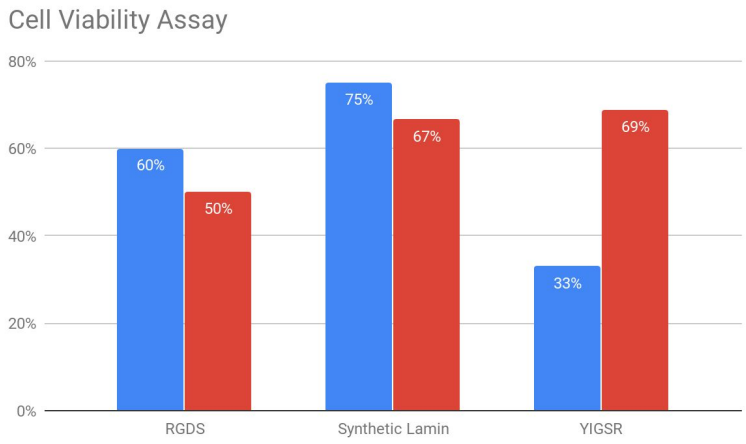
Probing the Requirements of a Bruch's Membrane for Retinal Pigment Epithelial Cells

Brenda Okereke, Priya Mistry, Kristopher White PhD, Ronke Olabisi, PhD
Biomedical Engineering, Rutgers University, Piscataway, NJ USA

Introduction: Age related macular degeneration (AMD) is the leading cause of blindness in developed countries. AMD occurs due to dysfunction of the retinal pigment epithelial (RPE) cell basement membrane, the Bruch's membrane. Previous work in the lab demonstrated that retinal pigment epithelial cells preferred stiff substrates to soft ones, and that RGD-conjugated polyethylene glycol (PEG) hydrogels alone were not sufficient to support long term RPE cell health.[1] There is evidence that epithelial and neural cells prefer laminin-derived peptides over fibronectin-derived peptides. Therefore, we examined the fate of RPE cells when seeded on PEG hydrogels conjugated with synthetic laminin peptides.

Materials and Methods: The PEGDA scaffolds were created based on parameters cited in previous studies that optimized the mechanical properties needed to replicate the Bruch's membrane.[1] Briefly, hydrogels were formed from a prepolymer solution containing 0.4 g/mL PEGDA (5 kDa), 10 µL Acetophenone/NVP solution (300 mg acetophenone/mL NVP), and 1 mL HEPES buffered saline (25 mM). The prepolymer solution was injected in glass molds with teflon spacers and exposed to UV light for 1 minute. The resulting hydrogels were 1 cm x 1 cm. RGDS, YIGSR peptide, and a synthetic laminin-derived peptide (EMD, item# SCR127) were conjugated to the surface of the PEGDA hydrogels. The peptides were first conjugated to acrylate-PEG-succinimidyl valerate (PEG-SVA) under basic conditions. The peptides were then immobilized onto the surface of the hydrogel via photoinitiated reaction of the free acrylate groups within the hydrogel and PEG-peptide conjugates. RPE cells were seeded onto the surface of the hydrogel at 10,100 cells/1 cm^2 gel. Cell survival was assayed after one day and two days of culture on the PEGDA hydrogels.



Results and Discussion:

Figure 1. Percentage of viable ARPE-19 cells on PEGDA hydrogels incorporating immobilized RGDS peptide, proprietary synthetic laminin peptide and YIGSR laminin-derived peptide after 1 day (blue) and 2 days (red).

There were very few cells adhered to gels by this point, but a live/dead assay was performed to assess cell viability. The live/dead assays of the ARPE-19 cells immobilized on PEGDA hydrogels with a RGDS binding motif featured a 60% cell viability on Day 1 and 50% cell viability on Day 2. The cell viability decreased and displayed similar results to previous studies[1]. The synthetic laminin also showed a decrease in cell viability after the first day after averaging around 4 cells on day 1 and 2-3 cells on day 2. The YIGSR showed an increase in cell viability averaging around 2 cells on day 1 and 11 cells on day 2. Overall the number of cells within the hydrogels is very low.

Conclusions: Few of the ARPE cells cultured adhered to PEGDA hydrogels as evidenced by the viability stains. Future studies will repeat the assays with different binding motifs for comparison and over a longer span of time.

Acknowledgements: The work presented here was conducted in part as a part of the educational component of the NSF CAREER Award (CBET-1752079).

References: 1. White CE et al. J Biomed Mater Res A. 2018 Nov;106(11):2871-2880