A PHYSIOLOGICALLY RELEVANT IN VITRO GUT BARRIER MODEL

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Introduction

The intestinal epithelium forms a highly selective barrier that regulates nutrient absorption and drug uptake, while preventing pathogen entry. Drug transport across this barrier occurs via transcellular and paracellular pathways. Paracellular transport is governed by tight junctions, whose integrity can be assessed using trans-epithelial electrical resistance (TEER) measurements[1]. The Caco-2 cell line, which is derived from human colorectal adenocarcinoma, is widely used as an in vitro gut barrier model; however, its TEER values (often 500–1000 Ω·cm²) are substantially higher than those measured in vivo in the small intestine (50–100 Ω·cm²), limiting physiological relevance [2].

Native intestinal mucus plays a crucial role in modulating barrier properties and drug diffusion[3]. This project, therefore, explored whether adding a mucus layer to Caco-2 monolayers could better replicate in vivo conditions for an in vitro model, enabling more accurate modelling of processes such as drug uptake.

Objective

To determine whether applying purified pig intestinal mucus to Caco-2 monolayers can reduce TEER values to in vivo intestinal levels (50–100 Ω·cm²), which would provide a more physiologically relevant in vitro gut barrier model.

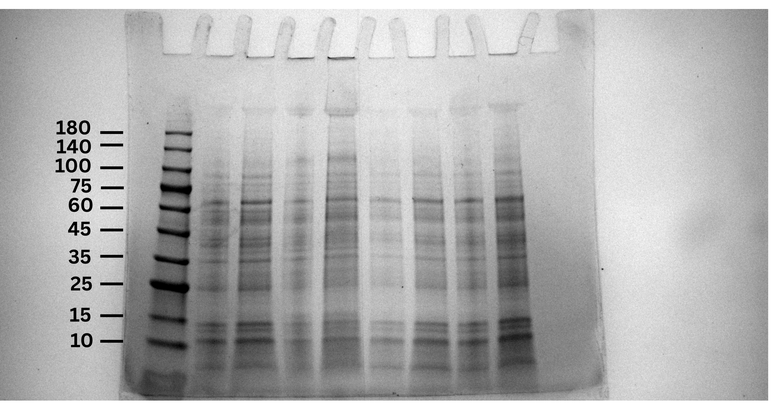

Figure 1: An imaged SDS-page gel, annotated with molecular weight markers

Methodology

01 Caco-2 cells were cultured under standard tissue culture conditions until confluent monolayers formed (21 days).

02 Native pig intestinal mucus was isolated and purified following a modified density-gradient centrifugation method[4]. The mucus was dialysed against membranes with different molecular weight cut offs. The 3 samples prepared were dialysed against: 12-14 kD, 100 kD, and both 12-14 & 100 kD.

03 The protein contents of the mucus and its dialysate (obtained during the purification process) were characterized using native SDS-PAGE[5].

04 Purified mucus was applied to the apical surface of the Caco-2 monolayers. 3 different mucus samples were used, which had been dialysed against different molecular weight cut off membranes.

05 TEER was measured hourly for 4 hours using an EVOM2 voltammeter to assess the cell tight junctions before and after mucus application.

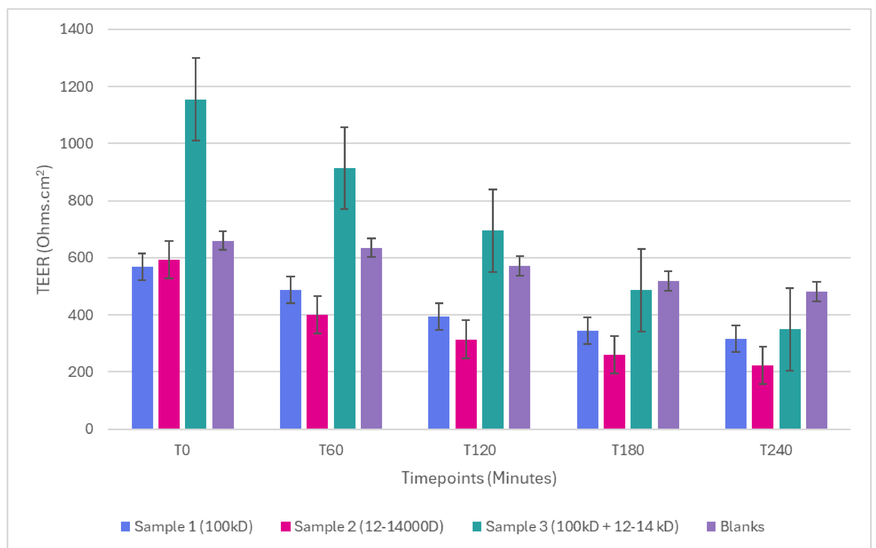

Figure 2: The change in TEER value over time

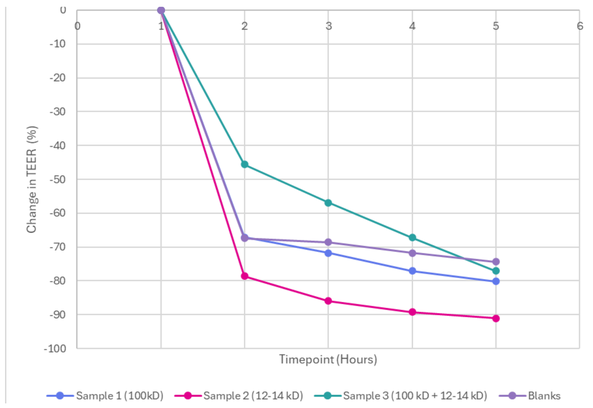

Figure 3: The percentage change in TEER value over time

Results and Analysis

- All conditions exhibited progressive decreases in TEER over time (greater negative % change at later timepoints) (see Figure 3). This indicates a reduction in the resistance of the epithelial barrier following the experimental procedure, suggesting change in the tight junctions
- The 12–14 kD fraction (Sample 2) consistently produced the greatest TEER reduction at every measured time point (≈ −86 to −91%) (see Figure 3), suggesting that low-molecular-weight mucus components (or associated contaminants/enzymes) have a pronounced impact on barrier integrity.
- The combined fraction (Sample 3) produced the smallest reduction (the least negative % change) (see Figure 3). It tended to mitigate the TEER loss relative to the smaller fraction and, at early timepoints, showed less decrease than the blanks.
- Variability (SD) is highest for Sample 2 and Sample 3 at later timepoints (see Figure 2), indicating heterogeneity between biological replicates or sample preparations.
- SDS-PAGE on the mucus fractions and dialysates demonstrated multiple protein bands across samples (incl. high-molecular-weight bands consistent with mucins and lower-molecular-weight bands) (see Figure 1). However, the gel does not identify which specific protein(s) correlate with the TEER changes, and those likely to be responsible for regulating the tight junctions.

Conclusion

Application of purified pig intestinal mucus fractions to Caco-2 monolayers produced a time-dependent reductions in TEER (see Figure 2). The data indicates that mucus composition, not simply mucus presence, strongly influences measured barrier properties. Protein identity is unresolved. Research to identify the molecules responsible for tight junction regulation will be continued including: repeats of the experiment with improved conditions to get enough data to statistically test, mass spectrometry to identify proteins present in the different samples of mucin, and further SDS page of a range of fractions in the mucus preparation stage.

Key Sources

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