

Chapter 13

Co-cultivation of Caco-2 and HT-29MTX

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Abstract The intestinal epithelium is one of the body's largest mucosal surfaces and it generates a physical barrier against the external environment. The majority of cells lining the epithelium are absorptive enterocytes with mainly metabolic and digestive functions. Hence, the diversity of functions the intestinal epithelium carries out depends on the presence of additional specialized intestinal epithelial cells (IEC). Secretory IEC, goblet cells, enteroendocrine cells and Paneth cells are specialized cells that participate in maintaining the digestive and barrier functions of the epithelium. Goblet cells release mucins, which give rise to a mucus layer on the epithelial surface that functions as physical and biochemical barrier for luminal content. The presence of the different epithelial cell types in an in vitro model will affect how well the model reflects the properties of the intestinal epithelium. We here describe a co-cultivation system of enterocytes and goblet cells, which are the two major epithelial cell types.

Keywords Co-culture • Enterocytes • Goblet cells • Transport • Absorption • Transepithelial electrical resistance (TEER)

13.1 Origin, Features and Mechanisms

The models described in this section include the cell lines Caco-2 and HT-29. Description of the origin, features and mechanisms of these cells are included in previous sections, and further information is found in Chap. 10 for Caco-2, Chap. 11 for HT-29.

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13.2 Stability/Consistency and Reproducibility

The stability and reproducibility of the co-culture model of Caco-2 and HT-29MTX is very similar to the monocultures. The cells are cultivated together during the whole polarization period of 21 days. The co-cultures should be used for experiments between day 21 and 25. See Chap. 10 for Caco-2 cells and for HT29MTX Chap. 11.

13.3 Relevance to the Human In Vivo Situation

The intestinal epithelium is the main barrier preventing molecules from the lumen (e.g. food and toxins) reaching the systemic circulation. The epithelium is composed of several cell types: enterocytes, goblet cells, Paneth cells, enteroendocrine cells and stem cells. However, absorptive and goblet cells constitute the two major cell types of the intestine. The cell lines Caco-2 and HT-29MTX are derived from intestinal absorptive and goblet cell types, respectively. The human intestinal cell line Caco-2 differentiates into enterocytes (Shah et al. 2006), and is extensively used as an *in vitro* model of the small intestine, particularly to determine the permeability of the intestinal barrier to drug and food components (Sambuy et al. 2005). Caco-2 is of human colonic origin; however, when grown in culture, the cells exhibit many properties of the small intestinal epithelium. They form a well-differentiated polarized monolayer of columnar absorptive cells that express brush border with typical small intestinal enzymes and transporters on their apical surface (Artursson 1991; Hilgendorf et al. 2000). A disadvantage of the Caco-2 model and other monocultures of epithelial cells are that they do not closely simulate the composition of the normal epithelial layer with several types of cells (Hidalgo 1996). The epithelial cell layer is separated from the luminal content by a mucus layer. The mucus layer acts as physical and chemical defence against food particles, chemicals, enzymes and host-secreted products such as bile acids, microbiota and microbial products (Johansson et al. 2008). Only goblet cells are mucus-secreting cells, hence a mucus layer will be lacking in the Caco-2 model. However, the HT-29MTX cells produce both the membrane bound MUC1 and the gel-forming MUC5 (for more information on HT-29 cells see Chap. 11). A co-cultivation of the two cell lines will therefore provide a model constituting the two cell types that are most represented in normal epithelium, enterocytes and goblet cells. In addition the mucin secretion from HT-29 will form a layer on top of the epithelial cells. Presence of mucus in the model system is important for estimation of intestinal permeability as the mucus acts as a barrier against the absorption of certain compounds, particularly those that are lipophilic (Behrens et al. 2001). The lack of mucus allows highly diffusible small molecules easy access to the cells, which often results in an overestimation of permeability of such compounds.

Even though the Caco-2 cells have been widely employed to measure drug and nutrient transport, this model has been criticized because the permeability of the Caco-2 monolayer to hydrophilic compounds, generally transported by paracellular mechanisms, are poor because of the relatively tight junction that are characteristic of these cells (Artursson et al. 2001). A pure Caco-2 cell model also has a overexpression of P-glycoprotein which may lead to higher secretion rates and consequently lower permeability in the absorptive direction (Anderle et al. 1998). The HT-29MTX cell line has less expression of tight junctions. The ability of mannitol to penetrate tight junctions in HT-29 monolayer is 50-fold higher than that of Caco-2 monolayers (Wikman et al. 1993). The permeability of a cell layer resulting from co-cultivation between Caco-2 and HT-29 are more in resemblance with that of the normal intestine. The permeability of the Caco-2/HT-29 co-culture model was correlated with fractions absorbed in humans for selected drugs, and it was found relatively good correlations (Walter et al. 1996).

13.4 General Protocol

Maintain Caco-2 and HT-29MTX cells as described in Chaps. 10 and 11 respectively.

The Caco-2 and HT-29MTX cells are grown separately. The two cell lines are mixed prior to seeding. The most physiological relevant ratios are between 90:10 and 75:25 (Caco-2/HT-29MTX). In this range, the best compromise between model response and the presence of mucus layer will be obtained. Co-cultures of Caco-2 and HT-29MTX cells are seeded onto cell inserts with 0.4 μm pores at a density of 3×10^5 cells per 0.9 cm^2 . The co-culture is maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10 % heat inactivated fetal bovine serum, 2 mM L-glutamine, 1 % non-essential amino acids and 100 U/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin, at 37 °C in 5 % CO_2 . The culture medium is changed every 2–3 days (both in apical and basolateral compartment) for 21 days.

When investigating intestinal transport and absorption using undigested or digested food compounds it is important to adjust pH (7.4) and osmolality (310 mOsm/kg before addition to the cell culture. Evaluation of transepithelial absorption is described in chapter 2, section 2.2.2 and calculation of the apparent permeability coefficient (P_{app}) is described in Chap. 10.

13.5 Assess Viability

The integrity of the cell layers should always be checked by measurement of TEER values, and filters with a TEER value below 200 $\Omega \text{ cm}^2$ should not be used for further experiments. A reference compound that is know to be transported over the

epithelium (LY, labetalol, propranolol, ranitidine, or colchicine) should always be measured concurrently in each permeability assay to ensure validity of the assay.

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13.6 Experimental Readout

The Caco-2/HT-29MTX co-culture model is widely used to study intestinal transport and absorption. An in vitro study of intestinal transport of inorganic and methylated arsenic species from in vitro digested rice, garlic and seaweed has compared the Caco-2 monolayer with various proportions of the Caco-2/HT-29MTX co-culture model (Calatayud et al. 2012). They concluded that arsenic absorption increased with increased proportion of HT-29MTX. Another report studying transport of methylmercury and inorganic mercury in various Caco-2 and HT-29MTX models showed that incorporation of HT-29MTX reduced the permeability for mercury (Vazquez et al. 2013). In this case, the layer of mucus secreted by HT-29MTX retained mercury. Iron bioavailability of in vitro digested food (white, red and soy beans, beef and fish) were investigated by the Caco-2/HT-29MTX model (Mahler et al. 2009). They report that addition of HT-29MTX significantly lowered the cell ferritin formation in the presence of high bioavailability iron digests.

The use of Caco-2/HT-29 co-culture to evaluate transport and bioavailability of glutathione-enriched baker's yeast also revealed an increase in transport rates when HT-29MTX was incorporated in the monolayer compared to only using Caco-2 cells (Musatti et al. 2013).

Bacterial adhesion and invasion of *Salmonella enterica* spp. has been studied using the Caco-2/HT-29 co-culture model (Dostal et al. 2014). Bacterial adhesion and invasion was determined by plating of serial dilutions of the disrupted cell suspension after an extensive washing procedure, on agar plates. In the case of invasion, the extracellular bacteria were killed by incubation with gentamicin for 45 min before disrupting the cells. TEER was measured for evaluation of tight junction disruption during bacterial invasion.

13.7 Advantages, Disadvantages and Limitations

The use of cell lines will give good reproducibility of the model system and there are several reports establishing good correlations between this model and human in vivo studies. The two cell lines are easily available and easy to cultivate. The model is quite easy to use and is efficient for screening purposes. The disadvantages of this model is that some of the transporters/carriers found in normal human intestinal epithelium are not expressed by the two cell lines, therefore in the case of transport

studies the expression of the necessary molecules should be checked. The ratio between the two cell types will be crucial for the formation of homogeneous mucin layer, which is of great importance for the relevance to the in vivo situation.

13.8 Conclusions

The co-culture of Caco-2 and HT-29MTX is a model that is useful for the investigation of transport over intestinal epithelium and for bacterial adhesion and invasion. For such applications the mucin layer and the permeability of the cell layer is crucial and co-culture will give results that are more in compliance with the in vivo situation than monocultures.

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