Examining the superstructure of collagen fibers

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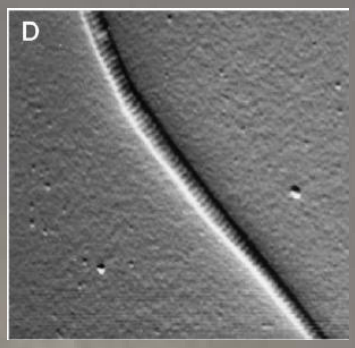
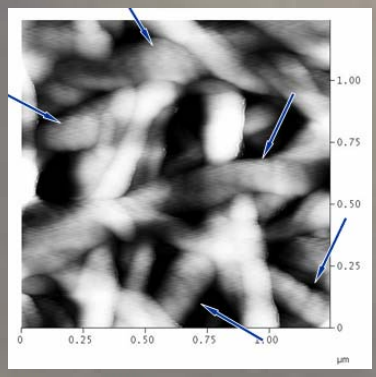
Background. When replicating the microenvironment of the corneal matrix, the artificial scaffold must resemble the natural cornea in every way. Collagen "banding" occurs every 67 nm on natural collagen fibers taken from many organs from many animals. We aim to compare electrospun collagen fibers with natural ones.

Approach. We use the atomic force microscope to examine electrospun collagen and Purecol natural collagen fibers.

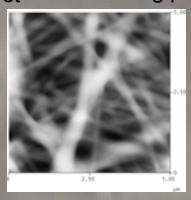
Results. Evidence of banding with a period of 67 nm is present in Purecol fibers. There is no conclusive evidence in electrospun fibers.



Collagen fiber showing banding perpendicular to the direction of the fiber. Source: Paige, M. et al. Fibrous Long Spacing Collagen Ultrastructure Elucidated by Atomic Force Microscopy. Biophysical Journal, 74. 3211-3216.



Arrows: Purecol fibers showing banding pattern.



Electrospun fibers seen under the AFM. Banding is not seen.

Future Work. Flatter samples will be prepared so that vertical resolution on the atomic force microscope increases.

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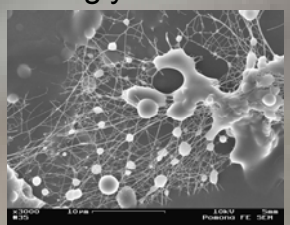
Electrospinning Collagen and Chondroitin Sulfate

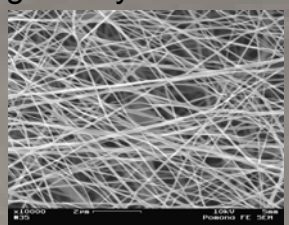
Background. In most tissue, chondroitin sulfate (CS) , an unbranched repeating disaccharide, forms macromolecules along with collagen called proteoglycans. Although the specific function of CS is unknown, it is thought to help regulate fiber spacing. Attempting to recreate the corneal matrix, we electrospun collagen and CS together.

Approach. We dissolve collagen-CS in acetic acid and load it into a syringe. The collection plate consists of two parallel copper strips mounted on a quartz plate. Roughly 10 kV are applied to the gap, which causes fibers to shoot out of the syringe. This process is called electrospinning. Trials were performed on 5%, 7.5%, and 10% CS.

Results. The addition of CS greatly lowered the viscosity of the solution, so parameters needed to be greatly changed from electrospinning collagen only. As seen from pictures below, 10% and 7.5% CS did not produce good fibers – they are spindly and not aligned. 5% CS produced fibers that strongly resemble collagen only fibers.



SEM image of 7.5% CS. Collagen formed droplets and a large dark mass, seen in top-left and bottom-right corners. 10% CS also exhibited these traits.



SEM image of 5% CS. From this picture, these fibers are nearly indistinguishable from collagen-only fibers.

Future work. An assay to determine the presence of CS must be found and used to verify that it is present.