Jonsson School Undergraduate Research Scholars Award

The Development of a Methodology to Fabricate Collagen Patches of Known Dimensions to Analyze Keratocyte Behavior

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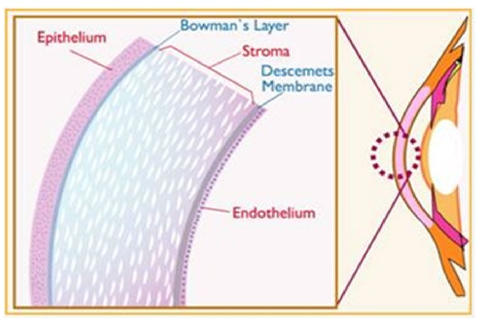
Abstract

Abnormal corneal wound healing is a significant medical problem that has been widely addressed in the bioengineering community. It is estimated by the Federal Occupational Health that there are ~ 2.5 million eye injuries in the United States each year, and about 40,000 corneal transplants occur annually, according to the National Eye Institute.

Following surgery or traumatic injury, corneal wound healing can lead to impairment of ocular function via scarring and corneal fibrosis. This fibrosis is caused by the activation of corneal keratocytes from their native quiescent state to an activated my fibroblastic phenotype. This transformation is tied to the release of platelet-derived growth factor (PDGF) upon damage of the basement membrane of the corneal epithelium. Thus, activated by PDGF, the keratocytes begin to secrete extracellular matrix (ECM) proteins and exert elongation forces to help repair the wound. Consequently, however, these behaviors can lead to a disordered tissue microstructure within the corneal stroma, which causes corneal hazing or a reduction in the transparency of the cornea. Although it is well known that the response of keratocytes in corneal wound healing is regulated by both soluble biochemical cues and insoluble biophysical cues (e.g. topography, ECM composition, elasticity) present in the local microenvironment, there are still significant gaps in our knowledge of the factors and mechanism(s) that lead to stromal fibrosis and corneal haze instead of normal wound repair.

Currently, there are no efficient technique that can recreate the orthogonal collagen patterning of the stroma, in the form of a patch *in vitro*. After laser eye surgery or a keratectomy, there are areas of collagen that have been severed and acellular zones. By creating collagen patches, the biomimicry of wound injury can be modeled for cellular responses due to the release of PDGF. When a section of collagen is severed (i.e. Photorefractive Keratectomy (PRK) or Laster In Situ Keratomileusis (LASIK)).

In this study, we answer the following question:
using microfluidic devices can we develop a technique to fabricate orthogonal collagen patterned patches *in vitro*?



"The Cornea." *Hybrid Cornea,* www.hybridcornea.org/aboutcornea.htm.

Figure 1. depicts the layers of the cornea, the relative thickness of each layer are, Epithelial ~50 um, Bowman's ~5 um, Stroma ~500 um, Descemet's membrane ~10 um, and Endothelial ~5 um.

Hypothesis

- By placing an orthogonal poly-dimethyl siloxane (PDMS) microfluidic device on a fabricated collagen line, the underlying collagen layer will be ripped off where it meets the stamp, producing a collagen area of known dimension.
- During normal wound healing *in vivo*, keratocytes are exposed to various stimuli that can have additive, antagonistic, synergistic, or potentiating effects on the keratocyte response.

Methods

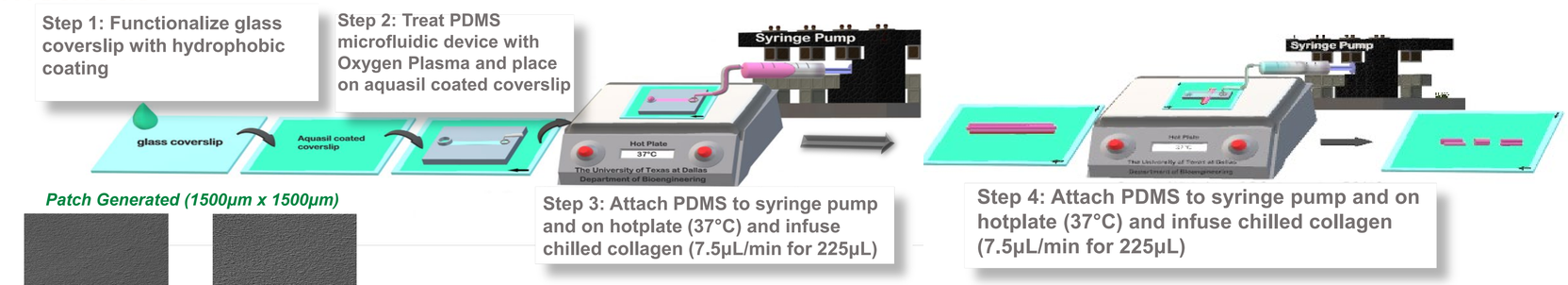


Figure 2. Schematic of the procedure for fabricating aligned collagen patches via microfluidic patterning. Developed using Paint-3D & Photoshop.

Patch Generated (1500µm x 1500µm)

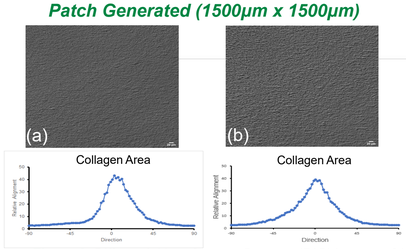


Figure 3. Differential Interference Contrast (DIC) images of (a) and (b) aligned collagen fibers, and the respective patch generated (1500µm x 1500µm) from removal of orthogonal microfluidic device formed under microfluidic patterning.

Response of Keratocytes to Aligned Collagen Fibers

To determine the response of keratocytes to aligned collagen fibers, we plated rabbit corneal keratocytes with 10,000 cells/mL and 50ng/mL PDGF (BB-subunit monomer), serum-free on top of the aligned fibrillar collagen patch substrates and characterized their response by optical and fluorescence microscopy. Figure B shows a Differential Interface Contrast (DIC) image of the cells and portrays that the cells align in the direction of the collagen fibers. Similarly, figure F shows a fluorescent image of the actin cytoskeleton of keratocytes seeded on the collagen fibers.

Atomic Force Microscopy

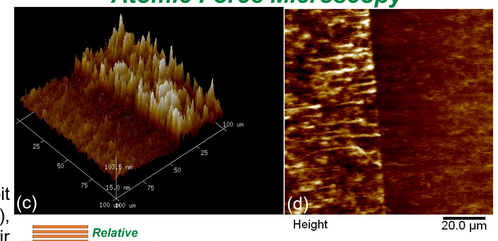


Figure 4. Depicts Atomic Force Microscopy (AFM) images of an aligned collagen patch formed with a constant shear rate of 150 s^{-1} of the green location. (c) Diagonal top view of the 3D topography of the edge of the collagen patch to emphasize the periodicity of the fibers. (d) Top view of the 3D topography of the edge of the collagen patch to show fiber density.

Patches Generated (1500µm x 1500µm & 1500µm x 50µm)

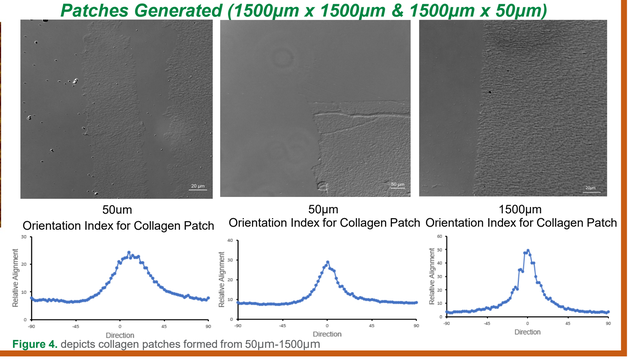


Figure 4. depicts collagen patches formed from 50µm-1500µm

Seeded Keratocytes

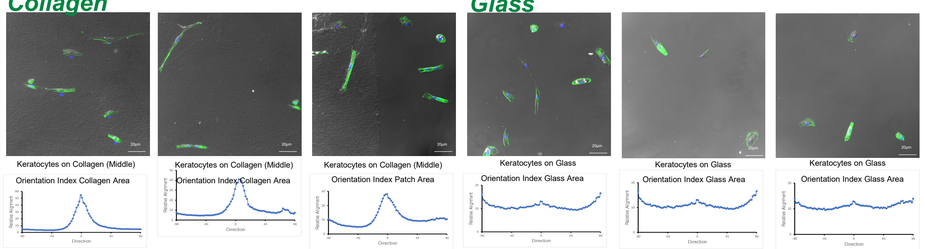


Figure 5. Differential Interference Contrast (DIC) and fluorescent images of aligned collagen fibers formed with their respective orientation index and alignment.

Discussion

$I(\emptyset)$, cell alignment as a function of cell radial angle, is quantified using Fourier Transform algorithms. The degree of cell alignment is calculated using an Orientation Index (OI) in the form of the equation:

$$\mathrm{OI}\,(\theta) = \{2 < \cos^2(\theta) > \, -1\} * 100\%$$

where,

$$\cos^2(\theta) = \; 1000 * \frac{\int_{-90}^{90} \mathrm{I}(\emptyset)\,\{\cos^2(\emptyset - \theta)\}\mathrm{d}\emptyset}{\int_{-90}^{90} \mathrm{I}(\emptyset)\mathrm{d}\emptyset}$$

With $I(\emptyset)$ centered at $\theta = 0°$ and evaluated over the direction of flow during deposition along the aligned collagen fibers at $\theta \in (-90°, 90°)$.

For cells completely aligned with collagen, the OI is equal to 100%; however, cells perpendicular to the collagen fibers have an OI of -100%.

Conclusion

- We have developed a novel method for creating patches of aligned collagen fibrils of well-defined widths (50-1500 um) that can serve as biomimicry of wound injury.
- We have demonstrated that when keratocytes are cultured on top of these collagen fibrils, there was co-alignment between the keratocytes and the collagen fibrils.
- These substrates can be used to further understand how topography regulates the adhesion and migration of corneal keratocytes during wound healing.

Future Goals

- Use TGF-β_1 to investigate its contractile forces and stress-fibers to further understand corneal wound healing.
- Analyze the interaction between stromal and epithelial wound healing as related to corneal wound healing.
- Investigate fibronectin as it relates to fibroblast adhesion to fibrin substrates and collagen matrices, as it pertains to corneal wound healing.

References

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Contact & Acknowledgments

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