Novel 3D Human Small Intestinal Tissue Model (EpiIntestinal™) to Assess Drug Permeation & Inflammation

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Presented at AAPS NERDG Annual Meeting, May 1, 2014, Farmington, CT

Abstract

Purpose: Drug transport and permeability across the small intestine (SMI) is a key parameter for determining bioavailability of orally administered drugs. The currently available *in vitro* models are highly dependent on immortalized cell lines (e.g. Caco-2) and have significant deficiencies for drug development applications. Therefore, we developed an *in vitro* SMI model (EpiIntestinal™) using normal human intestinal cells which closely recapitulates the physiology, 3D tissue architecture, and function of the small intestine for use in pharmaceutical development applications and inflammation.

Methods: Normal human primary SMI epithelial cells, fibroblasts, and endothelial cells were harvested from primary tissue and expanded in monolayer culture and seeded onto microporous membrane inserts to reconstruct the 3D SMI tissues. The 3D tissues were characterized phenotypically, (H&E, immunohistochemistry, and TEM), physiologically (TEER), and for gene expression (RT-PCR). Functionally, drug permeation was analyzed using LC-MS/MS and inflammatory responses were measured by BioPlex ELISA.

Results: Characterization of the in vitro SMI tissue model showed: 1) wall-to-wall tissue growth which makes it ideal for apical (lumen-like) application of drugs, 2) columnar epithelial cell morphology, 3) TEER in the physiological range of 60-180 Ω*cm^2 mimicking the SMI microenvironment in vivo, 4) formation of brush borders and tight junctions, 5) gene expression of the prominent efflux drug transporters such as P-glycoprotein (P-gp, MDR-1) and BCRP, and 6) extended life span (up to 30 days) in culture. Permeation studies of drugs (ranitidine and talinolol) showed efflux ratios >2 fold indicative of P-gp efflux transport. Exposure of the SMI tissue to proinflammatory cytokines (TNF-α and IFN-γ) induced upregulation of cytokines/chemokines such as IL-6, IL-8, and GRO-α.

Conclusions: The newly developed SMI tissue model will have application in drug safety and inflammation studies. Availability of an easy-to-use, economical, and reproducible in vitro reconstructed human SMI tissue will reduce animal use, minimize the number of test compounds dropped due to permeation problems in Caco-2, and significantly improve the prediction potential for drug absorption or inflammation in humans for candidate pharmaceutical compounds.

Methods & Results

Tissue preparation: Small intestinal (SMI) epithelial cells harvested from post-mortem donors following IRB approval. SMI cells were seeded onto cell culture inserts (partial thickness tissue, SMI-100) or onto a myofibroblast collagen-gel matrix (full-thickness tissue, SMI-100-FT), raised to the air liquid interface **(Figure 1)** and cultured in specially formulated culture medium designed to induce differentiation for 2 weeks.

Histology: To examine structural features, the small intestinal epithelial tissues were fixed in 10% formalin (overnight, room temperature), paraffin embedded, sectioned using a microtome, and stained with hematoxylin and eosin (H & E) according to standard procedures. The histological results revealed: 1) wall-to-wall growth of the epithelial layer, and 2) the presence of columnar epithelial cells similar to the in vivo counterpart **(Figure 2).**

Immunohistochemistry (IHC): Immuno-staining was performed following de-paraffinization of tissue sections and antigen retrieval. IHC staining showed expression of cytokeratin (CK)-19) and the efflux transporter, P-gp (Figure 3).

Tissue barrier integrity: The SMI tissue model barrier function was quantified using transepithelial electrical resistance (TEER) measurements. TEER measurements were made using an EVOM volt-ohmmeter equipped with an Endohm electrode chamber (World Precision Instruments, Sarasota, FL). TEER values (reported in Ohm*cm^2) were calculated by multiplying raw resistance measurements by the surface area of the tissue (0.6 cm^2). (Table 1) The barrier integrity of the tissue model was stable for up to 4 weeks of culture period as monitored by TEER and Lucifer Yellow leakage assay **(Figure 4).**

Ultrastructural features: Transmission electron microscopy (TEM) was used to examine ultrastructural features such as brush borders and tight junctions in the small intestinal epithelial tissues **(Figure 2).**

Drug transporters: RT-PCR was performed to investigate gene expression levels of efflux drug transporters (p-glycoprotein (MDR1), multidrug-resistance associated (MRP)-1 and 2 and the breast cancer resistance protein (BCRP) in the in vitro reconstructed human small intestinal tissue model at days 14 of the culture period. Results showed that the efflux drug transporters were expressed by the differentiated SMI tissue model **(Figure 5).**

Cytokine Release: To evaluate the inflammatory response of the SMI model to exogenous stimuli, tissues were exposed to IL-1β, TNF-α/IFN-γ for 24 hr. Following exposure, epithelial integrity was monitored by measuring TEER values (Figure 6). Culture supernatants were collected and analyzed for pro-inflammatory cytokine / chemokine release using Bio-Plex ELISA. The results showed increased secretion of IL-6, IL-8, and GRO-α following IL-1β and TNF-α /IFN-γ treatment **(Figure 7).**

Drug Permeation: Drug permeation was analyzed by LC-MS/MS. Functionally, drug permeation studies (ranitidine and talinolol) showed <2 fold A to B transport with an efflux ratio >2 fold indicative of P-gp transport **(Table 2).**

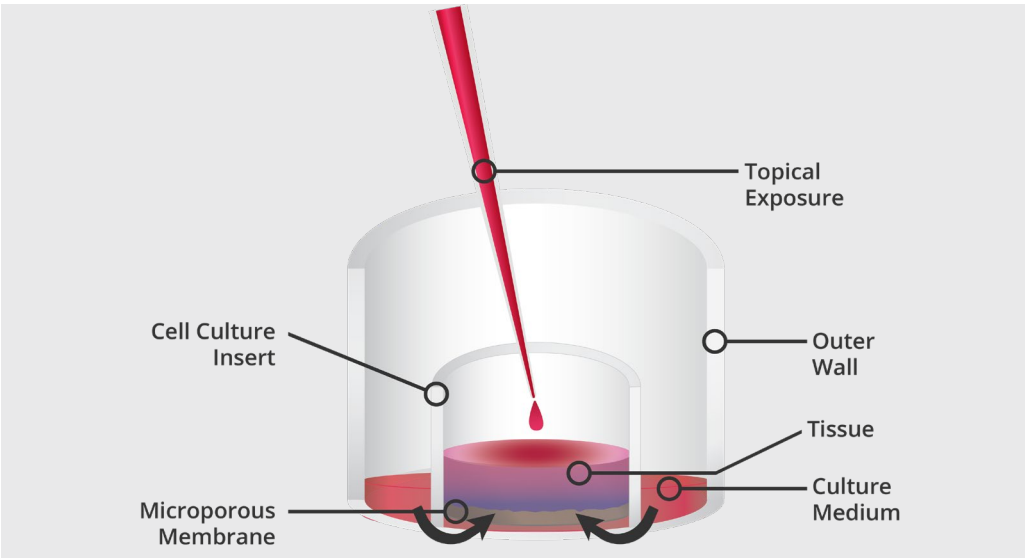


Figure 1: A schematic representation of the SMI tissue model. The tissue is "air-lifted" by culturing it in a microporous-membrane cell culture insert with the apical surface exposed to the atmosphere and is fed by medium diffusing through the membrane. Systemic exposure conditions are achieved by adding the test chemicals into the medium.

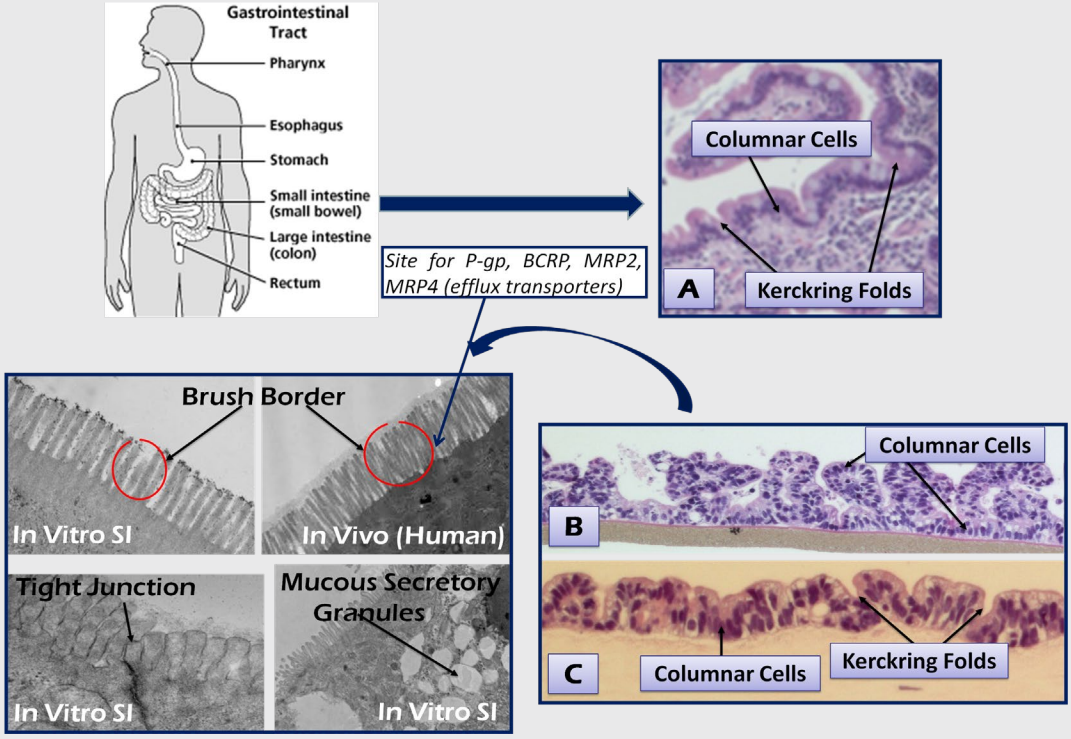


Figure 2: H&E staining showing the: **(A)** epithelium of explant small intestinal tissue and **(B)** partial thickness (PT) and **(C)** full-thickness (FT) EpiIntestinal tissue model. Micrographs of brush border membranes, tight junction formation and mucous secretory granules are also shown.

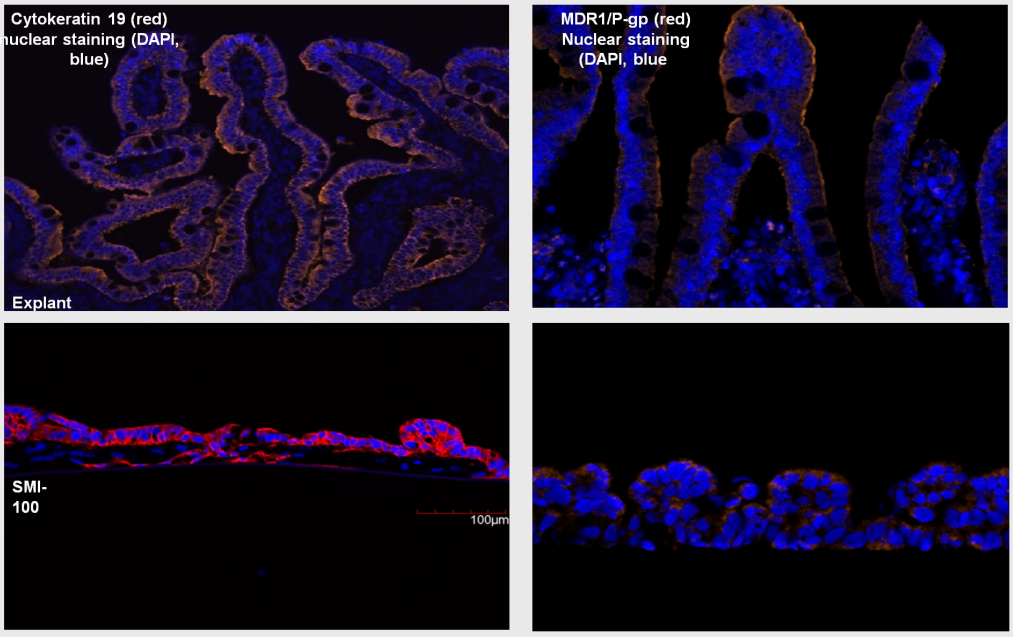


Figure 3: Immunohistochemistry showing cytokeratin 19 (CK19) and P-gp expression (orange) in the reconstructed or explant small intestinal tissue.

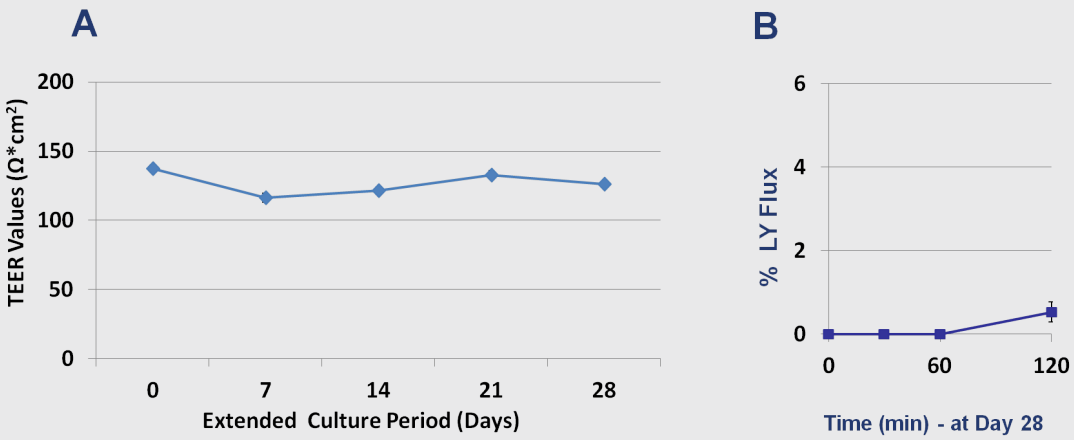


Figure 4: Stability of SMI-100 tissues in culture: to remove sloughed off epithelial layer from the mature EpiIntestinal (SMI-100), tissues were rinsed in PBS (pH 6.8) every week and cultured in extended culture system for up to 4 weeks. Barrier integrity was monitored by measuring TEER every week (A) and Lucifer Yellow (LY) leakage was measured at day 28 (B).

Tissue Lot # (all lots from same donor)	TEER values - Day 14	
	Ω* cm²	St. Dev.
17232	132	7.2
17241	136	1.7
17245	123	7.6
17249	114	11.0
17252	130	11.0
17242	146	10.2
17247	125	3.4
17253	139	4.2

Table 1: SMI-100 Tissue Reproducibility *Trans-Epithelial Electrical Resistance (TEER)*

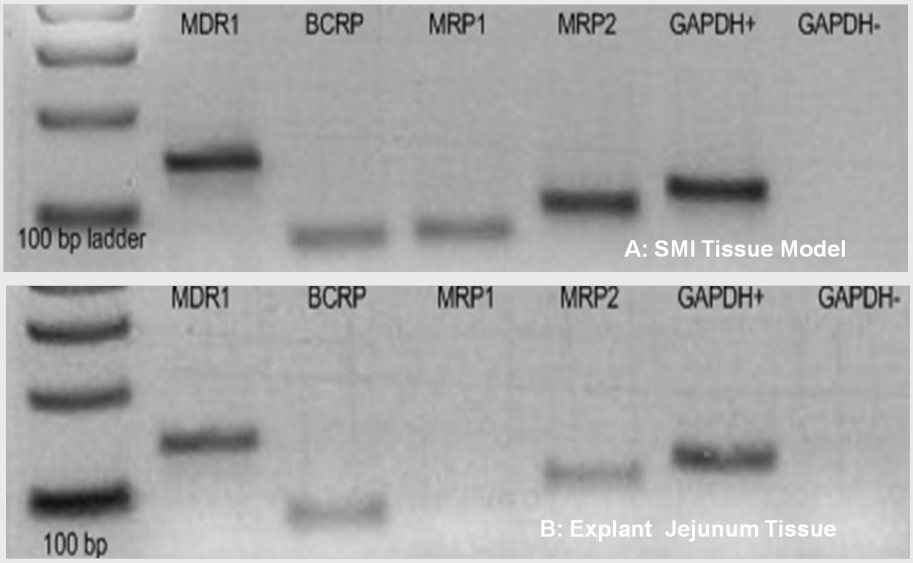


Figure 5: RT-PCR showing gene expression of efflux transporters: P-glycoprotein (MDR1), BCRP, MRP1, and MRP2 by the in EpiIntestinal tissue model (day 14) (**A**) and human small intestinal explant tissue (**B**).

Table 2: LC-MS/MS analysis showing permeation of model drugs in the SMI -100-FT tissues.

Intestinal Tissue	Test compound	Test conc (uM)	Assay duration (hr)	Mean Papp (A->B) (10^{-6} cm s^{-1})	Mean Papp (B->A) (10^{-6} cm s^{-1})	Asymmetry ratio	Comment
SMI-100-FT	Ranitidine	10	2	1.6	5	3.1	Low Permeability
	Warfarin	10	2	16.5	55.5	3.4	High Permeability
	Talinolol	10	2	1.1	9.3	8.5	Pgp Substrate
	Talinolol +Verapamil	10	2	1.8	4.1	2.2	Pgp Substrate

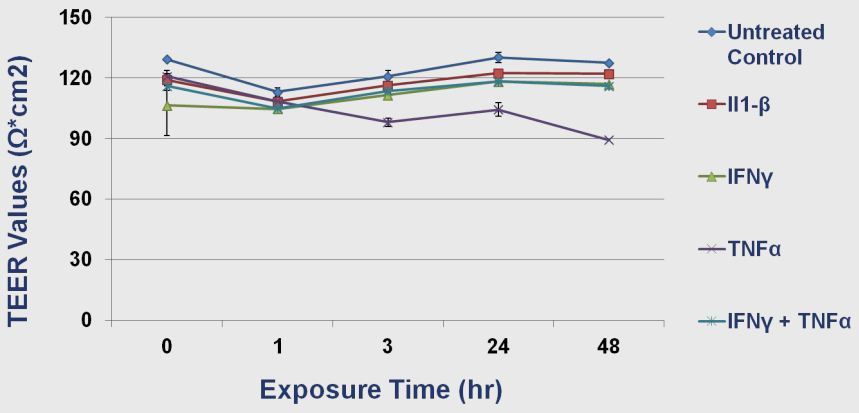
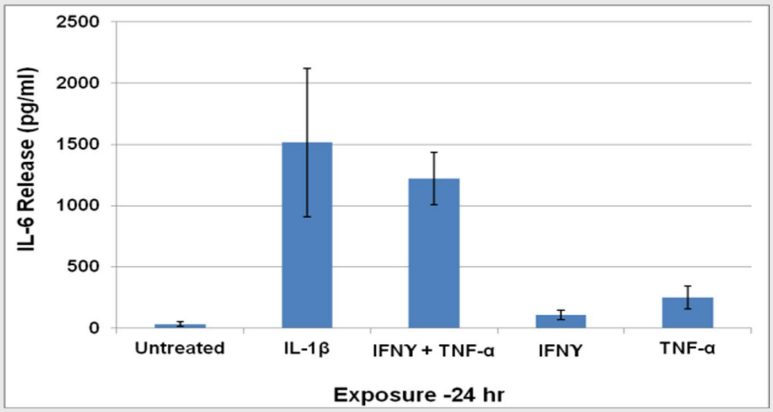
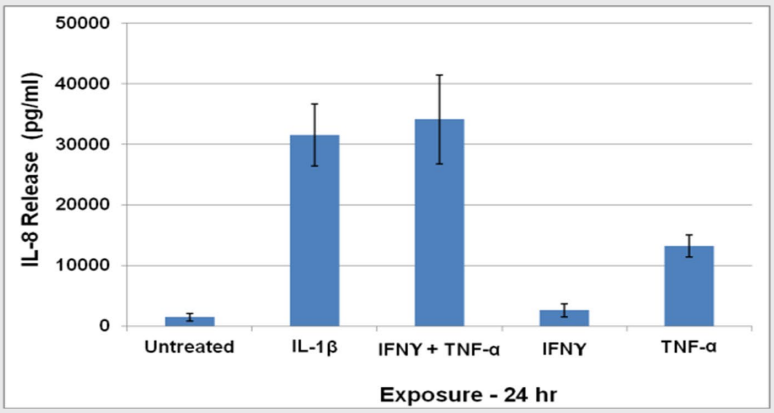


Figure 6: TEER values of EpiIntestinal (SMI-100) tissue following 48 hr exposure to IL-1β (5 ng/ml), TNF-α (40 ng/ml), IFN-γ (5 ng/ml), and TNF-α + IFN-γ. Medium treated wells were used as untreated controls





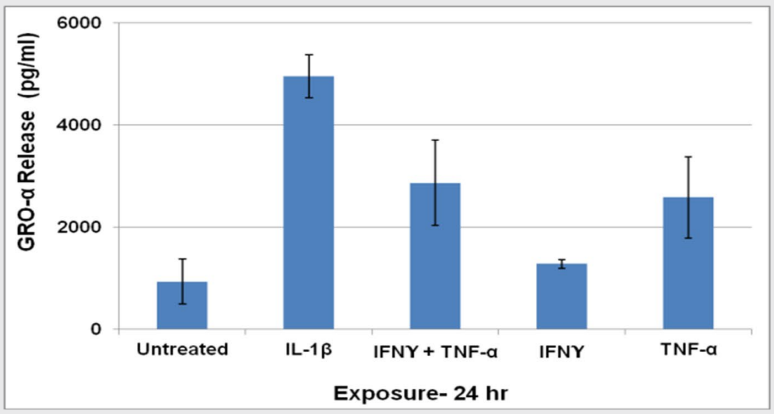


Figure 7: Cytokine release by EpiIntestinal (SMI-100-FT) tissues following 24 hr exposure to IL-1β (5 ng/ml) or TNF-α (40 ng/ml) + IFN-γ (5 ng/ml). Culture supernatants were collected and analyzed for IL-6, IL-8, and GRO-α release using Bio-Plex ELISA. Medium treated wells were used as untreated controls.

Summary and Conclusions

- Histological cross-sections show that EpiIntestinal tissues closely resemble native human SMI tissue (**Figure 2**).
- The EpiIntestinal tissue model forms brush borders, tight junctions, and mucous secretory granules (**Figure 2**) and expresses epithelial markers, cytokeratin 19 and P-glycoprotein (**Figure 3**).
- The SMI tissue model allows minimal Lucifer Yellow permeation (<4% leakage after 120 mins) and has a stable barrier over a 4-week period (**Figure 4**) and between tissue lots (**Table 1**). RT-PCR analysis shows that the EpiIntestinal tissue model expresses efflux drug transporters present in native small intestine tissue (**Figure 5**).
- The newly developed SMI tissue model will likely be useful for predicting drug absorption (**Table 2**) and inflammation of the small intestine caused by new drug formulations (**Figures 6-7**).
- The *in vitro* reconstructed human SMI tissue will minimize the number of test compounds dropped due to permeation problems in Caco-2 and significantly improve the prediction potential of drug absorption or inflammation in humans.