

Optimizing the Growth and Characterization of Retinal Pigment Epithelial Cells

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Introduction: Age related macular degeneration (AMD) is the leading cause of blindness in developed countries and accounts for nearly 10% of vision loss around the world. One major difficulty in studying AMD is modeling the retinal pigment epithelium (RPE) in vitro. The RPE is a single layer of cells that supports photoreceptors by providing nutrients and filtering waste products. RPE cells grow on the acellular Bruch's membrane, which sits directly superior to the choroid layer. RPE have a characteristic dark pigment and grow in tight polygonal cell junctions. When grown in tissue culture flasks, these characteristics are not exhibited. In this study, spider silk coated substrates were used to replicate the surface chemistry of Bruch's membrane and promote RPE growth, morphology, and differentiation.

Materials and Methods: RPE cells were cultured in a T25 cell culture flasks until a concentration of 4.5×10^5 cells/ mL was reached. Cells were maintained using DMEM-F12 nutrient medium with 10% FBS and 10,000 units/ mL penicillin. A six well plate was coated with three different spider silk proteins (MaSp1/MaSp2, MaSp1, and FLYS3) and inoculated with RPE cells. The cells were maintained using the same media for the first two days, then changed to a 5% FBS solution to prevent cell overgrowth. Cell confluency was measured daily using light microscopy; SEM images were captured after 13 weeks. Transepithelial electrical resistance (TEER) was measured weekly using a World Precision Instruments EVOM2 and STX2/Endohm 24Snap.

Results and Discussion: Cells grown on spider silk showed similar characteristics to those grown on standard tissue culture plates. Cells did not pigment in the time period measured, but had similar confluency and morphology (Figure 1). After 5 weeks, the TEER suggested that there was no significant difference in the tight junction formation between the coated and uncoated wells.

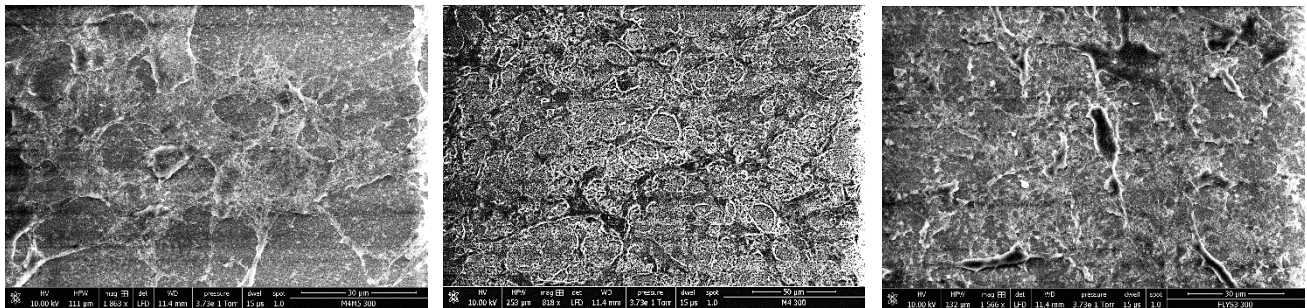


Figure 1 – RPE cell growth after 13 weeks on MaSp1/MaSp2, MaSp1, and FLYS3 proteins, respectively (SEM images).

Conclusion: The spider silk protein shows promise as a substrate for RPE cell growth. Further research may show advantages in cell characterization using spider silk compared to tissue culture flasks. Spider silk coated permeable membranes may lead to a polarized RPE monolayer. Layering protein additives (collagen I-V, fibronectin and vascular endothelial growth factor (VEG-F)) may lead to better RPE cell differentiation.