The microstructure of the corneal stroma is organized into lamellae 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 unmodified polyacrylamide. Here, we used microfluidic devices to create patterns of aligned type I collagen fibers on polyacrylamide hydrogels more closely mimicking the microstructure of the corneal 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.

Materials and Methods

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 then DTAF stained and imaged using a Zeiss confocal microscope at 40X with an oil objective. Despite the high alignment 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; we suspect to be nonspecific binding of monomeric collagen, as well large clumps of collagen.

Results

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.

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.

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