**Study Transportation of Drugs within Newly Established Colon Organoid Systems**

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**Statement of Purpose:** The development of 3D organoids in the small intestine has been a tremendous breakthrough in drug development and biological research. However, colonoid cultures are particularly challenging due to a lack of simple, cost-effective protocols for cultivation. Thus, there is a need to design an easy and cost-effective colonoid culture protocol for functional studies. Here, we describe intestinal homogenates containing many stem cell growth factors as a supplement to the culture medium for maintaining and replicating colonic stem cells. To evaluate the functionality of colonoids, we also performed permeability testing to study the transportation of drugs.

**Methods:** Colonoids were isolated from 129S6 mice colonized with the altered Schaedler flora (ASF) and housed in isolators at Iowa State University. The isolated colon crypts were obtained, seeded in Matrigel, and placed on a pre-warmed 24-well plate. The growth dynamics of colonoids were measured longitudinally over 6 days using a Leica DMi1 inverted microscope. Fixed tissues and colonoids were embedded in paraffin blocks and then sectioned for evaluation by H&E staining and immunohistochemistry. To perform the Fluorescein isothiocyanate dextran (FITC-DEX) staining of colonoids, 1 mg/mL of FITC-DEX 4000 Da (Sigma) and FITC-DEX 40000 Da (Sigma) were added to each well of organoids as one measure of intestinal permeability. To assess whether P-glycoprotein functionality was present in colonoids, 1 μM of Rhodamine123 (Rhod123) (Sigma) and 40 μM Verapamil hydrochloride (Across organics) was added to the wells. Last, the Cystic fibrosis transmembrane regulator (CFTR) conductance for chloride ion transport across the colonoid epithelium was evaluated.

**Results:** The H&E staining of colonoids grown with a 1:1 homogenate/CMGF+ mixture is shown in Figure 1. A smooth surface replaced the outward bud-like protrusions seen in small intestine organoids within colonoids. Moreover, colonoids demonstrated proliferation and a high survival rate after six passages cultured in the homogenate/CMGF+ mixture medium. For drug transport tests, the polyimmunoglobulin receptor (pIgR) is responsible for mediating the transcytosis of polymeric immunoglobulins across the intestinal epithelial layer. As shown in Figure 2, this receptor was expressed on the basolateral surface of colonoids. For the functionality and transport experiments, neither 4 kDa nor 40 kDa FITC-Dex diffused across the epithelium and into the lumen of the colonoids suggesting that the epithelial barrier was intact. Because of its importance in transporting xenobiotics out of epithelial cells, the fluorescent dye Rhod123 was used to investigate P-glycoprotein function. Next, verapamil was added to the colonoid system to inhibit the P-glycoprotein transport of Rhod123. Results indicated that Rhod123 transport was markedly reduced because verapamil inhibited the epithelial P-glycoprotein transporters on the apical membrane, and less Rhod123 accumulated in the lumen of the colonoids as compared to the same experimental conditions without verapamil.

**Conclusions:** We created a simple, cost-effective protocol for colonoid cultivation for more than 3 months. We performed permeability testing with suspensions of 4 kDa and 40 kDa FITC-DEX. The P-glycoprotein receptor was manipulated, as evidenced by its inhibition function. Forskolin treatment which affects chloride transport, confirmed the CFTR transporter. Our new colonoid model shows promise as a tool for high-throughput drug screening, toxicity testing, and oral drug development.

**References:**