiff PAAM gels (10 kPa). This assembly was then connected to a syringe pump and placed on a hot plate at 37 degrees Celsius. A solution of type I collagen was then infused through the microfluidic devices at a flow rate of 3.78 uL/min, which corresponds to a shear rate of 75 s^-1, for either 30 minutes, 20 minutes, or 5 minutes. The collagen solution was prepared on ice using an 8:1:1 ratio of bovine type I collagen, 10X MEM, and 0.1 M NaOH. The final concentration of the collagen solution was 1.6 mg/mL. The pH of the solution was kept within the range of 7.2-7.6 to optimize the polymerization of the collagen onto the substrates.

Results

The use of microfluidics to polymerize type I collagen fibrils has already been characterized on glass coverslips. However, we would like to fabricate aligned fibrils on PAAM gels of varying stiffness. In the following experiments, we polymerized collagen on stiff (10kPa) PAAM gels for various lengths of time. The gels were than DTAF stained and imaged using a Zeiss confocal microscope at 40X with an oil objective. Despite the high alignment we observed across the varying timepoints, the images taken below are not characteristic of the entire line. Throughout the length of the line, we observed high background noise, which we suspect to be nonspecific binding of monomeric collagen, as well large clumps of collagen.

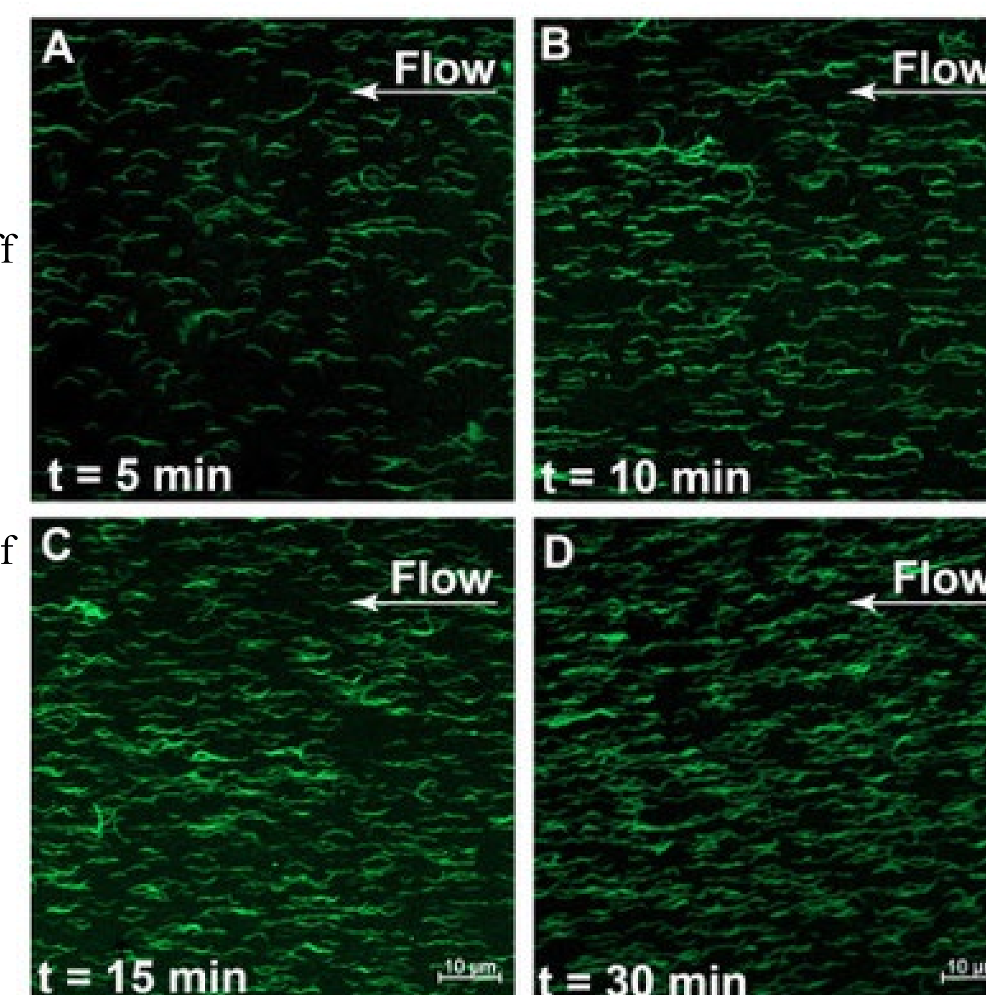


Figure 5: Collagen polymerization on glass substrates were found to be flow dependent. Type I collagen fibrils polymerized in the direction of the applied flow, displaying a highly aligned and an elongated structure, characteristic of collagen fibrils of the corneal microenvironment. Adapated from Lam et al. (in prep).

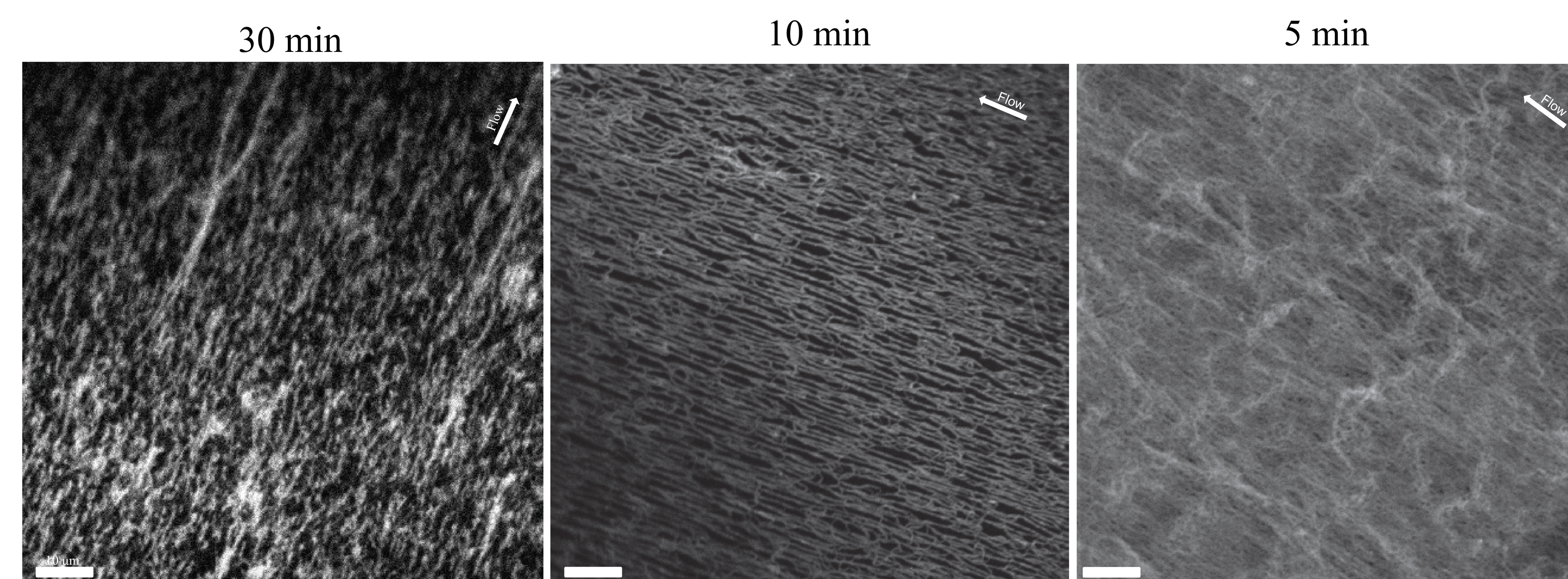
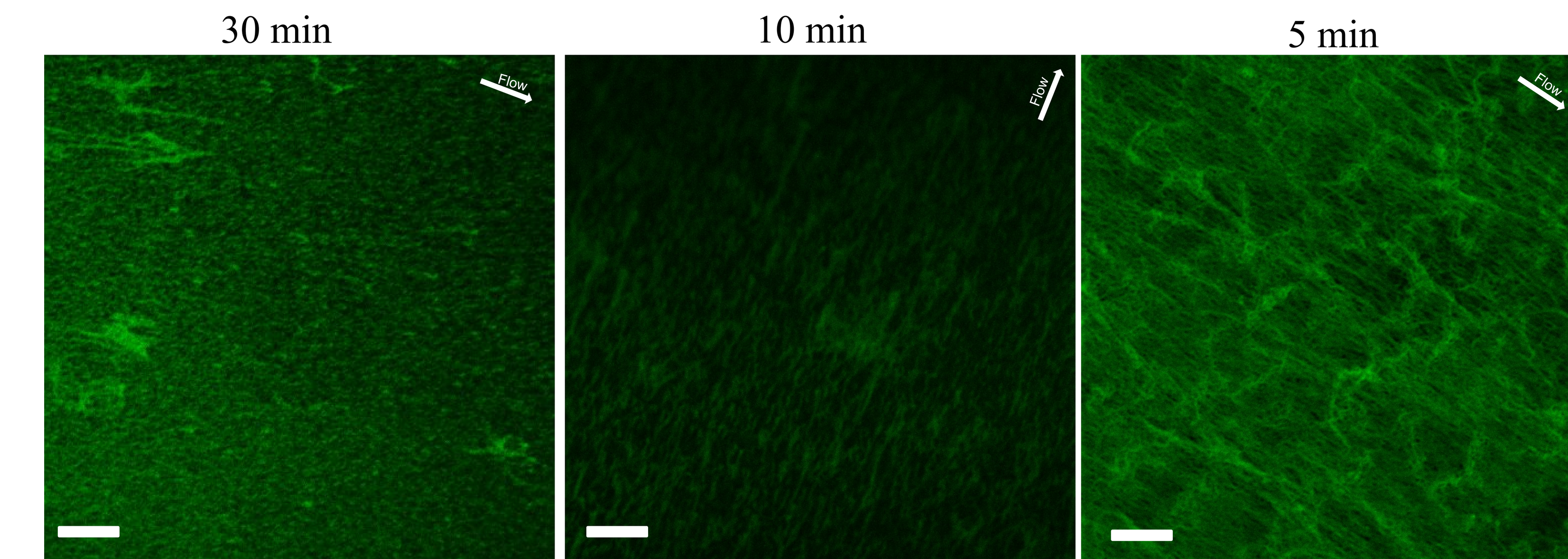


Figure 6: Aligned type I collagen fibers on sulfo-SANPAH treated PAAM gels. We observed highly aligned fibers at discrete locations along the gels. These regions, however, were not always characteristic of the overall substrate.

Results

Figure 5: The majority of the channel was less consistent with the highly aligned and elongated type I collagen fibrils cultured on glass substrates. On the stiff substrates, we found issues in producing a line with cohesive characteristics, without matting and clumping.



In order to reduce the amount of monomeric collagen binding to the gel, we removed the functionalization step of the PAAM protocol Sulfo-SANPAH binds to the surface of the gel and covalently binds to the type I collagen protein. By removing sulfo-SANPAH, we hypothesized that we could reduce the background noise.

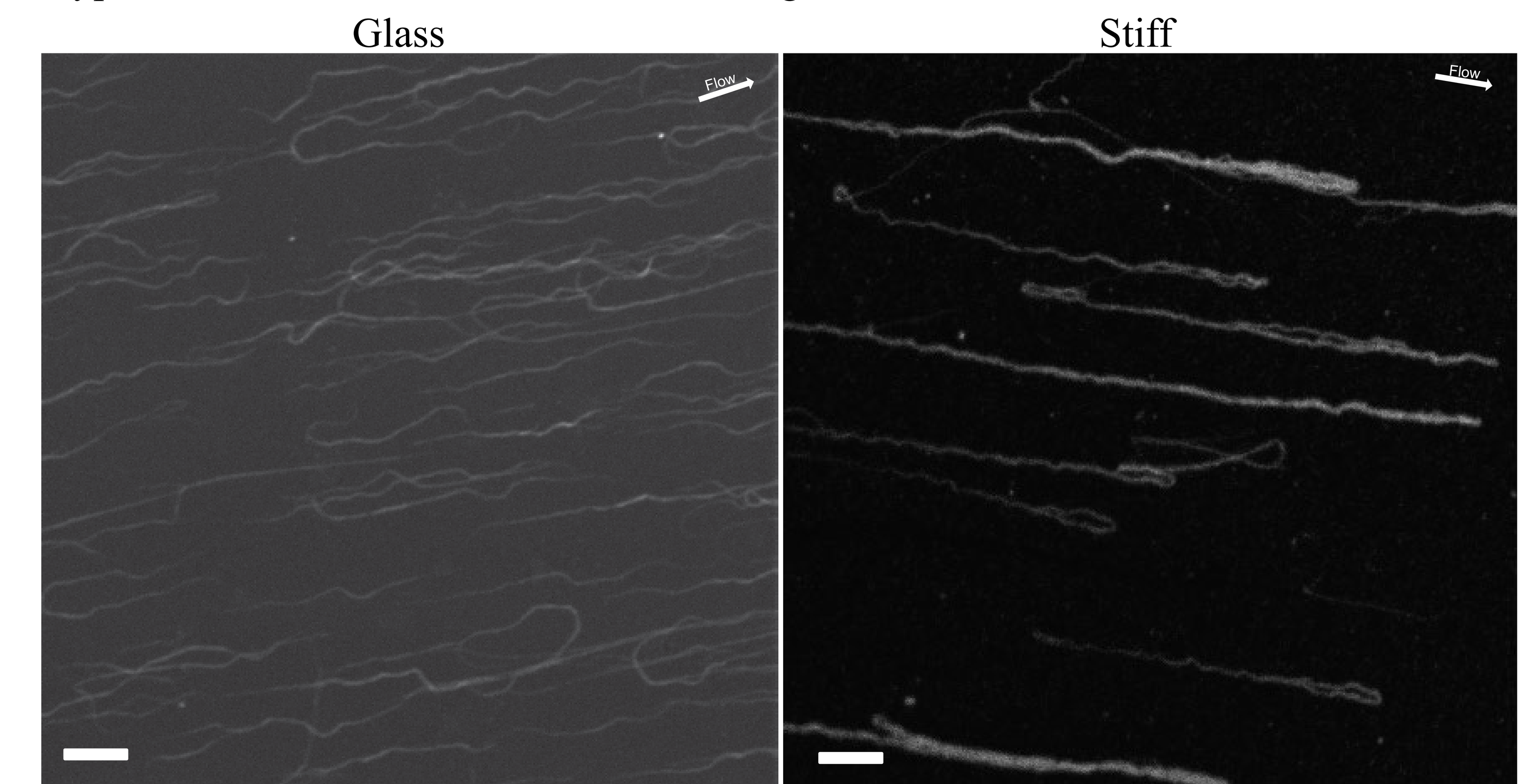


Figure 6: Collagen was polymerized on both stiff (10kPa) and glass (~Gpa) substrates for 20 minutes at the same shear rate. Though we saw a reduction in the background noise and highly aligned fibers, there were very few fibers on the stiff PAAM. The images above captured the only fibers on the entire length of the channel.

Conclusion

By optimizing both the time of collagen polymerization and the use of protein crosslinker, sulfo-SANPAH, we are able to reduce the amount of collagen matting on the substrates. However, we are still working towards creating more elongated, and aligned collagen fibers onto the stiff PAAM gels, which we have been unable to produce under the current experimental conditions. However, we suspect that by increasing the amount of time allowed for collagen polymerization without the presence of sulfo-SANPAH, we will observe less matting and more aligned fibers.

Acknowledgements

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