Characterizing the Growth of Retinal Pigment Epithelial Cells on Recombinant Spider Silk Membranes

Chase Paterson, Thomas Harris, Farhad Farjood, Dr. Randy Lewis, Dr. Elizabeth Vargis

I. Goals
- Investigate RPE cell growth and attachment on free-standing spider silk membranes
- Compare efficacy of two membrane fabrication methods and silk proteins

II. Introduction
Retinal Pigment Epithelium (RPE)
- Monolayer with tight, hexagonal cell junctions
- Grows on acellular Bruch’s Membrane (Figure 1)
- Provides nutrients to photoreceptors
- Filters waste out of eye
- Affected by age-related macular degeneration (AMD)

Bruch’s Membrane in vivo performs specific functions:
- Mechanical support for RPE
- Semipermeable
- Promotes barrier function in RPE

Previous results suggest that recombinant spider silk proteins isolated from transgenic goat milk can be used as a synthetic Bruch’s Membrane (Figure 2).
- Major Ampullate Spidroin 1 (M4)
- Major Ampullate Spidroin 2 (M5)

III. Methods
Membrane types: M4 spin coated (M4<sub>SC</sub>), M4 gravity (M4<sub>G</sub>), and 50:50 ratio of M4/M5 gravity (50:50<sub>G</sub>).

Transwell® membranes were cut out from the inserts and replaced with free-standing spider silk membranes. The membranes were mounted using a silicone ring as shown in Figure 4.

Figure 4 – Schematic of free-standing spider silk membrane.

Transwell<sup>®</sup> membranes were cut out from the inserts and replaced with free-standing spider silk membranes. The membranes were mounted using a silicone ring as shown in Figure 4.

RPE cell growth analysis after 2 and 7 days:
- Cellular dsDNA content analysis
- Immunocytochemical (ICC) staining – ZO-1 proteins

IV. DNA Assay Results

![DNA Content Graph]

Figure 5 – dsDNA content at 2 and 7 days. The spider silk membranes experienced a higher increase in growth after 2 days than the controls.

V. RPE Cell Morphology
ICC staining was performed to visualize RPE cell morphology and tight junction formation. These results showed a noticeable difference between spider silk membranes and the Transwell® control (Figure 6).

![ICC Images]

Figure 6 – ICC images of ZO-1 proteins at 7 days.

VI. Conclusions and Future Work
With good attachment, morphology, and growth of ARPE-19 cells, we can conclude that spider silk can be used as a synthetic Bruch’s membrane in an in vitro model of the retina and therefore advance research towards combating eye diseases like AMD.

Continuing and future research:
- Investigate the growth of primary porcine RPE cells on spider silk membranes to create a more representative model of the retina

VII. Acknowledgments