

Development of an *in vivo*-like *in vitro* Intestinal Epithelial Model

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INTRODUCTION: The gastrointestinal tract is one of the most important and convenient routes for drug administration. However current models for drug testing, mostly based around the use of established cell lines, are unrealistic of the multicellular structure of the intestine *in vivo*. According to some previous work with the intestinal epithelial cell line HCA-7, transepithelial resistance (TER) is increased in co-culture growth with intestinal myofibroblasts¹. Here we investigated changes in TER over an extended period of time and assessed the affect of co-culture growth at the gene expression level. A further intestinal cell line, Caco-2, was also investigated for its potential in the system. Additional primary intestinal cells were isolated from mice and the growth of these in co-culture was assessed.

METHODS: Isolation of myofibroblasts: Colonies of human myofibroblasts were established from human lamina propria cultured for 4 weeks². **Cell Lines:** HCA-7 and Caco-2 cells were maintained in culture in 10% FCS-DMEM. **Isolation of Primary Intestinal Cells:** Colons were excised from adult male mice. Luminal contents were flushed out using HBSS and the tissue was cut into 2mm² segments³. Collagenase-dispase digestion followed by a sedimentation procedure was used to produce a pure crypt preparation. **Co-culture:** Co-cultures were established on poly(ethylene terephthalate) filter supports. Myofibroblasts were trypsinised and resuspended in 10% FCS-DMEM. This cell suspension was seeded onto the inverted filter insert and allowed to adhere for 24 hours³. The filters were turned and grown in wells containing 10% FCS-DMEM. Epithelial cells or primary intestinal cells were seeded on the opposite side of the filter at a density of 3x10⁵ cells/ml. **TER Measurements:** An Epithelial Voltometer (World Precision Instruments) was used to measure TER across the membrane. **DNA Microarray:** Microarray analysis was carried out using epithelial RNA following co-culture.

RESULTS: Co-cultures of epithelial cell lines and intestinal myofibroblasts showed an enhanced TER by comparison with those in monoculture.

Intact crypts were successfully isolated from the intestinal tissue over a period of 1 – 2 hours of digestion. Spreading of the crypts in this system was observed. Cell ‘clusters’ rather than crypts were present after only 15 hours of culture. These were then maintained for periods of up to 3 weeks. Initial studies suggest that there is an interaction between the two cell types present in the co-culture.

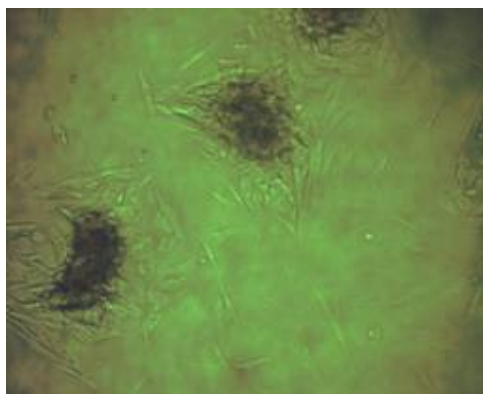


Fig. 1: Growth of intestinal crypts in co-culture with human myofibroblasts

DISCUSSION & CONCLUSION: The co-culture of primary intestinal crypts with intestinal myofibroblasts represents a novel way of recreating the intestinal environment *in vitro*. This system can be expanded upon in the future to create a 3D model of the intestine that can be used for pharmaceutical and potentially even medical benefits.

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