Reconstruction of novel 3D models of the three parts of small intestine for drug toxicity testing

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Abstract

Background: Gastrointestinal (GI) toxicity is a common adverse event that limits pharmaceutical development across diverse therapy areas. The mechanisms of drug induced GI toxicity are often poorly understood, in part due to the lack of physiologically relevant in vitro models that recapitulate the three segments of the small intestine. Traditional in vitro cell cultures utilize the immortalized human colorectal Caco-2 cell line. Even though Caco-2 has been in use for more than 5 decades, it has limitations since Caco-2 is a cancer derived cell-line which lacks the major drug transporters and drug metabolizing enzymes, does not produce fully polarized structural features, and is not predictive of GI toxicity,. To mimic the physiology and functionality of the human gut, we previously developed EpiIntestinal, a 3D in vitro small intestinal model utilizing cells derived from the ileum of normal human tissue. However, there is a need by the pharma industry for in vitro 3D models representing the other parts of the small intestine (duodenum and jejunum). Availability of models from the different parts of the small intestine will allow identification of the primary sight of absorption for drug candidates to predict efficacy, risk, and/or dose scheduling to mitigate risk.

Methods: State-of-the-art tissue culture and tissue engineering methods were used to reconstruct 3D GI models representing the duodenum, jejunum, and ileum of the human small intestine. The 3D tissue models were cultured using primary human cells and were characterized morphologically by histology and functionally by performing drug permeation studies. Gene expression levels of drug transporters and drug metabolizing enzymes were quantified by qPCR & protein quantification analyses by LC/MS. The activities of the transporters were evaluated using vinblastine as substrate for P-glycoprotein (P-gp) and Multi-drug resistant protein (MRP-3).

Results: The 3D tissues developed barrier function and structural features resembling villi similar to native intestinal tissue. mRNA analysis from the 3D tissue models agreed with published data showing high expressions of SLCO2B1, SLC16A1 and SLC15A1 by the three segments of the small intestine. Some of the genes transcribed to proteins include: 1) drug transporters such as P-gp (MDR1), multi-drug resistant protein (MRP-3), and breast cancer resistant protein (BCRP, ABCG2). P-gp, MRP-2, and ABCG2 expression in the ileum model was lower compared to the duodenum and jejunum models. In contrast, SLC15A1 Peptide transporter 1 (PepT-1) expression was greater in the ileum model compared to the others (Ileum > jejunum > duodenum). Expression of drug metabolizing enzymes such as CYP3A4, CYP2C9, UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), and carboxylesterase 1 (CES-1) was detected in all three regions at a comparable level. However, CYP2C9 expression was most pronounced in the jejunum (rank order was jejunum > duodenum > ileum). The efflux ratio for vinblastine decreased when MK571 (MRP inhibitor) and Elacridar (P-gp inhibitor) were added. Treatment of the tissues with Raloxifene (an anti-osteoporotic drug, CYP3A4 substrate) showed formation of raloxifene-6-glucuronide which could be inhibited by Zafirlukast, an inhibitor of the glucuronidase enzymes. This demonstrated the presence of the phase 2 glucuronidase enzymes in the models.

Conclusions: These results suggest that the reconstructed tissues from the three segments of the small intestine will serve as useful tools to predict both investigational and traditional GI drug safety and absorption in the GI tract. In addition, use of these models will reduce animal use and improve the pre-clinical drug development process.

Methods

Tissue preparation: Small intestinal (SMI) epithelial cells were isolated from primary tissue using proprietary techniques. The tissues were produced by seeding the SMI epithelial onto tissue culture inserts, raising them to the air liquid interface, and culturing them for 2 weeks in specially formulated culture medium to induce differentiation.

Histology: Tissues were fixed in 10% formalin (overnight, RT), paraffin embedded, sectioned using a microtome, and stained with H&E (**Figure 1**).

Gene analysis: Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed to investigate gene expression levels of: 1) drug transporters such as P-glycoprotein (P-gp, MDR1), multi-drug resistant protein (MRP-3), breast cancer resistant protein (BCRP, ABCG2), 2) peptide transporter 1 (PepT-1), 3) drug metabolizing enzymes such as CYP3A4, CYP2C9, UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), and carboxylesterase 1 (CES-1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization.

Protein expression: Protein expression was quantified in the samples via HPLC-MS/MS method at Genentech. Proteins included: 1) drug transporters such as P-glycoprotein (P-gp, MDR1), Multi-drug resistant protein (MRP-3), breast cancer resistant protein (BCRP, ABCG2), 2) Peptide transporter 1 (PepT-1), 3) drug metabolizing enzymes such as CYP3A4, CYP2C9, UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), and Carboxylesterase 1 (CES-1), and 4) Midazolam and its metabolite 1-Hydroxymidazolam.

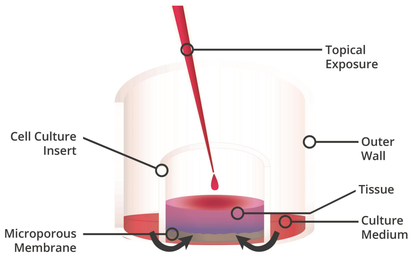

Figure 1: Schematic of the 3D primary human duodenum, jejunum, and Ileum tissue models grown in cell culture inserts at air liquid interface (topical tissue surface is exposed to air) allowing for topical or systemic exposure to test materials.

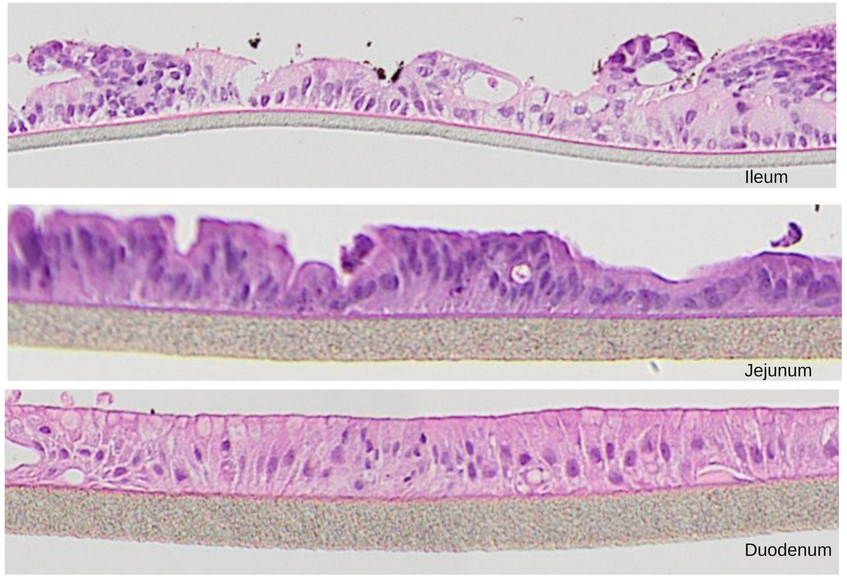

Figure 2: H&E stained histological cross-section of the three segments of the human small intestine tissues (duodenum, Jejunum, and Ileum) grown on microporous membrane support (pore diameter= 0.4 um)

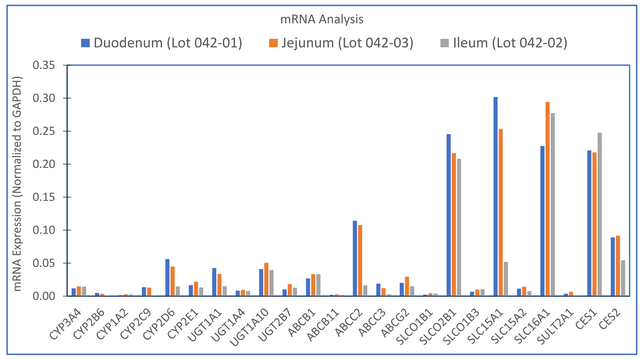

Figure 3: 3D Human intestinal tissue models showing gene expression levels (PCR) of drug metabolizing enzymes, efflux and peptide transporters, and Carboxylesterases.

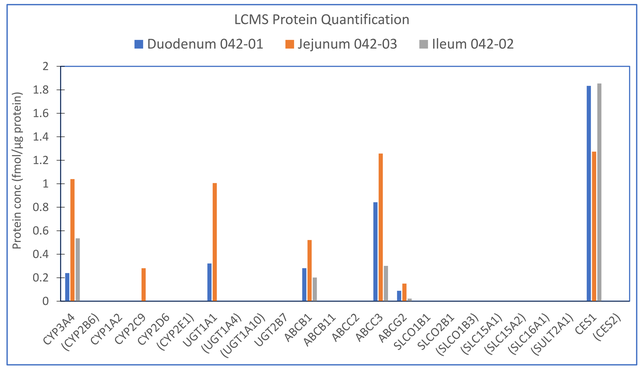

Figure 4: 3D Human intestinal tissue models showing protein expression levels (measured by LC-MS) of drug metabolizing enzymes, efflux and peptide transporters, and carboxylesterases.

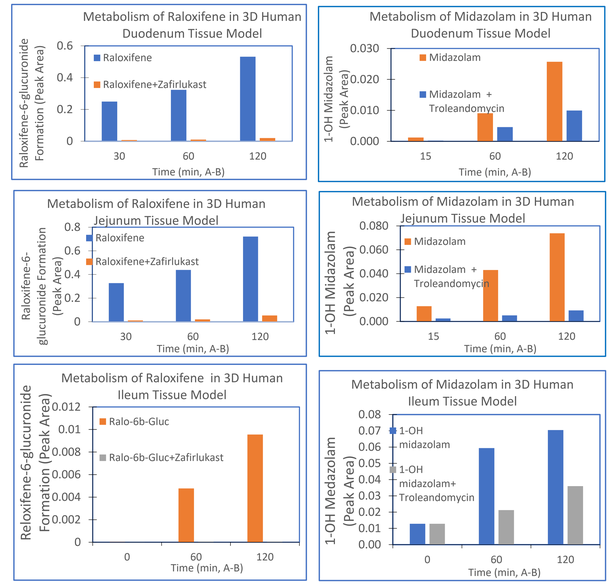

Figure 5: Metabolism of Raloxifene in the three segments of intestinal tissue models in the presence of inhibitor Zafirlukast. Raloxifene its metabolite raloxifene-6-β-glucuronide were quantified.

Figure 6: Metabolism of midazolam (substrate for CYP3A4) in the three segments of intestinal tissue models in the presence of the competitive CYP3A4 inhibitor Troleandomycin.

Table 1: Drug-Drug Interaction studies using small intestinal tissue (ileum)

		Papp (1x 10^{-6} cm/s)			
Compound	Transporter	A to B	B to A	Efflux Ratio	Comments
Digoxin	P-gp	4 + 3	20 + 8	5	-
Digoxin + Elacridar	-	15 + 5	8 + 1	0.6	Elacridar as a P-gp Inhibitor
Loperamide	P-gp	2 + 0.3	10 + 2	5	-
Loperamide + Elacridar	-	5 + 1	9 + 1	1.8	Elacridar as a P-gp Inhibitor
Sulfasalazine	BCRP	1 + 0.2	16 + 1	25	-
Sulfasalazine + Ko143	-	3 + 0.3	9 + 1	3	Ko143 as a BCRP inhibitor
Prazosin	BCRP	12 + 1	28 + 2	2.3	-
Prazosin+Ko143	-	18 + 1	25 + 2	1.4	Ko143 as a BCRP inhibitor
Vinblastine	P-gp, MRP2	1 + 0.2	33 + 3	31	-
Vinblastine+MK571	-	8 + 2	32 + 4	4	MK571 as a MRP2 inhibitor
Vinblastine+MK571+Elacridar	-	9 + 1	19 + 2	2	MRP2 & P-gp inhibited
Saquinavir	P-gp, OATP	2 + 0.4	11 + 2	5.5	-

Conclusions

- Intestinal tissues from the three segments (duodenum, jejunum, and ileum) were reconstructed on microporous insert membranes and have structural similarity to their in vivo counterparts (**Figures 1 and 2**).
- The 3D tissues are well polarized and stratified with villi-like structure formation (**Figure 3**).
- Similar to MatTek's standard intestinal ileum-derived tissue model (EpiIntestinal™), the 3D intestinal duodenum and jejunum models have high expression of: 1) SLCO2B1, SLC16A1 and SLC15A1, 2) drug transporters such as P-glycoprotein (PgP, MDR1), Multi-drug resistant protein (MRP-3), breast cancer resistant protein (BCRP, ABCG2), 3) drug metabolizing enzymes such as CYP3A4, CYP2C9, UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), and Carboxylesterase 1 (CES-1) (**Figures 3 and 4**).
- The efflux ratio for vinblastine decreased when the MK571 (MRP inhibitor) and Elacridar (P-gp inhibitor) were added with Vinblastine (**Table 1**)
- The intestinal tissue models can be used to: 1) examine drug metabolism and clearance and 2) investigate drug-drug interactions (**Figures 5 and 6**).
- The reconstructed tissues from the three segments of the small intestine will serve as useful tools to predict both investigational and traditional GI drug safety and absorption in the GI tract and reduce animal use during drug development.

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