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Comparison of the BD BioCoat™ Intestinal Epithelial Environment with the Conventional 21-Day Caco-2 System

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Introduction

Caco-2 cells are being increasingly used as an in vitro model for screening drug candidates for their intestinal absorption potential. The traditional method of culturing Caco-2 cells requires up to 3 weeks of culture and involves many labor intensive steps. The BD BioCoat Intestinal Epithelial Differentiation Environment (which provides a monolayer of Caco-2 cells ready for compound permeability screening in 3 days) has been reported to be a more convenient and productive system to use with Caco-2 cells to generate compound permeability data1. To further characterize the 3-day System and to compare it's performance with the 21-day Caco-2 System, several biological measures of Caco-2 cell differentiation were tested. Both the 3-day System and the 21-day System were tested for their ability to generate a monolayer of Caco-2 cells, upregulate Alkaline Phosphatase (AP) activity, express brush border peptidase activity, and show functional P-Glycoprotein.

Results indicate that the cell barrier formed in the 3-day System (Papp = 4-6 x 10^{-6} cm/sec) was 3-4 times more permeable to Mannitol than the 21-day System (Papp = 2-3 x 10^{-6} cm/sec). The 3-day System upregulates AP levels comparable to the 21-day System (from 3-6 fold higher than differentiated Caco-2 controls). Both the 3-day System and the 21-day System expressed similar levels of brush border peptidase activity. Both Systems allowed for the expression of P-Glycoprotein activity functioning in the proper directional orientation. So, the 3-day System is comparable to the 21-day System in that it upregulates AP levels and expresses functional P-Glycoprotein activity in the proper orientation.

Therefore, the 3-day System offers a more convenient and productive alternative to the 21-day System to generate compound permeability data while retaining many of the important properties of the 21-day System.

Materials and Methods

Cell Culture: 3-day System (Cat. No. 355057) - Cells were cultured as per manufacturer's instructions. To request a copy of the Guidelines for use, contact Technical Support at labware@bd.com. Briefly, cells were seeded at 4.65 x 10^5 cells/cm^2 in Mito+ Serum Extender onto BD BioCoat Fibrillar Collagen Cell Culture Inserts (1 µm PET) and incubated for 24 hours. Then, media was then changed to MITO+ Serum Extender and incubated for 48 hours. At that time, cells were rinsed 2-3 times with PBS and further analyzed. 21-day System - Inserts (1 µm PET) were coated with type I collagen and dried. Cells were seeded onto the coated inserts at 50,000 cells/cm^2 and cultured for 21 days in DMEM + 10% FBS with media changes every other day. After culturing, cells were rinsed 2-3 times with PBS and further analyzed.

Barrier Function Assessment: To test for barrier function of the Caco-2 monolayer cells cultured in the 21-day System, the 3-day System (with either serum or Mito+ Serum Extender), the undifferentiated Caco-2 System and the 3- day System with only fibrillar collagen were all tested with Mannitol to assess their level of barrier function. These experiments were done by adding a fixed amount of 3H-Labeled Mannitol in PBS to the insert and assessing how much Mannitol was able to diffuse through the monolayer after a fixed amount of time at 22°C. A Permeability Coefficient (Pc) was then calculated and reported as the data in **Figures 1a and 1b.**

Differentiation Markers: To test for markers of differentiation in the 3-day System and the 21-day System, activity of AP, Aminopeptidase A (APA), Aminopeptidase N (APN), and Dipeptidylpeptidase IV (DPP IV) were measured by adding the appropriate enzyme substrates directly to the inserts containing the cells and incubating the cells for a given time interval. AP activity was determined using pNPP and the peptidases were assayed by using the 7-amino-4 methylcoumarin derivatives of each enzyme substrate[2,3]. Results of these experiments are shown in **Figures 2-4**.

P-Glycoprotein Function: P Glycoprotein activity was assessed in the Caco-2 systems4 by placing equal concentrations of vinblastine (10 nm) above and below the cell monolayer. One chamber or the other had a quantity of ^{3}H-Labeled vinblastine added, and the system was incubated for up to 2 hours. Samples were then counted to determine the amount of vinblastine transported from one chamber to the other. In addition, to test for the specificity of transport of the vinblastine, verapamil, a partial antagonist of vinblastine transport was added at 0.1 mm to a group of inserts. The data obtained from these experiments is presented in **Figures 5 and 6**.

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Mannitol Permeability Coefficients of Caco-2 Cells

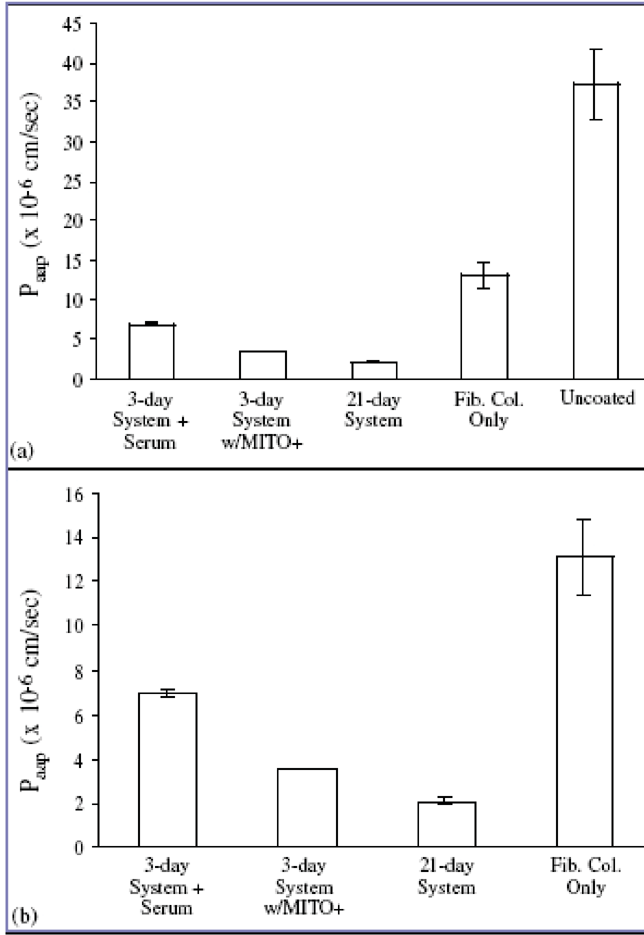

Figure 1a and 1b: "Fibrillar Collagen Only" samples did not use the differentiation media containing butyrate, but used normal growth media instead (DMEM + 10% FBS) for 3 days on BD BioCoat™ Fibrillar Collagen Cell Culture Inserts. Uncoated samples were grown for 3 days with no coating and normal growth media. Other culture conditions were as described above. Data shown is mean of n=3.

Alkaline Phosphatase Activity in Caco-2 Cells

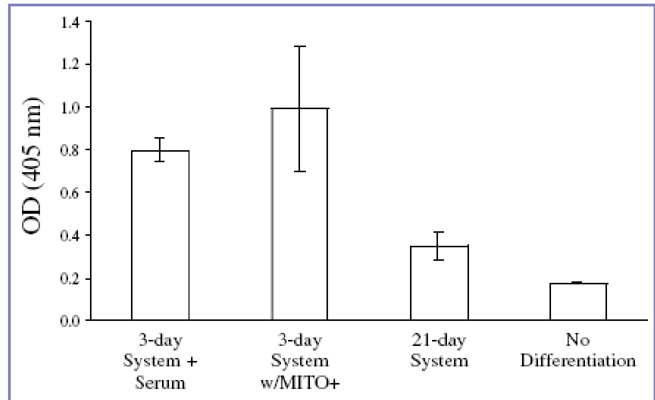

Figure 2: AP activity was assessed in Caco-2 cells grown in various culture conditions. The 3-day System contains BD BioCoat Fibrillar Collagen Cell Culture Inserts and butyrate containing differentiation media, supplemented with either 10% FBS or Mito+ Serum Extender as indicated. The 21-day System utilizes amorphous collagen coated inserts and normal growth media (DMEM + 10% FBS). The no differentiation system utilizes uncoated inserts and normal growth media and cells are cultured for 3 days. AP activity was measured using intact cells grown on inserts. pNPP was used as enzyme substrate and endpoint measurements were made at 20 minutes at 37°C. Data shown is mean of n=4.

Aminopeptidase N Activity in Caco-2 Cells

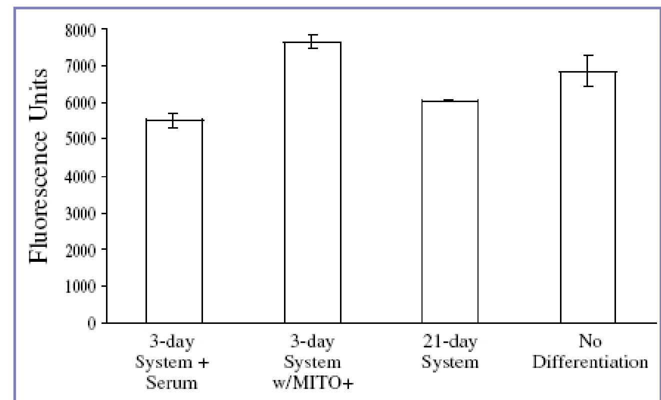

Figure 3: APN activity was assessed in Caco-2 cells grown in various culture conditions. APN activity was measured using intact cells on inserts. APN activity was measured using 200 µM Ala-7-amino-4-methylcoumarin as substrate. Activity was measured by detecting the fluorescence of the liberated 7-amino-4-methylcoumarin (excitation: 370 nm emission: 442 nm). Reactions were carried out for 60 minutes at 37°C. Data shown is mean of n=4.

Dipeptidyl Peptidase IV Activity in Caco-2 Cells

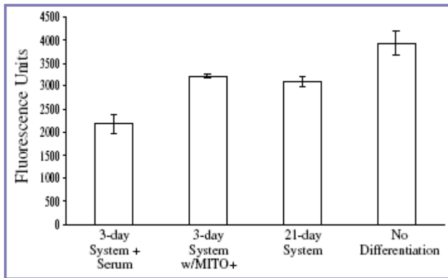

Figure 4: DPP IV activity was assessed in Caco-2 cells grown in various culture conditions. DPP IV activity was measured using intact cells on inserts. DPP IV activity was measured using 200 uM Gly-Pro-7-amino-4-methylcoumarin as substrate. Activity was measured by the fluorescence of the liberated 7-amino-4- methylcoumarin product (excitation:370 nm emission:442 nm). Reactions were carried out for 60 minutes at 37°C. Data shown is mean of n=4.

P-Glycoprotein Function in Caco-2 Cells

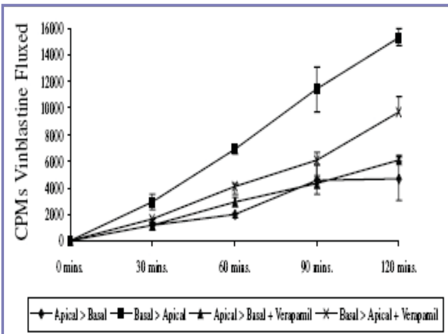

Figure 5: Caco-2 cells were cultured using the 3-day System supplemented with MITO+ Serum Extender. P-Glycoprotein function was assessed by placing 10 nm vinblastine solution in PBS at 22°C both above and below the insert. A small amount of 3H-Labeled Vinblastine was added to either the apical or basal side of the insert and samples were withdrawn from the non-labeled side of the insert and counted by scintillation counting. To inhibit the P-Glycoprotein with verapamil, 100 µm verapamil was added to the insert chambers. Data shown is mean of n=2.

P-Glycoprotein Function in Caco-2 Cells

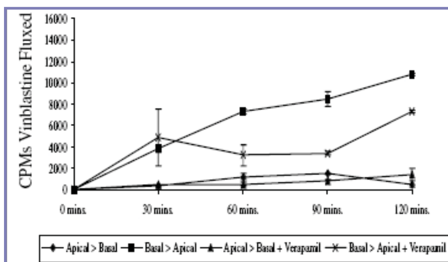

Figure 6: P-Glycoprotein function was measured as described in Figure 5. The culture system used here is the 21-day System as previously described. Data shown is mean of n=2.

Results/Conclusions

As seen in **Figures 1a and 1b**, the Mannitol Pc obtained in the 21-day System are lower (2-3 x 10^{-6} cm/sec) than those found in the 3-day Systems tested (4-6 x 10^{-6} cm/sec). By manipulating various components of the 3-day System, it can be seen that each of the components (BD BioCoat™ Fibrillar Collagen Cell Culture Inserts and Mito+ Serum Extender) contributes to the overall final cell barrier formation. The data in **Figure 2** shows that both the 3-day System and the 21-day System upregulates AP activity relative to the undifferentiated control. In the 21-day System we see about a 2-fold upregulation of enzyme activity. In the 3-day System with serum, we see a 4-fold upregulation of enzyme activity. While in the 3-day System with Mito+ Serum Extender, that upregulation is about 6-fold. So, all of the systems tested upregulate AP to some level. In fact, the 3-day Systems have even higher levels of upregulation than the 21-day System. As seen in **Figures 3 and 4**, neither APN nor DPP IV showed any upregulation relative to undifferentiated Caco-2 cells in our hands. The 3-day System was almost always at the same enzyme expression level as the 21-day System. Therefore, although we saw no upregulation of the peptidases in the 3- day BD BioCoat Intestinal Environment, we saw no upregulation in the 21-day System either. Finally, a negative control peptidase (APA) showed no activity in any Caco-2 System tested (data not shown). **Figures 5 and** 6 show that P-Glycoprotein function in the 21- day System and the 3-day System compares favorably. That is, the P-Glycoprotein is functioning in the proper orientation in both of the systems (BasalàApical not ApicalàBasal) and is functioning at comparable levels in the two systems. In addition, the vinblastine transport function of P-Glycoproteinis specific, as demonstrated by partial inhibition by verapamil.

Summary

To further characterize and compare the 3-day System with the 21-day System, we have looked at several important Caco-2 differentiation characteristics in the two systems. We have found that the 3-day System supports similar levels of differentiation marker enzyme activity, has properly functioning, appropriate P-Glycoprotein activity, and forms a monolayer with barrier function sufficient for screening of compounds for bioavailability. Therefore, the 3-day System offers a more productive alternative to the 21-day System to generate the data needed for compound permeability assessments, while retaining many of the performance characteristics of the 21-day System.
For detailed information on the 5-day modification of the 3-day Caco-2 System, consult Yamashita[5].

References

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