

Morphological Comparison of Caco-2 Cells in the Corning® BioCoat™ Intestinal Epithelial Cell Environment and the Traditional 21-day Caco-2 Culture System

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Introduction

We have compared Caco-2 cells cultured in the conventional 21-day system with Caco-2 cells cultured in the Corning BioCoat Intestinal Epithelial Cell Environment by transmission electron microscopy to identify and compare the ultrastructural characteristics of a differentiated monolayer of Caco-2 cells.

Historically, Caco-2 cells have been utilized in tissue culture to study compound transport. Recently, there has been an increased interest in utilizing the Caco-2 cell line as an *in vitro* model for screening drug candidates for their intestinal absorption potential. The conventional Caco 2 tissue culture model takes approximately 21 days to display the characteristics of a differentiated Caco-2 cell monolayer to perform as a transport barrier. Morphological comparison of the two systems shows that the 3-day Corning BioCoat Intestinal Epithelial Cell Environment has similar ultrastructural characteristics of the 21-day Caco-2 culture system.

Recently, the Corning BioCoat Intestinal Epithelial Cell Environment had been described as a useful alternative to the traditional 21-day Caco-2 culture system to assess a potential drug candidate's intestinal absorption characteristics.

To further characterize the Corning BioCoat Intestinal Epithelial Cell Environment and compare its performance to the traditional 21-day Caco-2 culture system, a series of electron micrographs were generated from samples of both the Caco-2 culture systems. Several morphological indicators of Caco-2 cell differentiation were examined in both of the systems. Cells were examined for the presence of microvilli-like surface specialization, indicative of Caco-2 cell differentiation to an enterocyte-like phenotype. In addition, cells were examined for cell/cell junctional complexes, and cell/cell interdigitation demonstrating the cell interactions necessary to generate cell monolayers with barrier function to compound transport.

Results indicate that many of the morphological features of Caco-2 cell differentiation found in traditional 21-day Caco-2 culture systems are also found in the Corning BioCoat Intestinal Epithelial Cell Environment. These features include apical micvillar surface specialization, cell/cell junctional complexes, and cellular interdigitation.

Finally, while the traditional 21-day culture system displayed multicellular layering effects, those effects were less apparent in the Corning BioCoat Intestinal Epithelial Cell Environment. So, the Corning BioCoat Intestinal Epithelial Cell Environment retains many of the morphological differentiation characteristics found in the traditional 21-day Caco-2 culture system, while providing for a more efficient system for using Caco-2 cells to generate compound permeability information

Materials and Methods

Cell Culture Conditions: 3-day System - Caco-2 cells were cultured as per manufacturers instructions. Briefly, cells were seeded at 5.0×10^5 cells/cm² in Mito+ Serum Extender supplemented media onto Corning BioCoat Fibrillar Collagen Cell Culture 1.0 µm PET Permeable Supports and incubated for 24 hours. Then, media was changed to Mito+ supplemented Entero-STIM Differentiation Media and incubated for 48 hours. At this point, cultures were rinsed with 2x changes of PBS. Primary fixation was in 2% glutaraldehyde in 0.2 M sodium Cacodylate buffer, pH 7.2 for 1 hour at room temperature. Specimens were rinsed in 0.2 M Sodium Cacodylate buffer, pH 7.2 two times and held in this buffer until processing into resin for electron microscopic evaluation. 21-Day System - Caco-2 cells were plated onto 1.0 µm PET collagen coated permeable supports. Cells were seeded at 50,000 cells/cm² and cultured for 21 days in DMEM + 10% FBS with media changes every other day. After completion of 21 days of culture, the permeable supports were rinsed in 2x with PBS at pH 7.2. Primary fixation was in 2% glutaraldehyde in 0.2 M Sodium Cacodylate buffer, pH 7.2 for 1 hour at room temperature. Specimens were rinsed in 0.2 M Sodium Cacodylate buffer, pH 7.2 two times and held in this buffer until processing into resin for electron microscopic evaluation.

Processing Procedure for Electron Microscopic Evaluation

- Post fixation of specimens was in 2% (wt/vol) OsO₄ in 0.2 M Sodium Cacodylate buffer, pH 7.2. After dehydration through a graded series of ethanol, the specimens were embedded in a firm recipe of Spurr's embedding resin (Polysciences-Warrington, PA).

The following recipe was used in mixing the Spurr's resin for infiltration and embedding:

10 ml - VCD 4Vinyl Cyclohexane Dioxide

6 ml - D.E.R. 736

26 ml - NSA Nonenylsuccinic Anhydride

0.4 ml - DNAE 2-dimethylaminoethanol

Gold sections were obtained using an LKB ultra-microtome. Sections stained with Uranyl Acetate and Lead Citrate were examined in a Hitachi 7100 transmission electron microscope.

Results and Discussion

As seen in **Figure 1**, the ultrastructural components of the Caco-2 cells in the 3-day Corning® BioCoat™ Intestinal Epithelial Cell Environment are consistent with differentiated barrier monolayer of Caco-2 cells. The differentiation characteristics of surface specialized microvilli, tight junction formation and interdigitation of the cell membranes are readily apparent. The presence of those morphological markers is indicative of a differentiated Caco-2 cell phenotype. **Figure 2** is a higher power micrograph Caco-2 cells cultured in the 3-day Corning BioCoat Intestinal Epithelial Cell Environment to better illustrate the morphological characteristics of tight cell/cell junctions (esp. desmosomes) present at the 3-day period. **Figure 3** is a micrograph of Caco-2 cultured cells using the conventional 21-day method. The Caco-2 cells display surface specialized microvilli, interdigitation of cell membranes, tight junction formation, multiple cell layers are present. **Figures 4 and 5** are high-power micrographs of the 3-day Corning BioCoat Intestinal Epithelial Cell Environment and 21-day Caco-2 cell culture conditions, respectively. In comparison, both sets of culture conditions allow for development of cell/cell junctional complexes which are indistinguishable.

Conclusions

The 3-day Corning BioCoat Intestinal Epithelial Cell Environment supports the timely differentiation and effective barrier function of Caco-2 cells. We have shown that the 3-day Corning BioCoat Intestinal Epithelial Cell Environment allows Caco-2 cells to mimic many of the morphologic characteristics of the 21-day systems. Results indicate that many of the morphological features of Caco-2 cell differentiation found in the traditional 21-day system are also found in the Corning BioCoat Intestinal Epithelial Cell Environment. These features include apical microvillular surface specialization, cell/cell junctional complexes, and cellular interdigitation. Finally, while the traditional 21-day system displayed multicellular layering effects, those effects were less apparent in the Corning BioCoat Intestinal Epithelial Cell Environment. So, the Corning BioCoat Intestinal Epithelial Cell Environment retains many of the morphological differentiation characteristics found in the traditional 21-day system, while providing for a more efficient system for using Caco-2 cells to generate compound permeability information.

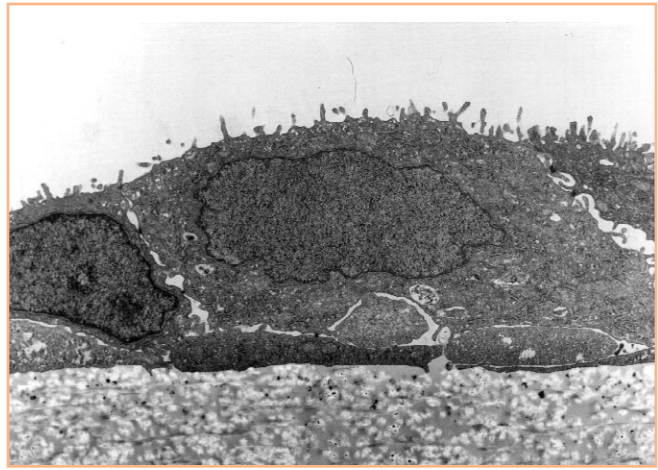


Figure 1: Electron micrograph of a Caco-2 cell monolayer cultured using the 3-day with the Corning BioCoat Intestinal Epithelial Cell Environment. Surface specializations of microvilli, interdigitation of cell processes, and tight junction formation (desmosomes) are readily apparent in this differentiated monolayer. Previous work has shown that Caco-2 cells cultured in the Corning BioCoat Intestinal Epithelial Cell Environment function as a differentiated Caco-2 cell barrier functioning monolayer. (Magnification: 14,400x.)

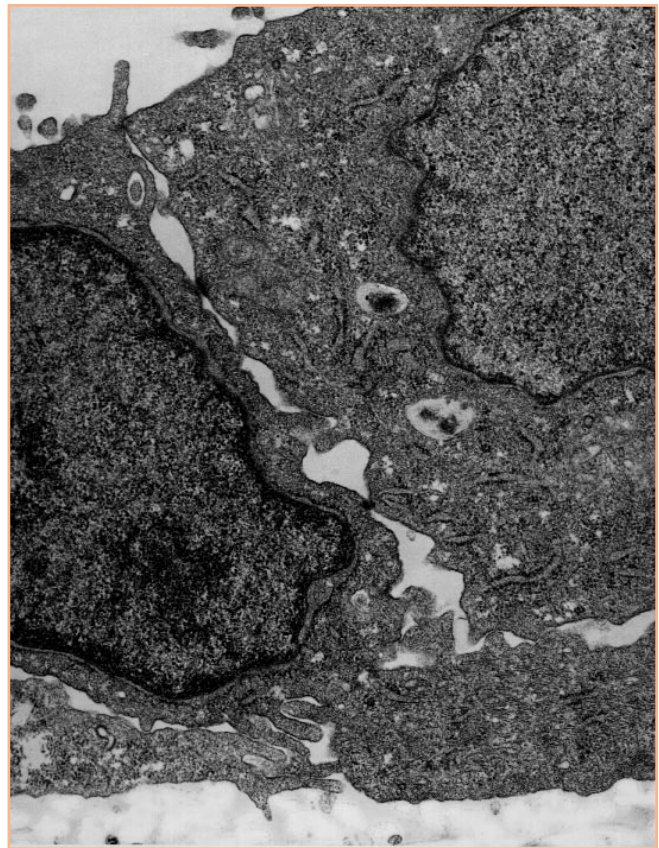


Figure 2: High power micrograph of Caco-2 cells cultured in the Corning BioCoat Intestinal Epithelial Cell Differentiation Environment. This micrograph illustrates surface specialization of microvilli, tight junction formation between cells and interdigitation of cell processes. These morphological features are indicative of differentiated Caco-2 cells capable of forming a barrier functioning monolayer. (Magnification: 41,000x.)

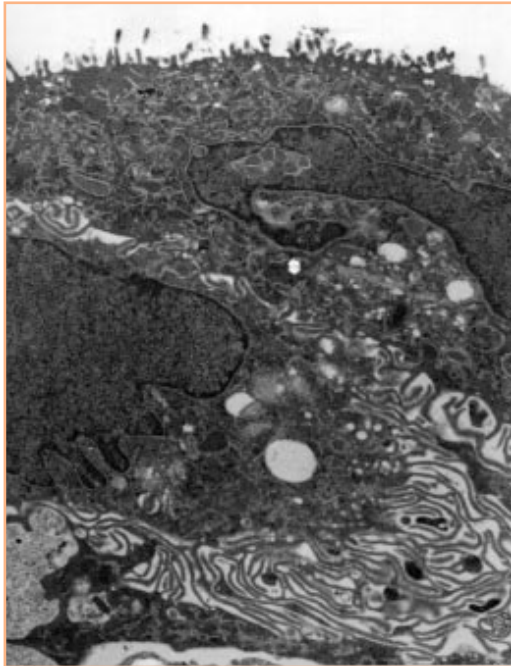


Figure 3: Representative micrograph of Caco-2 cells cultured in the conventional 21-day system. Please note the surface specialization's of microvilli, the extensive inter-digitization of cell processes and the presence of tight junctions. There is extensive piling or multilayers of cells present in the 21-day culture. (Magnification: 14,400x.)



Figure 4: A highpower micrograph of a 3-day Caco-2 cell culture illustrating cell-to-cell junctions and cellular interdigitation achieved in the Corning® BioCoat™ Intestinal Epithelial Cell Environment. (Magnification: 21,200x.) Insert: This illustration shows the fibrillar collagen fibrils that comprise the coating on PET 1.0 μm membrane. Notice the wide spaced collagen fibrills and the periodicity of the collagen. (Magnification: 15,800x.)

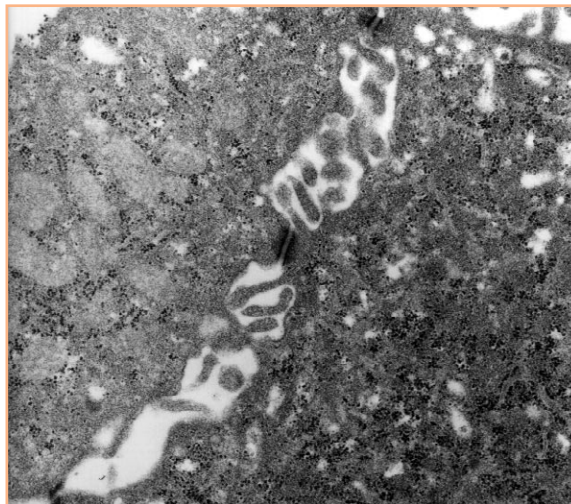


Figure 5: A high-power micrograph of a conventional 21-day Caco-2 cell culture illustrating the cell-to-cell junctions and cellular interdigitation. (Magnification: 41,400x.)

Corning acquired the BioCoat™ brand.
For information, visit www.corning.com/discoverylabware.

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