# Human 3D Colon Tissue Model for Toxicity and Microbiome Studies

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## Abstract

**Background:** The human colon plays host to a wide variety of microbes living together in an ecosystem. Any damage or injury to the colonic epithelium disturbs this ecosystem which can in turn lead to inflammation and disease. The colonic epithelium is a dynamic structure with a self-renewing capacity and serves as an organ for reabsorption of water, electrolytes, and bacterial metabolites. Recently, MatTek has developed EpiColon, a polarized and well-differentiated in vitro human colon epithelial model with distinct luminal and basolateral sides. The aim of this study is to characterize the EpiColon human tissue model generated using epithelial cells from the human ascending colon to study: 1) toxicants, 2) microbiome, 3) safety and efficacy of colorectal care products and anti-microbial agents, and 4) inflammation.

**Methods:** This study characterizes the structural features of a novel in vitro tissue model reconstructed from normal human primary colon epithelial cells (CEC) with or without fibroblasts. Briefly, primary cells were isolated from a healthy human ascending colon, expanded in monolayer culture, and seeded onto microporous membrane inserts and cultured at the air-liquid interface (ALI) to form a well stratified 3D organotypic CEC tissues. The tissues were characterized for polarity (H&E staining), barrier integrity (transepithelial electrical resistance, TEER) measurement, presence of mucin producing goblet cells (Periodic acid–Schiff; PAS staining), brush border formation (Villin staining), drug transporters and drug metabolizing enzymes (qPCR), and functionality in toxicological studies using Indomethacin (0.01-0.5mg/mL) and SN38 (20µM) following apical exposure for 24 hr. Finally, the immune response of the tissue was probed by induction with cytokines and Toll-like receptor ligands.

**Results:** Analysis of the 3D colon tissue model revealed: 1) wall-to-wall tissue growth in the cell culture insert, 2) an epithelial layer with in vivo like morphology, 3) a physiological TEER value of >200 Ω*cm$^2$ mimicking the colon microenvironment, and 4) surface marker expression of CK19 (epithelial cell marker), Alcian blue PAS staining (mucous producing goblet cells), and villin (brush border formation). Gene expression analysis revealed expression of drug transporters such as efflux transporters (PgP, MRP-1, 3, 5), sodium/glucose cotransporter (SLC5A1), and organic anion transporters (OAT) 2A1, 2B1, 3A1, and 4A1. Additionally, the tissue model showed strong gene expression of both Phase I and Phase II drug metabolizing enzymes such as CYP2C18, CYP2C19, CYP 1A1, CYP 2B6, CYP2R1, CYP2S1, CYP3A4, CYP3A5, CYPs4F2, Carboxylesterases 1-3, glutathione S-transferases, and UDP glucuronosyltransferase. Exposure of the CEC tissue to dextran sulfate sodium salt (DSS) and SN38 showed a toxicity as evidenced by decreased TEER values compared to untreated controls. Induction of the colon tissue model with cytokines (TNF-α and IL-β) and a Toll-like receptor ligand (LPS) induced release of inflammatory cytokines IL-6 and IL-8.

**Conclusions:** This novel human cell-based CEC tissue model will be a useful tool for: 1) pre-clinical assessment of chemicals/candidate drugs for their toxicity potential, 2) studying the colon microbiome and bacterial metabolites, and 3) monitoring mucosal inflammation induced by chemical/compound insult. The CEC model can be added to the toolbox of 3D gastrointestinal tract tissue models to predict drug/chemical safety and efficacy, disease modeling, and microbiome studies. Ultimately this model will play a role in reducing animal testing and improving the preclinical drug development process.

## Methods

**Tissue preparation:** Human colon epithelial cells (CEC) were either purchased from a commercial vendor or isolated at MatTek and using a modified cell expansion medium. Colon epithelial cells (**Figures 1- 3**) were seeded onto collagen-coated cell culture inserts and raised to the air-liquid interface, at which point they were cultured until fully polarized and stratified using specially formulated culture medium.

**Histology & Immunostaining:** EpiColon cultures 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 (**Figure 1**). Unstained slides were used for immunohistochemistry. Periodic acid-Schiff (PAS) staining was used to detect mucin in tissues (**Figure 2**).

**Gene expression:** Expression of genes encoding specific drug metabolizing enzymes was evaluated by qRT-PCR using the RT2 qPCR primer assays (Qiagen, Germantown, MD).

**Cytokine analysis:** Release of IL-6 and IL-8 following Induction with cytokines (TNF-α and IL-β) and a Toll-like receptor ligand (LPS) was quantified using commerciality available ELISA kits, as per the manufacturer's recommended protocols.

**Toxicity**: EpiColon tissues were exposed to dextran sulfate sodium (DSS) an irritant which is known to induce colitis in mice, Indomethacin, and SN38. Tissues were exposed basolaterally for 48 hr. SN38, known to cause toxicity in the gut, was used as a positive control.

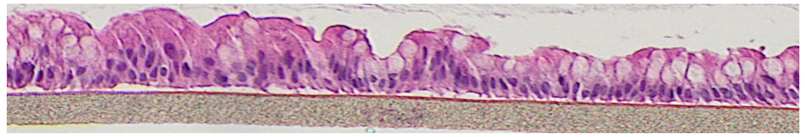

**Figure 1: H&E staining showing architectural features of EpiColon** on day 12 of the culture period.

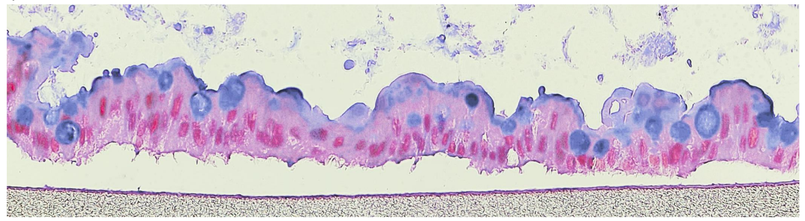

**Figure 2: PAS staining of EpiColon.** Mucus producing goblet cells are shown in blue.

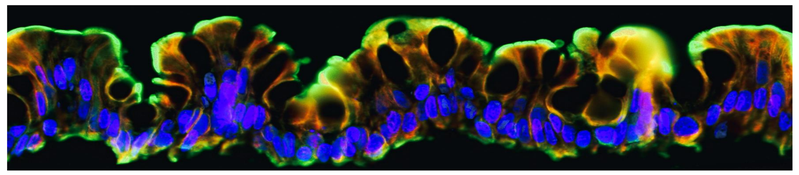

**Figure 3: Immunohistochemistry of EpiColon** showing brush border formation as indicated by villin staining (apical, green) on day 12 of the culture period. Tissues were also stained for cytokeratin 19 (orange) and nuclei were with DAPI (blue).

**Table 1: Gene Expression (RT-qPCR)** of Phase I drug metabolizing enzymes in the EpiColon tissue model on day 12 of the culture period.

| Target | Gene Name: Metabolic Phase I Enzymes | Cq Values |
|---|---|---|
| CEL | Carboxyl ester lipase (bile salt-stimulated lipase) | 32.88 |
| CYP1A1 | Cytochrome P450, family 1, subfamily A, polypeptide 1 | 29.88 |
| CYP27A1 | Cytochrome P450, family 27, subfamily A, polypeptide 1 | 31.30 |
| CYP2B6 | Cytochrome P450, family 2, subfamily B, polypeptide 6 | 27.16 |
| CYP2C18 | Cytochrome P450, family 2, subfamily C, polypeptide 18 | 28.89 |
| CYP2C19 | Cytochrome P450, family 2, subfamily C, polypeptide 19 | 27.84 |
| CYP2C9 | Cytochrome P450, family 2, subfamily C, polypeptide 9 | 31.65 |
| CYP2D6 | Cytochrome P450, family 2, subfamily D, polypeptide 6 | 30.19 |
| CYP2R1 | Cytochrome P450, family 2, subfamily R, polypeptide 1 | 29.51 |
| CYP2S1 | Cytochrome P450, family 2, subfamily S, polypeptide 1 | 24.56 |
| CYP3A4 | Cytochrome P450, family 3, subfamily A, polypeptide 4 | 25.36 |
| CYP3A5 | Cytochrome P450, family 3, subfamily A, polypeptide 5 | 22.45 |
| CYP4F11 | Cytochrome P450, family 4, subfamily F, polypeptide 11 | 27.13 |
| CYP4F12 | Cytochrome P450, family 4, subfamily F, polypeptide 12 | 31.43 |
| CYP4F2 | Cytochrome P450, family 4, subfamily F, polypeptide 2 | 26.89 |
| CYP4F3 | Cytochrome P450, family 4, subfamily F, polypeptide 3 | 32.63 |
| ESD | Esterase D | 31.17 |
| FMO4 | FMO4 Flavin containing monooxygenase 4 | 30.00 |
| FMO5 | Flavin containing monooxygenase 5 | 26.08 |
| HSD17B10 | Hydroxysteroid (17-beta) dehydrogenase 10 | 32.07 |
| MAOA | Monoamine oxidase A | 27.46 |
| MAOB | MAOB Monoamine oxidase B | 31.32 |
| Actb | Actin Beta | 19.32 |

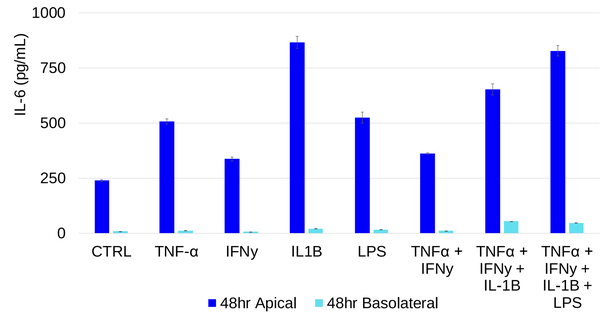


**Figure 4: IL-6 release by EpiColon** following 48hr apical or basolateral treatment with inflammatory cytokines

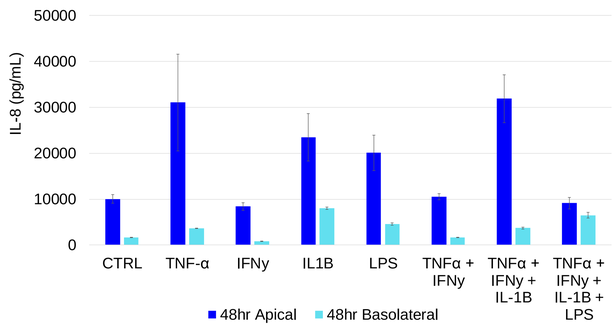


**Figure 5: IL-8 release by EpiColon** following 48hr apical or basolateral treatment with inflammatory cytokines

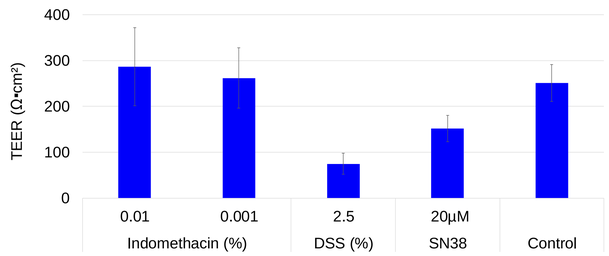


**Figure 6: TEER of EpiColon** Barrier integrity (TEER) of EpiColon decreases following basolateral exposure to Dextran Sulfate Sodium (DSS), and SN-38 (metabolite of the pro-drug Irinotecan) for 48 hr.

## Conclusions

- EpiColon is a reconstructed 3D human colon tissue model which possesses structural architecture and features similar to human colon tissues (**Figures 1-3).**
- Similar to native colon tissues, the 3D colon tissue model produces mucin on the apical surface of the epithelium (**Figure 2**).
- RT-qPCR shows expression of drug metabolizing CYP450 enzymes (**Table 1**).
- Exposure to a Toll-like receptor ligand (LPS) induced release of inflammatory cytokines IL-6 and IL-8 (**Figures 4-5**)
- Exposure of colon tissue to DSS and SN38 for 48 hr showed reduction in TEER (toxicity) (**Figure 6**)
- Our data suggest that the EpiColon 3D human colon tissue model will be a useful tool to study the effects of colorectal care products on toxicity, inflammation, and the colorectal microbiome.