In Vitro Reconstructed 3d Models Of Human Duodenum, Jejunum And Ileum

E. Reis[1*], J.Markus[1], L.Hudecova[1], Z. Stevens[2], J. Cheong[3], M. Klausner[2], A.Armento[2], S. Ayehunie[2]

1 MatTek In Vitro Life Science Laboratories, s.r.o., Bratislava, Slovakia
2 MatTek Corporation, Ashland, Massachusetts, United States of America
3 Genentech, San Francisco, California, United States of America

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Introduction

The study of gastrointestinal (GI) toxicity is limited due to the lack of physiologically relevant in vitro models that recapitulate the role of specific parts of the GI tract. For example, traditional in vitro cell cultures approach utilizes immortalized human Caco-2 cell line cultured for about 21 days to mimic properties of small intestine mucosa and assess ADME properties of drugs. However, these models are limited by the fact that they originate from cancer cells, have unphysiological expression and/or functionality of major drug transporters and drug metabolizing enzymes, do not have fully polarized structural features, and are not predictive of GI toxicity even though they have been in use for more than 5 decades. To mimic the physiology and functionality of the human gut, we established the small intestinal model from primary human cells, which recapitulates many aspects of small intestine biology. The only pitfall of this model is that it only consists of cells derived from jejunum. Here we present development of 3 new models of small intestine utilizing primary cells from different intestine regions in order to provide further physiological relevance and expand ability to pinpoint differences between different regions of small intestine mucosa (duodenum, jejunum, and ileum).

The newly developed 3D tissues mimic morphology of normal intestinal epithelium with structural features resembling villi and physiological-like barrier function. Gene expression analyses revealed differences in the expression of genes encoding transporter proteins (ABC family, peptide transporters) and drug metabolizing enzymes in various regions. On the other hand, expressions of some drug metabolizing enzymes such as CYP3A4, CYP2C9, UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), and Carboxylesterase 1 (CES-1) were maintained in each segment at a comparative level. Our preliminary experiments aimed at drug absorption and metabolism revealed that the permeation of Vinblastine was affected by inhibition of MRP and/or P-gP transporters. The activity of metabolic enzymes (phase II glucuronidase enzymes) was suggested by the presence of raloxifene-6-glucuronide metabolite following the treatment of tissues with raloxifene, which was sensitive to inhibition with glucuronidase inhibitor. These results suggest that the reconstructed tissues from the three segments of the small intestine may 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.

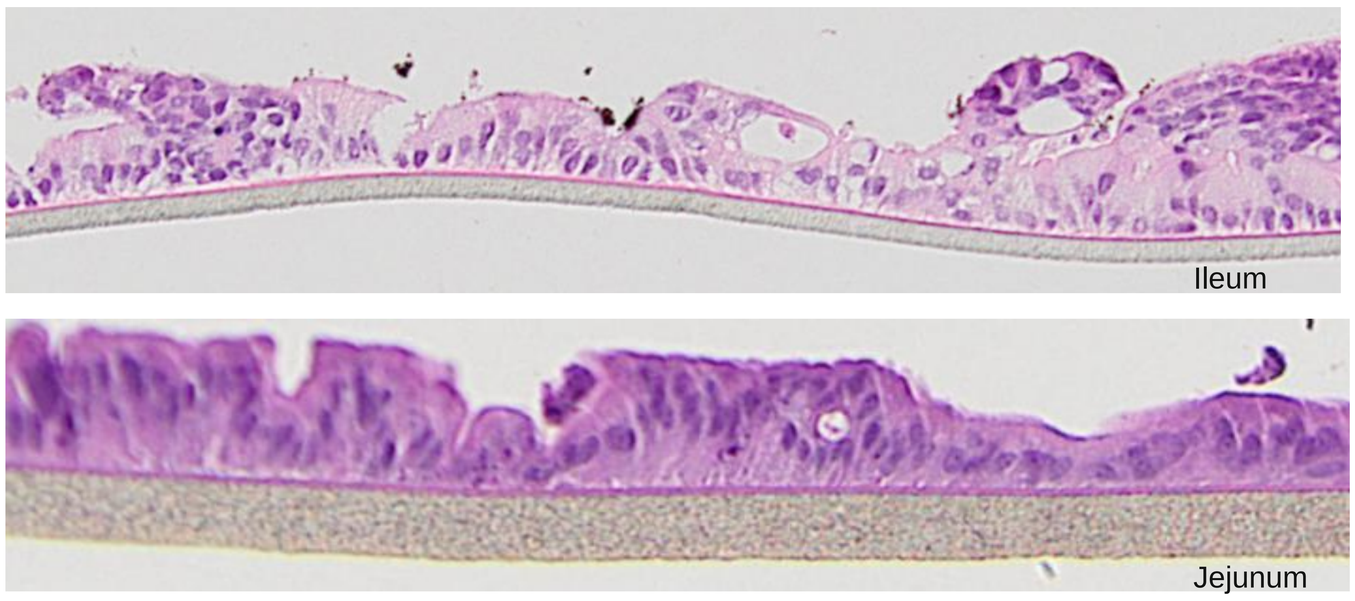

Figure 2: H&E stained histological cross-section of the three segments of the human small intestine tissues (duodenum, Jejunum, and Ileum) grown on microporous membrane support (pore diameter= 0.4 um)

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 expression 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.

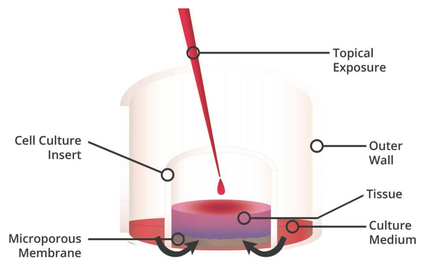

Figure 1: Schematic of the 3D primary human duodenum, jejunum, and Ileum tissue models grown in cell culture inserts at air liquid interface (topical tissue surface is exposed to air) allowing for topical or systemic exposure to test materials.

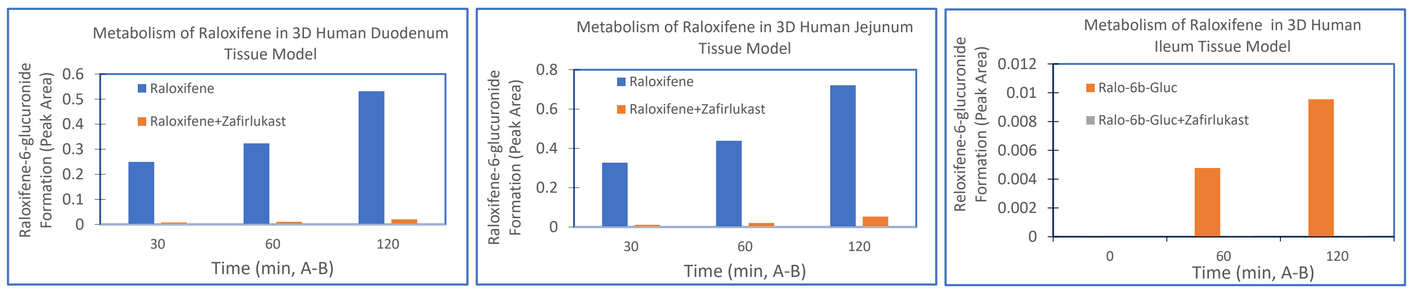

Figure 5: Metabolism of Raloxifene in the three segments of intestinal tissue models in the presence of inhibitor Zafirlukast. Raloxifene its metabolite raloxifene-6-β-glucuronide were quantified.

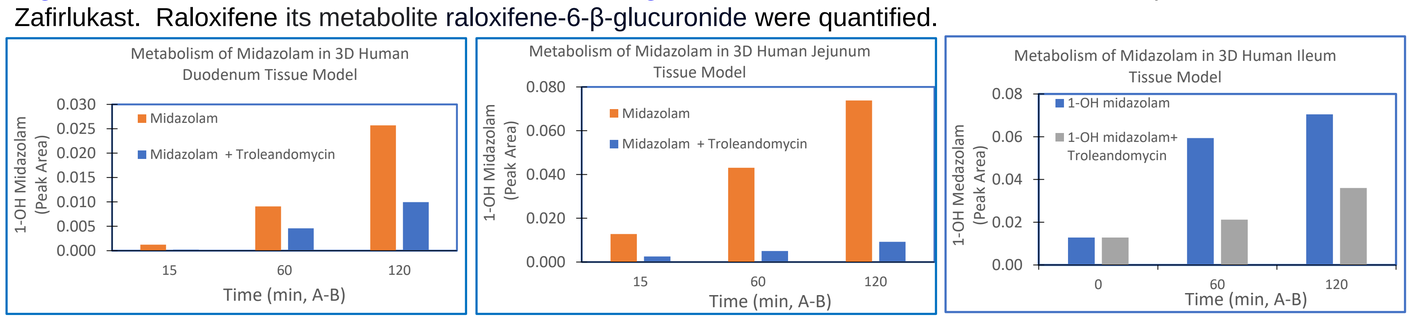

Figure 6: Metabolism of midazolam (substrate for CYP3A4) in the three segments of intestinal tissue models in the presence of the competitive CYP3A4 inhibitor Troleandomycin.

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	

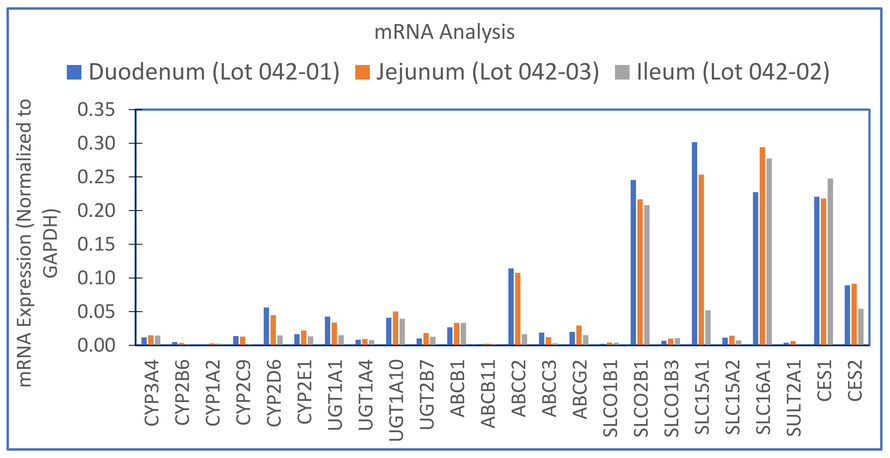

Figure 3: 3D Human intestinal tissue models showing gene expression levels (PCR) of drug metabolizing enzymes, efflux and peptide transporters, and Carboxylesterases.

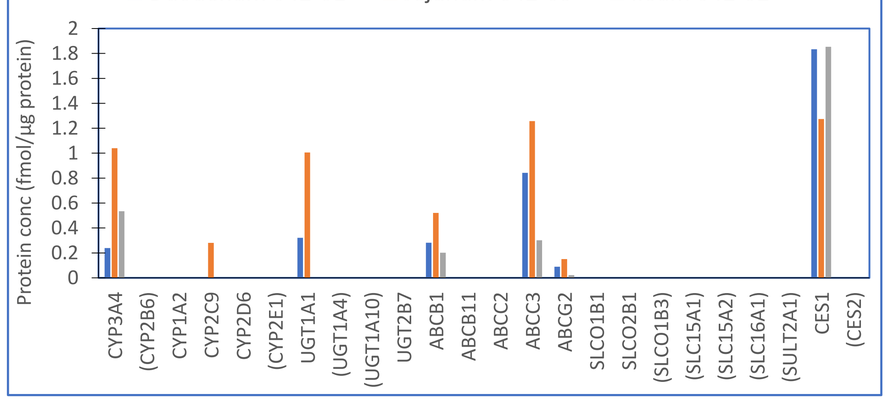

Figure 4: 3D Human intestinal tissue models showing protein expression levels (measured by LC-MS) of drug metabolizing enzymes, efflux and peptide transporters, and carboxylesterases.

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.

200 Homer Ave., Ashland, MA USA

MatTek Corporation
www.mattek.com

Mlynské Nivy 73, Bratislava, Slovakia