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Effects of ECM Proteins on Barrier Formation in Caco-2 Cells

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CORNING

Introduction

In vivo, several types of epithelial cells exhibit polarized morphology when fully mature. Enterocytes, the absorptive cells of the intestine, are one example of this type. Enterocytes are responsible for the uptake of material from the intestinal lumen, and it is the differential permeability of these cells to various materials that control absorption. The apical surface of the enterocyte faces the interior of the gut and is characterized by numerous microvilli which increase the surface area available for transport. The basal surface, which faces away from the interior of the gut, is in contact with extracellular matrix (ECM). This ECM plays a complex role in a number of cellular functions, including biogenesis of cell polarity and cellular differentiation. Differentiated intestinal epithelia have been used extensively as a model system for studying a number of phenomena including: intestinal transport (i.e., drugs, cholesterol), mechanisms of bacterial and viral infection, and enzyme induction and regulation. The majority of studies being done on transport, infection and enzyme regulation utilize established intestinal epithelial cell lines, such as Caco-2 cells. This model has some disadvantages, including the requirement for medium supplementation with animal serum and the lengthy culture periods necessary to achieve an enterocyte-like differentiated phenotype. Using Caco-2 cells, the establishment of mature barrier and transport functions *in vitro* has been reported to take between two to four weeks. We report that ECM proteins and other key media components affect the rate at which Caco-2 cells differentiate. Using a unique three-dimensional ECM substrate and an optimized serum-free medium, the culture time required to form a tight monolayer with barrier function can be decreased from 2-4 weeks to 3 days.

Materials and Methods

Cells and Culture Conditions: Caco-2 cells were purchased from ATCC and routinely maintained on Falcon® tissue culture plates (Corning) in DMEM (Gibco) with 20% Fetal Bovine Serum (KC Biological) with gentamycin (BioWhittaker). Flasks which were 90% confluent were harvested with trypsin/EDTA, neutralized with serum-containing medium, and centrifuged. The cell pellet was resuspended in a serum-free medium consisting of DMEM and Mito+ Serum Extender (Corning) and seeded onto 24-well size Falcon Cell Culture Permeable Supports (Corning) at $6x10^5$ cells/cm^2 (200,000 cells/permeable support). The permeable supports contain a track-etched polyethylene terephthalate (PET) 1.0 µm microporous membrane with fibronectin, laminin, collagen type I, or fibrillar collagen type I. The medium was replaced every 48 hours. The seeding medium was replaced 24 hours after cell seeding with optimized differentiation medium in experiments which required the use of differentiation medium; the medium was replaced every 48 hours thereafter.

TEER Measurements: Transepithelial electrical resistance (TEER) was measured utilizing the Endohm Tissue

Resistance Measurement Chamber (World Precision Instruments) at the time indicated. Measurements were corrected for baseline readings (permeable supports without cells) and were reported as ohm*cm2.

Mannitol Permeability Measurements: Apical to basal permeability assays were conducted as follows:

1. 1 ml of DPBS was added to each well of a 24-well plate.
2. The permeable support was placed in the well (permeable support housing) and 300 µl of test substance was added to the upper compartment of the permeable support.
3. At predetermined time intervals, the permeable support was shifted to a new well containing fresh DPBS and the concentration of the test substance in the first well determined by scintillation counting or spectrophotometry.

Basal to apical permeability assays were conducted as follows:

1. 1 ml of DPBS was added to each well of a 24-well plate.
2. The permeable support was placed in the well (permeable support housing) and 300 µl of test substance was added to the lower compartment of the permeable support.
3. At predetermined time intervals, 200 µl was removed from the upper compartment of the permeable support and immediately replaced with fresh DPBS.
4. The permeable support was shifted to a new well containing 1 ml of test substance.
5. The concentration of test substance in the sampled material was determined by scintillation counting or spectrophotometry.

Test materials were dissolved in DPBS. Permeability coefficients were calculated by the formula given below:

$$\left(\frac{\text{Volume Receptor Chamber}}{\text{Area Monolayer}^* \times \text{Concentration in Donor Chamber}}\right)\left(\frac{\text{Concentration Change in Receptor Chamber}}{\text{Change in Time}}\right)$$

SEM Analysis: Scanning electron micrographs were prepared as described (Technical Bulletin #405, available from Corning upon request).

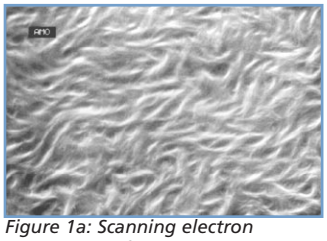


Figure 1a: Scanning electron micrograph of rat tail collagen type I (5,000x). Note the apparent amorphous structure of collagen.

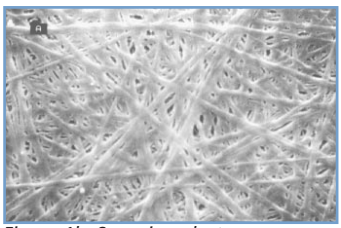


Figure 1b: Scanning electron micrograph of fibrillar collagen type I (5,000x). Note the organized structure with large collagen fibers.

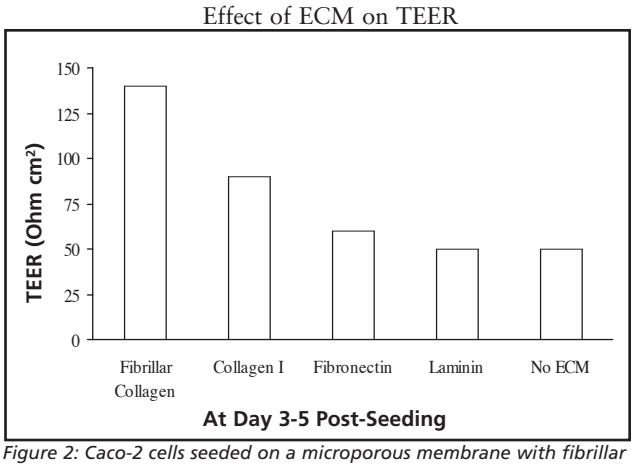


Figure 2: Caco-2 cells seeded on a microporous membrane with fibrillar collagen type I formed a monolayer with higher TEER readings than cells on membranes with other ECMs or without ECM (during the first week post-seeding). The results suggest that barrier formation by Caco-2 cells is accelerated on fibrillar collagen.

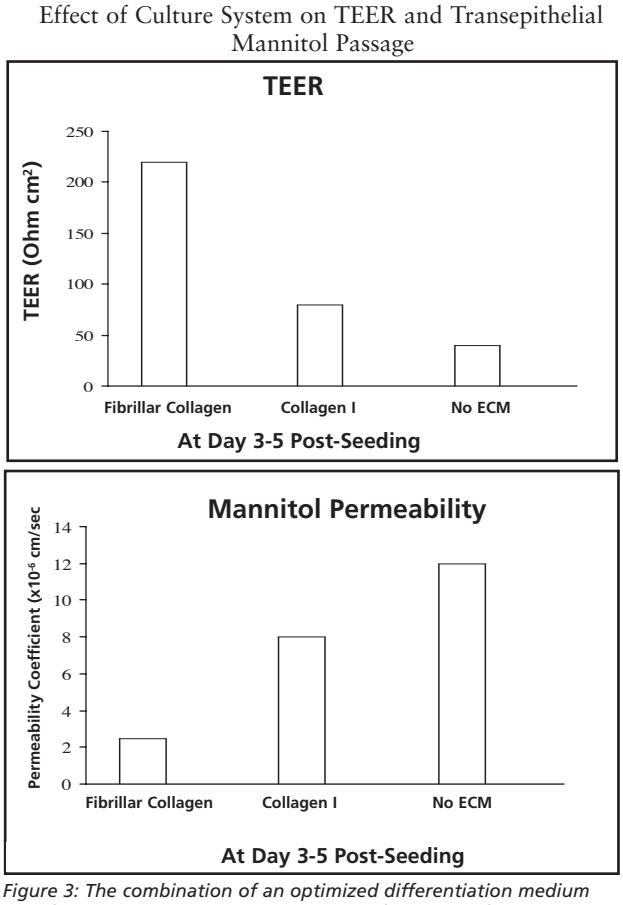
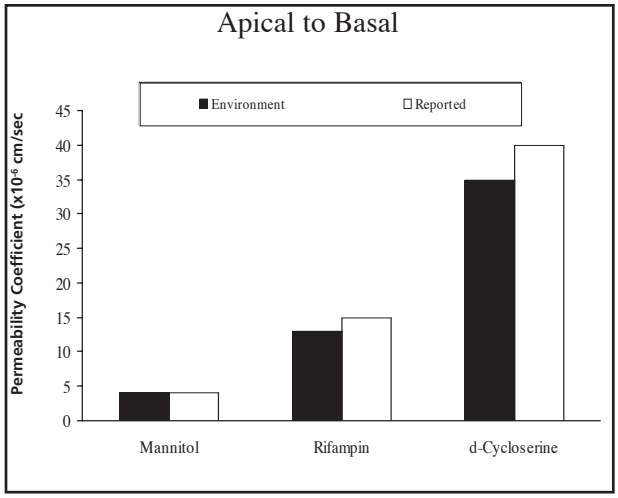


Figure 3: The combination of an optimized differentiation medium with fibrillar collagen resulted; in the rapid formation of a tight Caco-2 monolayer. Mature TEER readings ([top] >150 ohm cm²) and mature permeability coefficients ([bottom] <4x10⁻⁶ cm/sec) were achieved in 3 days, versus 2-4 weeks reported in the literature for cells cultured on amorphous collagen or without ECM[1,2].



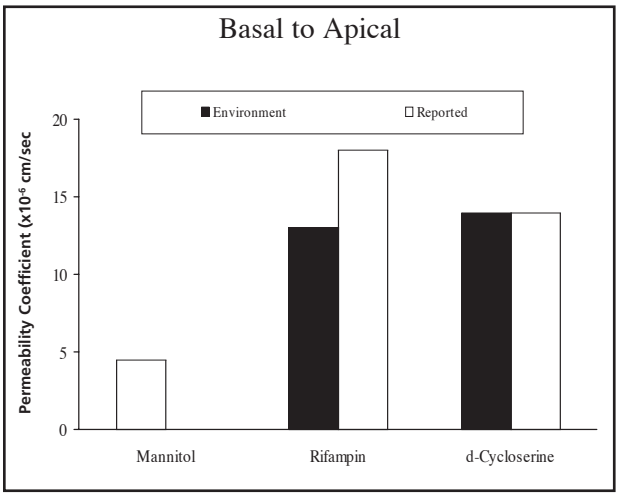


Figure 4: Permeability coefficients (Apical to Basal [top] and Basal to Apical [bottom]) for several compounds were determined for Caco-2 monolayers cultured in the environment and compared to those reported in the literature[1,2]. While the reported valued required culture time of 2-4 weeks (empty bars), we achieved similar values with the environment in only 3 days (solid bars).

Effect of Fiber Structure on Transepithelial Mannitol Passage

Amphoruous Collagen

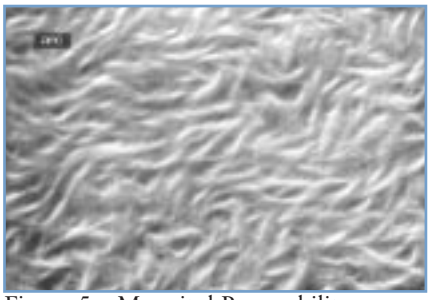


Figure 5a: Mannitol Permeability Coefficient = 5.7×10^{-6} cm/sec.

Small Collagen Fibers

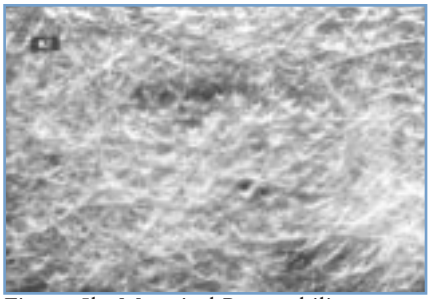


Figure 5b: Mannitol Permeability Coefficient = 4.4×10^{-6} cm/sec.

Large Collagen Fibers

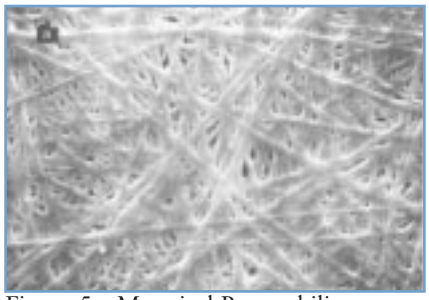


Figure 5c: Mannitol Permeability Coefficient = 2.5×10^{-6} cm/sec.

Figure 5: To examine the effect of fiber structure on mannitol permeability, a series of fiber sizes were produced by varying the manufacturing process of fibrillar collagen. Mannitol permeability coefficients were determined 3 days post-seeding. The results indicate that a gradual decrease in mannitol permeability is correlated with a gradual increase in collagen fiber sizes suggesting that large fibers promote rapid monolayer formation by Caco-2 cells.

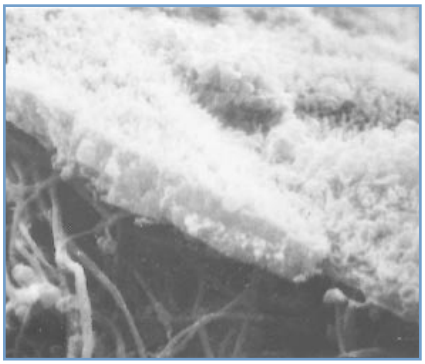


Figure 6: Scanning electron micrograph of Caco-2 cells grown on a 1 µm microporous membrane containing fibrillar collagen type I. Note the numerous microvilli on the apical surface and the large collagen fibers on the basal surface.

Conclusion

- Fibrillar collagen accelerates the development of a tight monolayer by Caco-2 cells, as determined by TEER and mannitol permeability measurements.
- The Corning® BioCoat™ Intestinal Epithelial Differentiation Environment (a combination of fibrillar collagen and an optimized, serum-free differentiation medium) promotes the development of mature barrier 3 days postseeding, as opposed to 2-4 weeks.
- The structure of the three-dimensional fibrillar collagen matrix is crucial to achieve accelerated monolayer formation by Caco-2 cells.

Discussion

Differentiation of Caco-2 cells can be influenced by interactions with other cells, ECM or soluble factors. The effect of cell-matrix interactions on Caco-2 cell differentiation, in particular on barrier formation, has been determined by TEER and mannitol permeability measurements. The extent of differentiation was dependent not only on the type of ECM (**Figure 2**), but also on the structure of the collagen type I fiber (**Figure 5**). The importance of cell-solute interactions has also been demonstrated (**Figures 3 and 4**): in addition to an optimized ECM, an optimized medium further accelerated differentiation, compared to routinly used culture conditions. The Corning BioCoat Intestinal Epithelium Differentiation Environment can be used to construct an *in vitro* intestinal model for a number of applications. The collagen-containing membranes are easily removed from their housing (e.g., for scintillation counting) and can be processed by standard procedures for either light or electron microscopy. Cells grown on fibrillar collagen can be recovered by conventional methods (such as trypsin treatment) for further biochemical anlayses.

Applications:

- Exploration of transepithelial passage of orally administered drugs to help predict bioavailability of new therapeutic agents in humans[1-3]
- Studies of intestinal transport mechanisms for various nutrients, metabolites and trace elements, and of compounds that interfere with this process[4-6]
- Investigation of the mechanisms involved in the entry, replication, and expression of infectious agents of the intestinal tract[7]
- Studies of structural and/or functional changes in the intestinal epithelium associated with diseases, such as Crohn's disease, rheumatoid arthritis, or drug-induced intestinal inflammation[8-10]

References

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