Organization and differentiation of retinal progenitor cells on microfabricated poly(e-caprolactone) thin films

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Statement of Purpose: Recent advances in regenerative medicine have increased interests in the application of stem cell-based therapies to cure retinal degenerative diseases. These diseases, including retinitis pigmentosa and age-related macular degeneration, lead to a permanent loss of photoreceptor cells in the retina and cause blindness. Studies have demonstrated that dying retinal tissue can be replaced by retinal progenitor cells (RPCs) that differentiate into new adult retinal tissue following delivery to the sub-retinal space. However, existing methods do not allow transplanted cells to undergo the necessary morphologic developments, lamination, or extensive integration with the host retina necessary to cure these diseases. New methods are needed to develop a mechanism or device that can confer organizational and instructional cues to these grafted cells to promote cell survival, differentiation and integration. In this research, micro and nano-electro-mechanical systems (MEMS/NEMS) processing techniques were used to create biodegradable thin-film scaffolds to guide the differentiation and organization of stem cells for retinal tissue engineering.

Methods: The initial step in this 2D film making process was to create a silicon master mold containing the desired surface features, posts and ridge grooves. In making the patterned master mold, a silicon oxide wafer was spun with S1805 positive photoresist, which was then exposed with ultraviolet light through a chrome mask (L-Edit, Toppan) with the designed features. Reactive ion etching was performed to achieve an aspect ratio for ridge groove patterns of 1-to-1. After wafer completion, a PCL (polycaprolactone) solution was spun on the master mold, oven baked, and subsequently lifted-off the wafer. The film was then hard-baked, sterilized and used to culture RPCs. After a set period, RPCs were examined for morphology and orientation at predetermined position by scanning electron microscopy (SEM), and for their differentiation potential using immunocytochemistry.

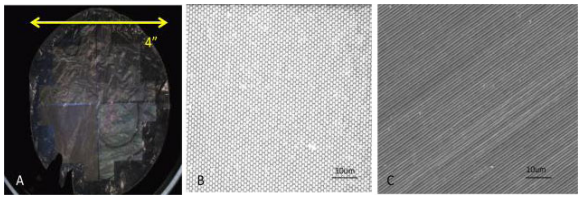

Figure 1. (A) Thin 6µm PCL film after lift off from wafer. (B) SEM of post structures on film. (C) SEM of ridge-groove features transferred into the PCL thin film

Results: The novel templating process developed was able to imprint these ridge grooves and posts structures into biodegradable polycaprolactone (PCL) thin films (5 - 10 um thick) with minimal deformation. This specific type of PCL material was chosen due to its low melt temperature, adaptability to microfabrication processing, as well as its mechanical and bioresorptive properties. Furthermore, PCL thin films have been shown to be well tolerated long term when transplanted in the subretinal space of pigs. Results showed sub-micron topographical features can induce cell orientation /alignment and morphological changes. The cell alignment angle was established by the angle between the vector of cell elongation direction and predetermined direction of topography. In analyzing cell morphology, an initial indicator of cell differentiation, the focus was primarily on the effect of sub-micron patterns on cell elongation. Elongation in this case is defined as the degree to which a cell body stretches in one direction:

$$E = (A_{maj} - A_{min})/(A_{maj} + A_{min})$$

where A_{maj} is the length of the major axis while A_{min} is the length of the minor axis. The cells on blank film with no patterns had an average of 0.48 elongations with the cells on posts and ridge grooves having an average of 0.23 and 0.58 respectively. Cell areas were also calculated with the range of values for all three types of films being roughly equal. While RPCs cultured on post structures demonstrated an early upregulation of differentiation markers, including rhodopsin and recoverin, RPCs cultured on a ridge-groove topography developed substantial elongation and parallel alignment in addition to upregulation.

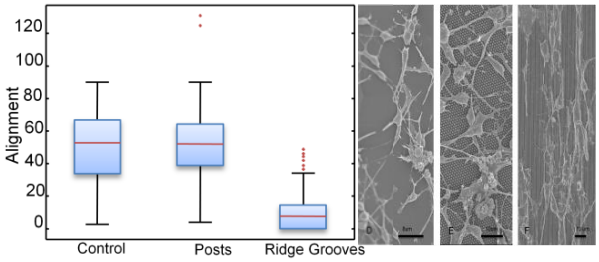

Figure 2 This chart measures the cell body alignments to submicron structures in all three types. (D), (E), (F). SEM of RPCs on control blank polymers, posts and ridge grooves.

Conclusion: From the above results with mouse RPCs, the use of sub-micron patterned PCL thin films holds great potential to enhance RPC organization and cell survival after transplantation to the retina. The hope is that this unique structured PCL thin-film platform can provide a means to organize and differentiate RPCs in a controlled manner and offers potential as a clinical treatment for retinal degenerative diseases.

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