Modeling the Permeability of a Stria Vascularis Endothelial Monolayer Using Transwells™

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Introduction

The Stria Vascularis cell line makes up the cells within the inner ear vasculature. In particular, they make up what is known as the blood labyrinth barrier (BLB). The BLB is made up of three cell types; endothelial (EC), pericyte (PC), and paravascular marcrophage-like melanocytes (PVM/M). Together the cells regulate the intake of nutrients from the blood into the inner ear. The relative permeability of the BLB is of interest to study the movement and permeability to drugs that target the inner ear. This area, however, is relatively under-researched.

To change this, a model must be created. This is done using Transwell™ cell culture inserts. To do this, Stria Vascularis endothelial cells (SVECs) were harvested and sent by the University of Oregon for use. They are seeded onto a Transwell membrane and allowed to form a monolayer within 5 days. To measure permeability, fluorephore isothiocyanate (FITC) labeled dextran at pre-determined molecular weights of 70 and 150 kDa will be used and be allowed to pass through the monolayer. A second set of samples will be take after 8 days of seeding to determine if there is any change in the permeability of the membrane. Permeability coefficents can then be calculated and used to further future drug research as a reference.

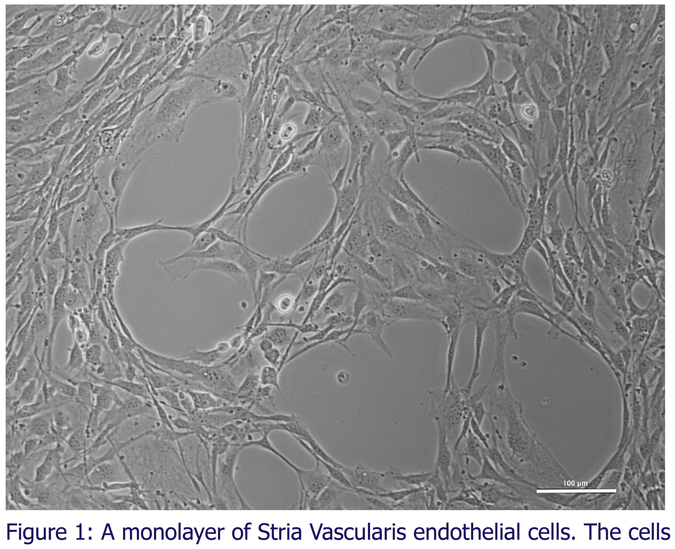

Figure 1: A monolayer of Stria Vascularis endothelial cells. The cells can be seen forming circular formations. These could be the cells attempts at creating some kind of vasculature within the monolayer.

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Methods

Twelve 24 well plate Transwell™ cell culture inserts were coated with 5 µg/mL of type 1 rat tail collagen. The apical side of the inserts were then seeded at a cell density of 4 x 10^4 with 0.1 mL of a endothelial cell media (ECM) supplemented with 5% FBS and 1% ECGS. The basolateral side was then filled with 0.6 mL of ECM. Six wells were designated for day 5 experiments and the other six were designated to day 8 experiments. Each set was subdivided into three wells for 70 kDa and 150 kDa FITC-dextran. Once the wanted day was reached (5 or 8 days), 5 mg/mL of either 70 kDa or 150 kDa FITC-dextran was placed into the apical chamber of the insert. At 5, 30, 60, and 120 minutes, 75 µL samples were taken out of the basolateral chamber and placed into a black opaque 96 well plate.

A standard curve was created using known concentrations of 70 and 150 kDa FITC-dextran. The standards were placed in triplicate into the same well as the experimental samples. Concentrations of 0.05, 0.25, 0.5, 1, 5, 10, and 75 µg/mL were used to create the curve.

The 96 well plate containing the samples was then placed into a fluorimeter set to excite the FITC at 485 nm and read the emissions at 523 nm. The readings were then normalized against samples of DPBS that were placed in the plate.

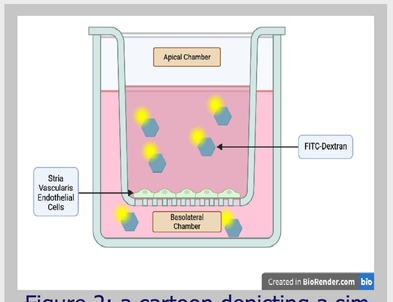

Figure 2: a cartoon depicting a simplified depiction of the permeability assay using FITC-Dextran.

Molecular flux was then calculated by dividing the calculated FITC-dextran concentration by the time. The permeability coefficient was then calculated using the following formula:

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A+C_0}$$

- $\frac{dQ}{dt}$ = molecular flux (C/t)
- A = area of membrane (cm^2)
- C_0 = initial concentration (C)

Results

The standards were plotted and an equation was determined. Using these equations FITC-dextran concentrations were determined for each time set (5, 30, 60, and 120 min) and divided by the time to obtain a molecular flux value for each set. For each flux value, a P(app) was calculated using the equation in the methods. The permeability coefficients were then compared to each the other values to determine if there is any significant change between data sets. To do this, two tailed t-tests were conducted using a significance value of 0.05.

P_{app} (5, 70)	3.07E-04	+/- 1.15E-04	38%	cm/min
P_{app} (5, 150)	4.25E-04	+/- 1.21E-04	28%	cm/min
P_{app} (8, 70)	1.42E-04	+/- 1.26E-05	9%	cm/min
P_{app} (5, 150)	1.59E-04	+/- 2.74E-05	17%	cm/min

P-values were calculated between day 5 molecular weights of the same dextran and day 8 molecular weights of the same dextran. Additional p-values were calculated between the molecular weights within the same set were calculated to determine if there was significant difference between the permeability of 70 kDa and 150 kDa FITC-dextran. The statistical analysis determined that there was a significant difference between the permeability of the 150 kDa FITC-dextran between day 5 and day 8. All other t-tests resulted in p-values above the significance level of 0.05 indicating a lack of evidence to disprove any kind of change had occured.

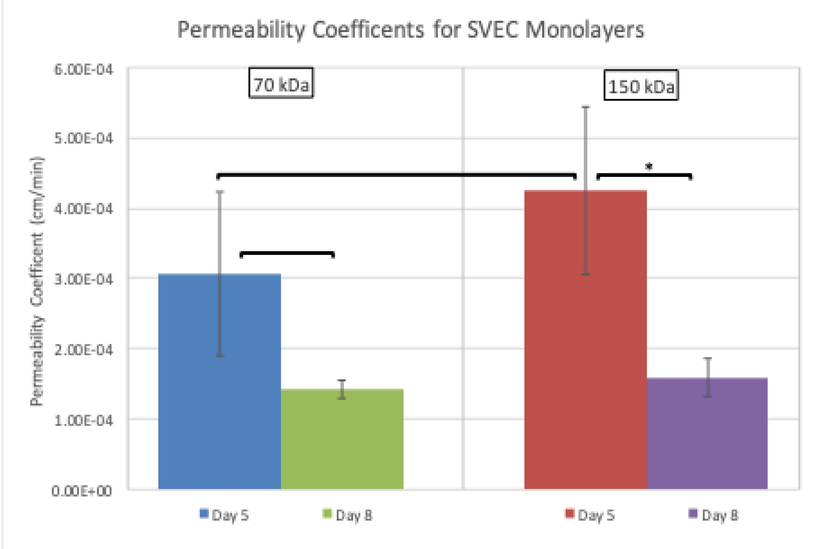

Figure 3: a bar graph comparing the permeability coefficients of the different sets. Each set can be compared to its own molecular weight. The permeability can be seen to decrease as the monolayer has time to develop. The p-values are depicted as a bar above the graphs. * = $p \leq 0.05$

Conclusion

The p-values calculated using the t-tests concluded that there was a statistically significant difference between day 5 and day 8 150 kDa FITC-dextran. The data also shows a congregation of the two molecular weights to a similar permeability coefficient. This suggests that the monolayer was not fully developed by day 5. The observed drop in permeability could be due to the formations of stronger tight junctions by the SVECs. This could also explain the large error seen within the day 5 set as the differences between the development of the monolayers could not be accounted for.

Further permeability studies are needed to create a model of the BLB. Future experiments would involve allowing the monolayer to develop longer to determine when permeability plateaus. Extremes of permeabilities would also be considered by identifying permeabilities coefficients for 10 kDa and 500 kDa FITC-dextran. While the monolayers are forming, transepithelial electrical resistance (TEER) would be measured to quantify tight junction formation as well as permeability to ions.

The data collected from the assays conducted, as well as future assays, will contribute to the development of a model for the BLB.

References and Acknowledgments

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