2017

Optimizing the Growth and Characterization of Retinal Pigment Epithelial Cells

Lori Caldwell
Utah State University

Optimizing the Growth and Characterization of Retinal Pigment Epithelial Cells

Lori Caldwell
Utah State University

Dr. Elizabeth Vargis
Utah State University

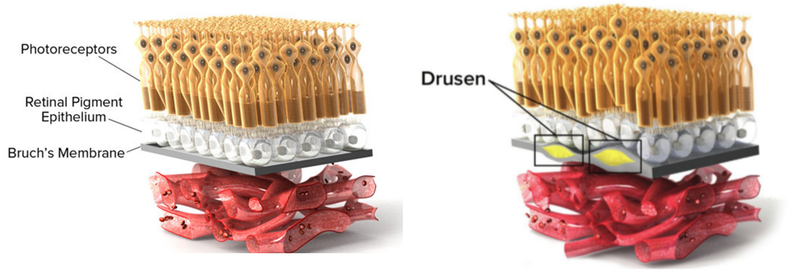
Introduction

Retinal Pigment Epithelium (RPE):
- Single layer of cells
- Attached to acellular Bruch's Membrane (Figure 1)
- Provides nutrients to photoreceptors
- Filters waste out of eye

RPE cells *in vivo* exhibit distinct characteristics to perform their function:
- Pigment absorbs excess light entering the eye
- Tight polygonal cell junctions provide a blood vitreous barrier that prevents large molecules from entering the eye

Bruch's Membrane failing is one cause of age-related macular degeneration.
- Causes lipid build up, overgrown blood vessels, and photoreceptor death (Figure 1)

Figure 1 – Markers of AMD



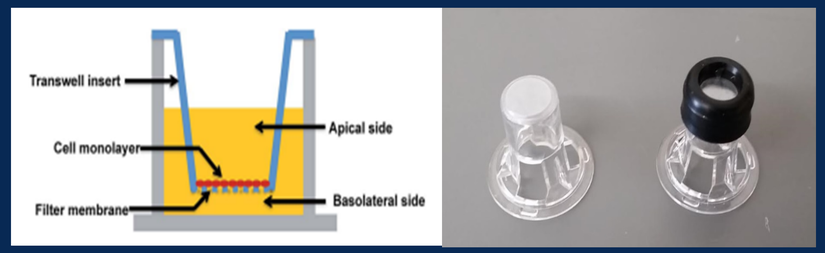
In a healthy eye, the RPE forms a single flat layer below the photoreceptors. In AMD, drusen forms disrupting the vital functions of the RPE leading to vision loss.

Methods

Free standing membranes replaced the polycarbonate membrane in a Transwell tissue culture plate (Figure 2). Cells were cultured in DMEM-F12 with 10% added FBS over three weeks.

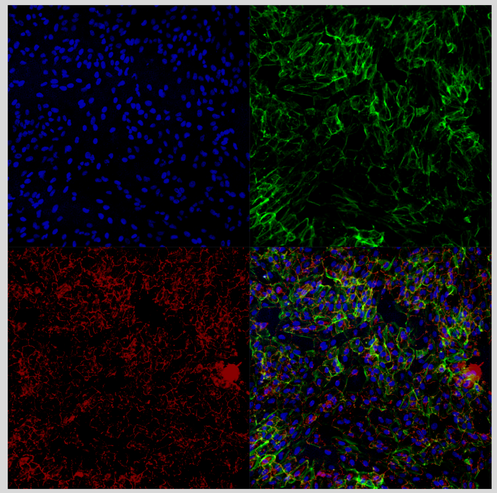
1. Free standing membranes were built using spider silk protein
2. ARPE-19 cells were grown on the free standing membranes
3. Viability measurements were taken periodically throughout the experiment

Figure 2 – Spider silk free standing membrane



The polycarbonate membrane was removed from a standard Transwell insert and replaced with a membrane fabricated from spider silk proteins.

Figure 3 – RPE cell imaging



Blue = Nuclear stain (verifies life). Green = F-actin stain (tight junction formation). Red = ZO1 stain (tight junction formation). Bottom right is a combining image of each stain.

Results

Cells grown on spider silk showed similar growth characteristics to those seeded on standard tissue culture plates. Cells did not pigment in the time period measured and had reduced confluency as well as morphology consistent with distressed cells. The loose cell junctions were due to topography, flexibility in the fabricated membrane, and discontinuity of protein thickness (Figure 3).

Conclusion

The spider silk protein shows promise as a substrate for RPE cell growth. Further research may show advantages in cell characterization and imaging using spider silk compared to Transwell culture plates.

Future research may include:
- Collagen I-V, Fibronectin, Vascular Endothelial Growth Factor (VEG-F), and RGD (Arginine – Glycine – Asparagine).
- Layer proteins according to physiology of Bruch's Membrane

UtahStateUniversity

Lori Caldwell
Utah State University
Biological Engineering
12lcaldwell@gmail.com