Optimization of The Culture System On Apical-Basal Polarization of IEC-6 Rat Small Intestinal Epithelial Cells

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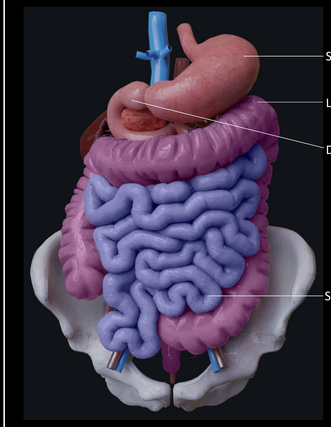
Introduction

Small Intestine

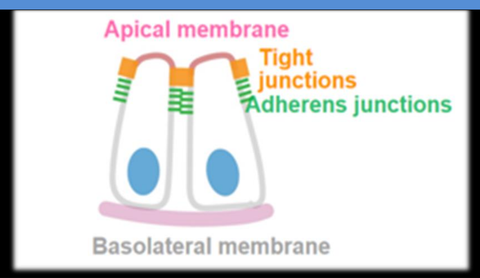


Functions:
- Enzymatic digestion of consumed nutrients
- Intraluminal bacteria barrier
- Moves food along gastrointestinal tract
- Secretion of digestive mucus
- Absorbs water

Epithelial Cells

Functions:
- Cover all body surfaces/body cavities/hollow organs
- Form epithelial tissue preventing inimical pathogens
- Act as rapidly renewing cells preserving the epithelial lining along the gastrointestinal tract

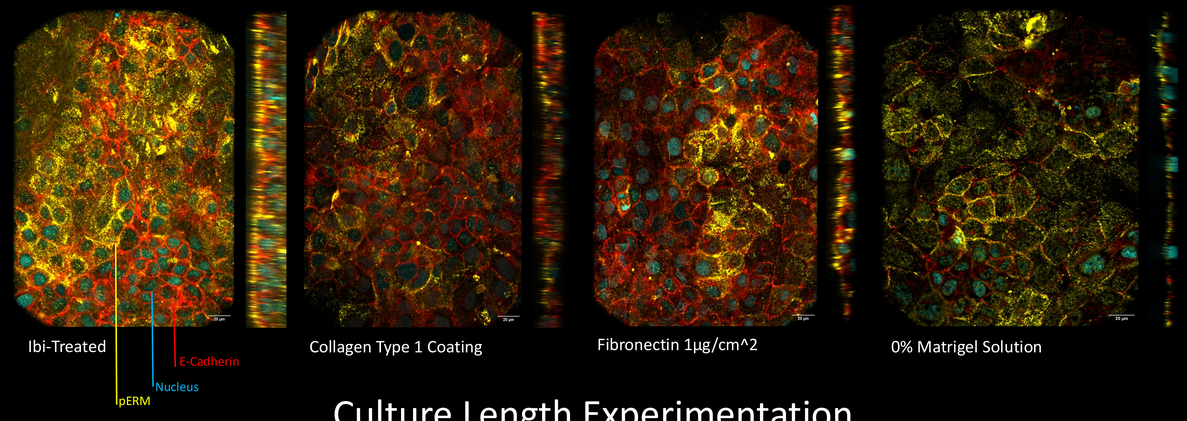
Apical-Basal Polarization



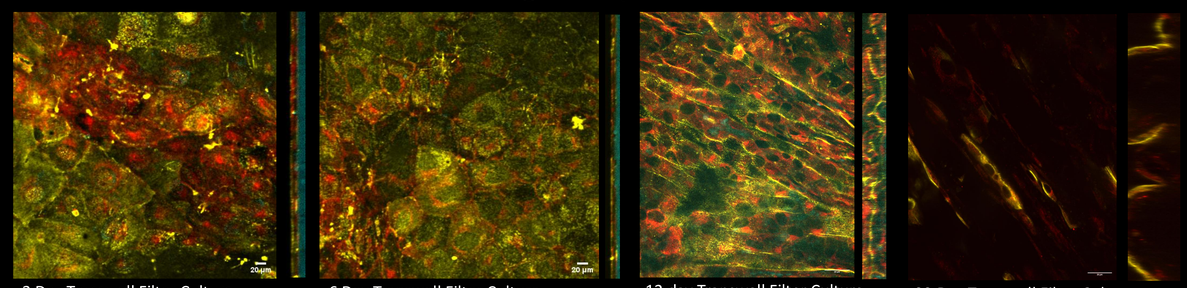
Apical-basal polarity is the asymmetric distribution of intracellular organelles as well as plasma membrane domains including apical and basolateral membranes, and plays an essential role in the formation of the epithelial-specific sheet architecture. The epithelial sheet structure depends mainly on the transmembrane protein E-cadherin on adherens junctions, whereas the apical microvilli structures rely on the expression and activity of Ezrin/Radixin/Moesin (ERM).

Data Analysis

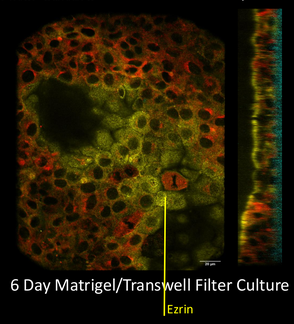
Culture Medium Experimentation



Culture Length Experimentation

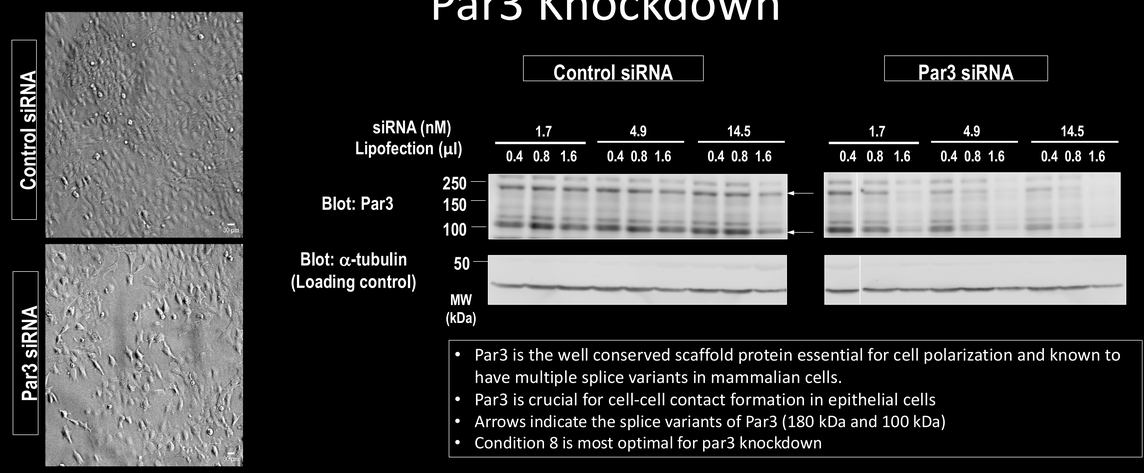


Initial Culture System Conditions
- Type 1 collagen coating
- Ibi-treated collagen non-coating
- Fibronectin 1 µg/cm^2
- Fibronectin 5 µg/cm^2
- 0%, 2%, and 5% Matrigel solutions



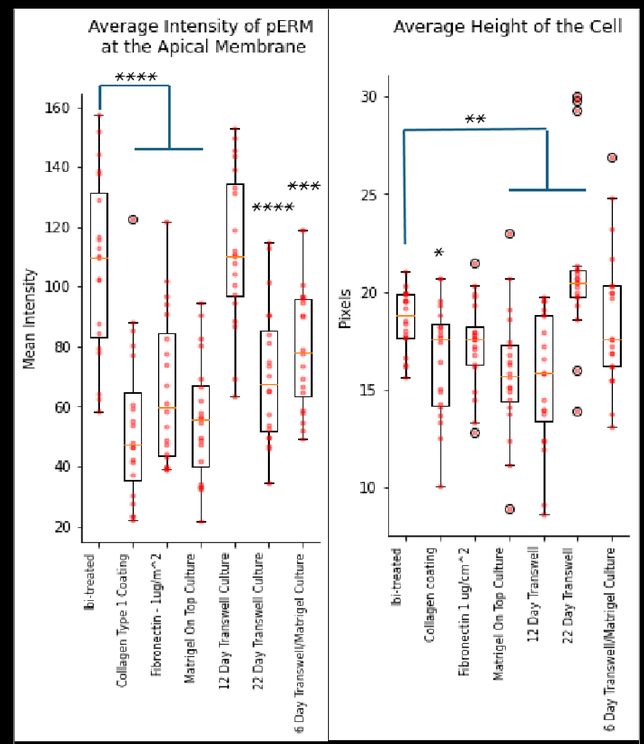
Multi-Day Transwell Filtration Trials
- 1, 3, 6, 12, 18, 20, and 22 day cell culture in transwell filter
- 6 Day Transwell filter culture in conjunction with a matrigel coating
- Matrigel "on top method"

Par3 Knockdown



- Par3 is the well conserved scaffold protein essential for cell polarization and known to have multiple splice variants in mammalian cells.
- Par3 is crucial for cell-cell contact formation in epithelial cells
- Arrows indicate the splice variants of Par3 (180 kDa and 100 kDa)
- Condition 8 is most optimal for par3 knockdown

Quantification



Statistical Analysis

Kruscal Waillis P-Value: 2.96*10^-11
U Whitney-WillCoxsan (Pairwise)
- ib-treatment is statistically different from all other conditions except 12 day Transwell filter

Height P-Value: 1.25e-6
- ibi-treatment is statistically different from 2, 4, 5, 6
- 12 day Transfell filter is different from 1, 3, 6, 7

Conclusions

Based upon the fluorescent intensity of phosphorylated ERM, which indicates the activity of the protein, the ibi-treated cell culture system and the 12 day transwell filter are most optimal for the development of apical-basal polarity. Furthermore, the ibi-treated system created columnar cells which were taller, on average, than the other culture systems. While ibi-treating and transwell filter were best for optimizing apical-basal polarization, it was also interesting to analyze the cellular architecture of the 12-day and 22-day transwell filter culture system. I observed a folded structure that may reflect the intestinal villi, which was upheld beyond the 12 day system into 22 days. With regards to the optimizing the RNAi method using a siRNA targeting the polarity protein Par3, condition 8 was optimal for desired mRNA depletion.

Acknowledgements

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