Microfabrication of an Artificial Bruch's Membrane for the Treatment of Age-Related Macular Degeneration

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Statement of Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries for people over 60 years of age.[1] AMD-related vision impairment is due to photoreceptor loss which is preceded by the degradation of Bruch's Membrane and death of the underlying retinal pigment epithelium (RPE). AMD is a complex and multi-factorial disease divided into two distinct types, dry and wet, both of which currently lack ideal therapeutic treatments. Whereas healthy patients have an RPE-Bruch's Membrane complex that acts both as a barrier and metabolic mediator, patients with AMD have either an extracellular build-up called drusen in dry AMD or insufficient barrier function leading to unbridled neovascularization in wet AMD. This research utilizes a novel polymer microfabrication processes to develop a porous micro-patterned poly(ε-caprolactone) (PCL) thin film scaffold (approximately 10μm thick) to act as an artificial Bruch's Membrane. This thin film was specifically designed at a scale that would maintain open channels of diffusion for gas, nutrient, and waste exchange while preventing cellular translocation.

Methods: The artificial Bruch's Membrane was fabricated by spin-assisted polymer molding on a micro-patterned wafer. The master mold was created using a combined photolithography-reactive ion etching (RIE) process initially developed for microelectomechanical systems to create high-aspect ratio cylindrical features. A darkfield quartz photomask with circles of 1 and 2μm was designed using L-Edit (Tanner Research, Inc., Monrovia, CA) and commercially manufactured (Toppan Photomasks, Inc., Round Rock, TX) prior to wafer processing. A 500nm layer of silicon dioxide was thermally grown on a wafer that was subsequently coated with hexamethyldisilazane and maN-2403 (Micro Resist Technology, Berlin, Germany), a negative photoresist, and exposed through the photomask to UV light. Unexposed resist was developed away and a combination of isotropic RIE and deep RIE (Bosch process) were used to achieve cylindrical features. A thin layer of PCL was deposited on the surface of the wafer using spin-assisted molding and then removed to yield a PCL film with pores of uniform size and distribution. Cell culture experiments were performed by seeding human RPE cells at confluency on the top of the porous scaffold or a non-porous PCL thin film control in modified transwell inserts (Corning, Inc., Lowell, MA). Experiments were concluded at 1 and 2 weeks and assessed for cell morphology and migration.

Results: Etching using a combination of RIE and Bosch processing resulted in highly uniform cylindrical needles with high aspect ratio greater than 10:1 (Figure 1A). These features were 794 ± 31nm in diameter at their tip

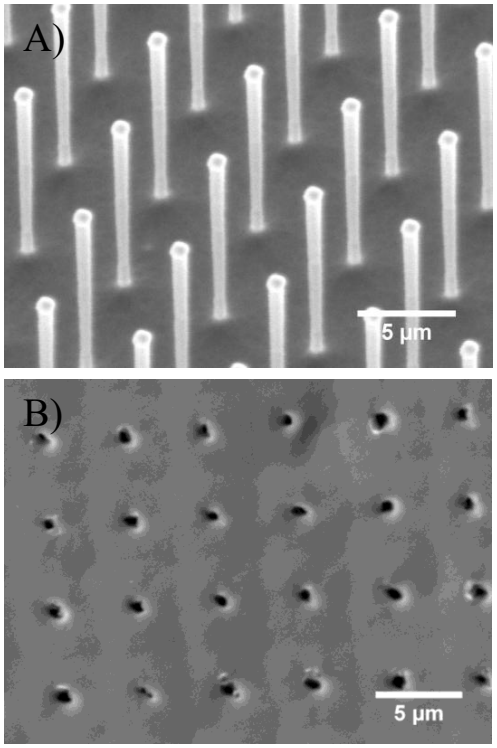

Figure 1. A) Cylindrical high aspect ratio needles on the silicon master mold, B) The porous PCL thin film scaffold with uniform pore size and spacing. Both images produced using a scanning electron microscope.

on the mold and formed slightly smaller pores 673 ± 56nm in PCL thin film (Figure 1B). RPE cells cultured on the novel scaffold formed a confluent monolayer by one week of culture which was maintained through the 2 week time point. Further, cells were unable to travel across the scaffold through the sub-micron pores.

Conclusions: A scaffold with well-defined, consistent pore size and shape was successfully produced using micro-fabrication and spin-assisted molding. RPE cells cultured on the scaffold formed a confluent, complete monolayer representative of what is found in vivo. In addition, pores in the scaffold were sufficiently small to prevent migration through the film, a favorable result for its potential in vivo use. These results suggest that this scaffold meets the initial design requirements of a sub-retinal implant for treating AMD, and therefore merits further exploration to elucidate the complex cellular interactions between the RPE and scaffold.

References: [1]Prasad PS. Maturitas. 2010;66:46-50.