Caco-2 Intestinal Epithelial Model Utilizing Collagen Based Substrates Patterned with Crypt-like Topography

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Statement of Purpose: Caco-2 cell, a human colonic cell line, spontaneously differentiates into a small intestinal epithelial cell-like phenotype and represents a useful tool for studying oral drug absorption across intestinal barrier[1]. However, the current Caco-2 model, which often involves culturing cells on a porous polycarbonate (PC) membrane, fails to predict intestinal permeability of some drugs, mainly due to lack of expression of key proteins, such as drug metabolism enzymes (e.g., cytochrome P450 3A4) and drug efflux transporters (e.g., breast cancer related protein (BCRP)), and tighter junctions between cells[2]. Modification of the Caco-2 cell model by developing a more biologically relevant cell culture substrate would enable better drug absorption prediction and be highly beneficial to drug discovery and development.

Enterocytes rest on basement membrane (BM), which is mainly composed of laminin (Ln), type IV collagen, and fibronectin (Fn); and an interstitial matrix, which is mainly composed of type I collagen[3]. Topologically, the intestinal extracellular matrix is folded into finger-like projections (villi) and well-like invaginations (crypts, ~100 μm in height, and ~ 50-100 μm in diameter) between the villi[4]. The migration, proliferation, differentiation, and function of small intestinal epithelial cells vary with position relative to crypt-villus structures. In this study, a type I collagen based small intestinal cell culture substrate with biomimetic (intestinal crypt-like) topology was fabricated. The substrate was coated with BM protein (i.e. laminin, fibronectin) and used as a culture substrate for up to 21 days. Caco-2 adhesion, proliferation, differentiation, and tight junction formation were studied.

Methods: A type I collagen based cell cultured substrate was fabricated by soft lithography (Fig. 1). Briefly, a poly-dimethylsiloxane (PDMS) mold with micro-well array was fabricated. A 5 mg/ml collagen type I solution was deposited on top of the PDMS mold and allowed to air dry overnight. The resulting collagen membrane was lifted off and thermo-crosslinked. After adhering to a circular insert, the collagen substrate was sterilized with 70% ethanol, washed with PBS, and then coated with either Fn (50 μg/ml) or Ln (10 μg/ml).

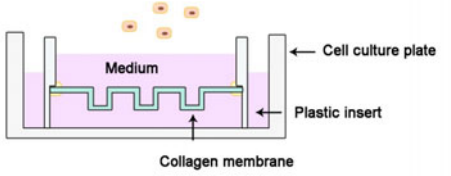

Figure 1. Collagen based intestinal cell culture system with biomimetic topography.

Caco-2 cells were cultured at 37°C and 5% CO_2 for 3-21 days. The adhesion and viability of Caco-2 cells were assessed using fluorescence microscopy by labeling cell nuclei with propidium iodide, or cells with Live/Dead dye. The metabolic activities were assessed by MTT assay and normalized by total cell count. Cell differentiation was measured by monitoring the activities of two cell differentiation markers, alkaline phosphatase (ALP), and alanine aminopeptidase (AAP). The tightness of cell-cell junctions was assessed by measuring trans-epithelial electric resistance (TEER).

Results: A collagen based intestinal cell culture substrate with both micron scale intestinal crypt-like topography (50, 400 μm in diameter, ~80 μm in depth), as well as nanometer scale fibril and porous topography was fabricated. Caco-2 cells form loosest cell-cell junctions when cultured on patterned collagen based substrate as comparing to flat collagen, and PC substrate (Fig. 2). It was found that Caco-2 cells maintain good cell layer integrity after culture for 21 days on Ln coated collagen (TEER value of monolayer is 400 Ω cm^2), while Caco-2 cell layers cultured on Fn coated collagen started to disintegrate at day 14, as TEER value decreased from 360 Ω cm^2 at day 14 to 200 Ω cm^2 at day 21. It was also found that Caco-2 cells attached better and maintained higher viability on LN coated collagen than on Fn coated collagen.

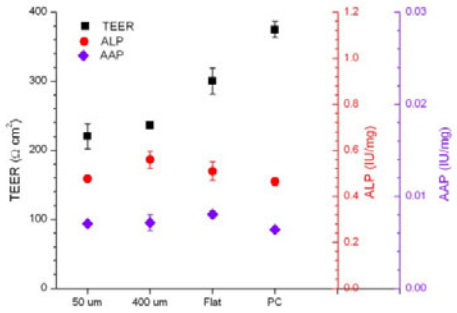

Figure 2. TEER, ALP activities, and AAP activities of Caco-2 cell layer grown on collagen membrane patterned with 50 um or 400 um wells, and also coated with laminin for 21 days.

Caco-2 cells cultured on Ln coated substrate were more differentiated than cells cultured on Fn coated substrate. Cells cultured on Fn coated collagen substrate reached peak ALP and AAP activities at day 7 in culture, while cells cultured on Ln coated collagen substrate reached peak ALP and AAP activities at day 21 in culture. Crypt-like topography was found affecting AAP activities, but not ALP activities of cells grown on Ln coated collagen (Fig. 2). The MTT assay results suggested that cells reached maximum metabolic activity around day 7 on Fn coated collagen substrates, while cells reached maximum metabolic activity at day 3 on Ln coated collagen substrate.

Conclusions: In summary, it was found that both topography and extracellular matrix protein coating (i.e. LN and FN) significantly affect Caco-2 behavior on collagen substrates. Micron-scale crypt-like topography has an effect on Caco-2 cell differentiation, as well as tight junction formation.

Reference: [1] Delie F. and Rubas W., Crit Rev Ther Drug Carrier Syst. 14(3), 221,1997. [2] Waterbeemd H.D., Lennernas H, and Artursson P., J Pharm Sci, 79(6), 1990. [3] Teller I.C. and Beaulieu J.F., Expert Rev Mol Med, 3(24), 1, 2001. [4] Weiss L., Histology, cell and tissue biology, Elsevier, 1983.