Engineering Bruch's membrane using polyethylene glycol diacrylate hydrogels

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Statement of Purpose: Age-related macular degeneration (AMD) is a disease which results in loss of vision due to damage to the macula, the center part of the retina. AMD occurs in two different stages: dry and wet stages. The dry stage, the more common of the two, is when the sensitive tissues of the macula becomes thin and slowly loses its function. The wet stage is caused by the growth of abnormal blood vessels in the choriocapillaries through the Bruch's membrane. Regeneration of the Bruch's membrane can be a substitute treatment to this disease. To tissue engineer the membrane, a poly(ethylene glycol) (PEG) hydrogel is developed and physical and biological properties are characterized. PEG photopolymerizable hydrogels will be used to stimulate retinal pigment epithelium (RPE).

Methods and Materials:

Cell Maintenance: The Bruch's membrane consists of 5 layers including the basement membrane of the Retinal Pigment Epithelium (RPE). The retinal pigment epithelium transports metabolic waste from the photoreceptors across Bruch's membrane to the choroid. RPE cells are cultured in DMEM media with 5% fetal bovine serum. Cells are maintained at 37°C with 5% CO_2

Hydrogel Preparation: PEGDA is dissolved in PBS at 37°C. PEGDA is then incubated for an additional 30 minutes to help remove bubbles. PEGDA hydrogels are prepared by dissolving 0.1 mg/ml polymer in 1 ml PBS. The photoinitiating acetophenone solution (10µl/ml) is then added and allowed to mix through diffusion for an hour. The prepolymer solutions are then photopolymerized by exposure to UV light (365nm, 10 mW/cm^2) which converts it to hydrogels.

UV cell death: RPE cells were seeded in 96-well plate at 15,000 cells/ml (n=5), and waited until wells were confluent. They were exposed to UV light for 30 secs, 1 min, 2 mins, and 3 mins at 7 cm and were compared to cells not exposed to UV light. Cell death was measured by MTS assay right after time of exposure.

RPE attachment on hydrogels with RGDS: Hydrogels are prepared as previously described but with 1:1 ratio of acryoyl-PEG-RGDS. After initial swelling in PBS for 24 hrs, RPE cells are seeded on surface at 15,000 cells/ml (n=3). Control include: cells on the 96-well plate, cell on hydrogel without RGDS, and cells on hydrogel with RGDS.

Results: Prepared hydrogels are clear and transparent. Hydrogels initially swelled to equilibrium over 24 hours. The RPE cell attachment was significantly greater on hydrogels with RGDS adhesion ligands as compared to control, cells on a plate, and just PEG (Figure 1). After exposure to UV light, it was observed that there was no cell death from the given time constraints (Figure 2).

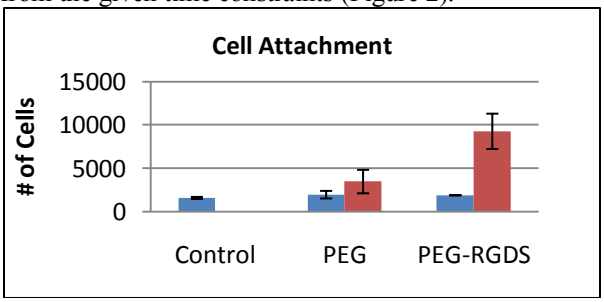

Figure 1: Cell attachment over 24 hours. Blue represents cells on plate and red represents cells on the hydrogel.

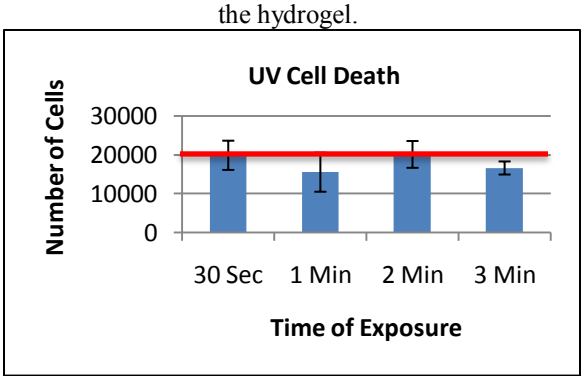

Figure 2: Cell death via exposure toUV, the cell number was about the same as the cells not exposed to UV light, therefore the cell death was at minimal.

Conclusion: PEGDA hydrogels maintain their structure and composition over 3 weeks. RPE cells are stable when exposed to the wavelength of light needed for hydrogel formation. PEGDA hydrogels support RPE attachment. In conclusion, it is believed that this biomatrix is a suitable biomaterial to use for the regeneration of Bruch's membrane. Future studies will include further assessment of cell morphology and function on these hydrogels for use in reconstructing the Bruch's membrane.

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References:

1. JM Seddon, Epidemiology of age-related macular degeneration. (AP Schachat, S Ryan eds.) *Retina*, 3rd ed. St. Louis, MO: Mosby; 2001;1039-50.
2. AM Fine, Earliest symptoms caused by neovascular membranes in the macular. Arch Ophthal. 1986;104:513-4.