Bioengineering Polycaprolactone Scaffolds for Growing Human Trabecular Meshwork Cells

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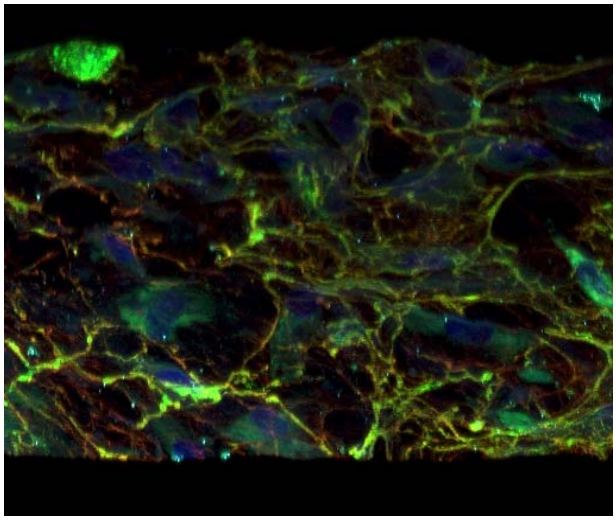
Statement of Purpose: Glaucoma is amongst the foremost causes of irreversible blindness worldwide. The only modifiable risk factor for this disease is elevated intraocular pressure. The reduced outflow of aqueous humor through the trabecular meshwork leads to increased intraocular pressure causing permanent damage to the optic nerve. The human trabecular meshwork (HTM), a thin tissue located beneath the eye, is responsible for maintaining the flow of aqueous humor. Under normal conditions, the trabecular meshwork maintains a healthy outflow of aqueous humor, providing normal intraocular pressure; however, in patients diagnosed with glaucoma, the trabecular meshwork is distorted and does not perform this duty properly, increasing intraocular pressure. The HTM is an intricate three-dimensional (3D) structure, consisting of HTM cells and their associated extracellular matrix (ECM), including interwoven collagen beams and perforated sheets arranged in a laminar pattern. Previously, our lab has bioengineered an "Artificial HTM" on microfabricated porous SU-8 scaffolds, demonstrating in vivo-like phenotype of HTM cells and responding to pharmacological agents in a similar fashion to the one seen ex vivo. Expanding on this work, this study aims to develop an implantable in vitro HTM tissue that can be use as potential treatment for early-stage open-angle glaucoma. Using a novel sacrificial layer technique, we successfully microfabricated thin, porous polycaprolactone (PCL) discs; a material which is biodegradable, biocompatible, and FDA approved, to use as a scaffold for the HTM tissue. The proper in vivo biological characteristics of the tissue were further verified.

Methods: PCL scaffolds were fabricated using a novel sacrificial layer technique. This technique allowed us to create well-defined patterned PCL scaffolds. Two different patterns, grids and. hexagons, with pores ranging 9-12 μm were chosen for this work because they simulate the trabecular beams on the HTM. Primary HTM cells were cultured on these scaffolds for 7-14 days. Additionally the effect of collagen-like coating was compared. Cell morphology and attachment was assessed by scanning electron microscopy. Protein expression and HTM specific markers were evaluated using immunocytochemistry. Real-time polymerase chain reaction (RT-PCR) and western blot were used to further assess which scaffold pattern enhances HTM specific marker expression and ECM proteins secretion.

Results: Primary HTM cells we successfully cultured on PCL scaffold of grids and hexagons patterns as demonstrated by scanning electron microscopy. HTM cells were able to successfully attach and proliferate on the scaffolds. Due to the hydrophobicity of the PCL, and to enhance cell attachment, scaffolds were coated with gelatin. Greater percentage of cell attachment was observed on gelatin-coated PCL scaffold. The efficacy of cell growth between grid vs. hexagonal patterns was also compared, demonstrating that grid patterned PCL scaffolds allows for enhanced cell attachment and proliferation. Additionally, on the grid patterned scaffold HTM cells appeared elongated and aligned. Furthermore, the cells expressed HTM-specific markers, myocilin and αβ-crystalline, along with proper ECM deposition of collagen type IV, fibronectin and laminin. Through z-stack confocal imaging, we were able to confirm the 3D organization of the HTM cells along with their secreted ECM.



Tilted angle view of HTM cells on PCL scaffold with grid features. Blue: DAPI (nuclei), green: collagen IV, red: fibronectin, cyan: laminin. Cells express ECM proteins properly.

Conclusions: The principal purpose of this study was to assess whether primary HTM cells could be successfully grown on PCL scaffolds. The research discussed has led to the conclusion that polycaprolactone is a suitable platform for the culturing of human trabecular meshwork cells. The cells exhibit HTM specific markers and grew in a morphologically correct fashion. Analysis was conducted to determine whether grid or hexagonal patterned PCL was more suitable but this is still not yet confirmed. We have confirmed that a greater number of cells attach to the scaffolds which are gelatin coated. Impending studies will include figures providing quantitative data on protein content using either western blot or PCR. Protein expression will be comparted for each criterion. This will provide better detail as to if the cells are behaving as they would in vivo