A Synthetic Hydrogel Scaffold to Mimic the Bruch's Membrane for Retinal Tissue Engineering

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Statement of Purpose: Dry Age-related Macular Degeneration (AMD) is one of the leading causes of blindness in developed countries. However, there is little known about the mechanisms of this disease and few treatment options for patients after onset. [1] The retinal pigment epithelium (RPE), a highly functional cell monolayer, is known to be dysfunctional in AMD causing neural retinal death and eventual blindness. Cell replacement therapy has shown promise in the treatment of AMD, particularly cell transplantation with a support scaffold. However, many current scaffolds show cell dedifferentiation into fibroblast-like cells and immunogenic reaction in vivo. The RPE receives mechanical support from the acellular Bruch's Membrane (BM), with an approximate modulus of 1.0 MPa. [2] We hypothesize that current scaffold issues arise from scaffold properties that do not match the native BM. We present the development of a mechanically tunable synthetic polymer scaffold, tailored to match the native mechanical properties of the BM, in order to understand how the intrinsic elasticity of support scaffolds affects cell attachment, viability, proliferation, and expression.

Methods: Polyethylene glyclol diacrylate (PEGDA) of varying molecular weights from 3.4 kDa to 20 kDa were used to prepare prepolymer solutions. These solutions were photopolymerized into hydrogel sheets in a glass mold using long wavelength UV light (365 nm, 10 mW/cm^2). Following swelling for 24 hours, the hydrogels were subjected to uniaxial tensile testing to determine Young's Modulus of the polymer scaffold. Following mechanical testing, a low modulus scaffold (60 kPa) and a scaffold of modulus that mimics the BM (1100 kPa) were chosen for cell studies. ARPE-19 cells, an immortalized RPE line, were seeded (10^4 cells/cm^2) and analyzed during culture for cell viability, adhesion, expression, and function.

Results: By varying both the molecular weight and concentration of PEGDA in prepolymer solutions, scaffolds with a range of moduli from 60 kPa to 1200 kPa were obtained. Hydrogels formed from 3.4 and 5 kDa PEGDA at 2x relative concentrations (40% w/v) produced scaffolds with a modulus approximately equal to that of a native porcine BM (~1.0 MPa; Fig. 1). Although there was no significant difference in cell viability between low and high moduli scaffolds, there were significant increases in metabolic activity (Figs 2, 3). At days 7 and 14, the metabolic activity from cells seeded on the high moduli scaffold showed significantly greater increases than cells seeded on low moduli scaffolds. Preliminary cell expression analysis of retinaldehyde binding protein-1 (CRALBP) and Collagen Type I (COL-I) (normalized to GAPDH) indicates that the expression of CRALBP on scaffolds is lower than the expression on tissue culture plastic (TCPS); however, the expression of COL-I on scaffolds relative to TCPS is 2 to 2.5 fold higher.

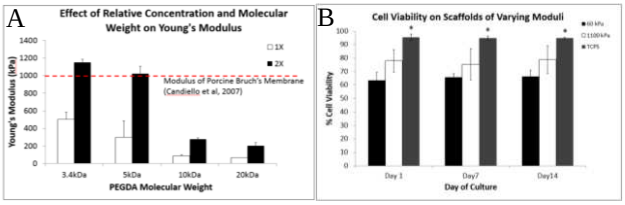

Fig. 1: **A.** Effect of hydrogel formulation on scaffold elastic modulus. Higher concentrations of low molecular weight PEGDA produced the stiffest hydrogels **B.** Effect of scaffold modulus on cell viability. Stiffer scaffolds had higher cell viability, approaching TCPS. Asterisks indicate statistical significance; $p<0.05$. Error bars show standard deviation.

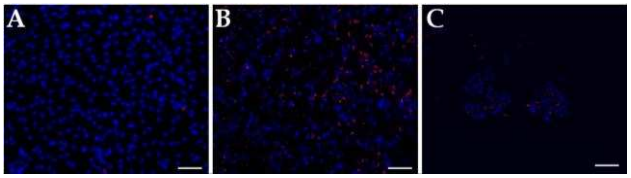

Fig. 2: **A.** ARPE-19 cells cultured on TCPS; **B.** 1100 kPa scaffold; and **C.** 60 kPa scaffold at day 7 of culture stained for nuclei (blue; Hoescht) and dead (red; ethidium homodimer1) cells. Cells tended to form small clusters with fewer regions of continuous monolayer on the low moduli scaffolds. Scale bars = 100 μm.

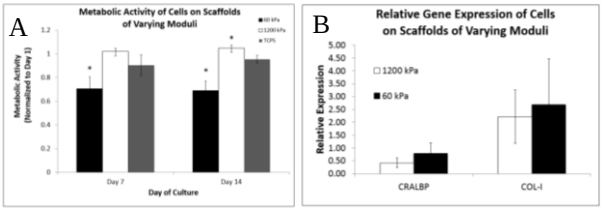

Fig. 3: **A.** Effect of scaffold modulus on cell metabolic activity and **B.** gene expression. Higher modulus scaffolds increased cell metabolic activity and COL-I expression. Asterisks indicate statistical significance; $p<0.05$. Error bars show standard deviation.

Conclusions: We were able to achieve a scaffold with an elastic modulus equivalent to that of the native Bruch's Membrane. Scaffold elastic modulus had measurable effects on cell viability, cellular metabolic activity, and gene expression. Future studies will modify the topology of these hydrogels in addition to incorporating bioactive moieties to further develop the hydrogel as a biomimic of the Bruch's Membrane.

References:

1. Bunche,C. BMC Public Health 2006, 6:58.
2. Chan WH. Young's modulus of Bruch's membrane: implications for AMD. Invest Ophthalmol Vis Sci 2007;48:2187