Isolation, Culture and Functionality Studies of 3D Colonoids Using the Original Niches

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Statement of Purpose: The development of 3D organoids of the small intestine has been a tremendous breakthrough in drug development and biological research. However, the 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 method for colonoid culture *ex vivo*. Here, we describe intestinal homogenate using the original niches of stem cell growth factors as a culture medium for maintenance and replication of the colonoids. Colonoids generated by this cultivation protocol demonstrated substantial proliferation and differentiation, preserving their structure for more than three months. To evaluate the functionality of colonoids, we also performed permeability testing and transplantation studies *in vivo*.

Methods: Colonoids were isolated from 129S6 mice colonized with the altered Schaedler flora (ASF) and housed in isolators at Iowa State University. Following humane euthanasia by CO asphyxiation, murine colons were removed, the feces evacuated within the lumen, and tissues were cut into small pieces and washed with PBS several times. After several washing steps, the upper one-half of the tissue-PBS mixture was removed, 30 mL of ethylenediaminetetraacetic acid (EDTA) was added. After that, isolated colon crypts were obtained. Next, the crypts were seeded in Matrigel and placed on a pre-warmed 24-well plate. For making the colonic homogenate, murine small intestine and colons were excised following euthanasia. Intestinal tissues were cut horizontally and washed with ice-cold PBS three times, then cut into small pieces. Basal medium was added to the tissue using a 1:20 W/V ratio and was homogenized with an Omni blender (OMNI TH, OMNI International) for 2 minutes. The supernatant was aspirated and transferred to a new tube. 10 % FBS was added to the supernatant, and the solution was filter sterilized with a 0.2 µm filter. The solution was stored frozen at -20 ℃ until further use. For histopathology (H&E) and immunohistochemistry (IHC), small intestine and colon tissue were incubated in 10% formalin (Fisher) for 24h then stored in 70% ethanol. 8-10 wells of organoids and colonoids were fixed in 10% formalin for 7 minutes and stored in 70% ethanol. Fixed tissues and colonoids were embedded in paraffin blocks then sectioned for evaluation by H&E staining and immunohistochemistry. We further performed the Fluorescein isothiocyanate dextran (FITC-DEX) staining of colonoids, the Efflux Transporter inhibition by Verapamil, the Cystic fibrosis transmembrane regulator (CFTR) conductance for chloride ion transport, and the transplantation trials *in vivo*.

Results: We performed permeability testing with suspensions of 4 kDa and 40 kDa colloids and observed that neither could permeate the epithelial barrier. The P-glycoprotein receptor, a vital drug efflux pump mediating potential drug toxicity, was functional since its expression was inhibited by verapamil as measured by Rhodamin 123 fluorescence. Forskolin treatment (resulted in organoid swelling, which confirmed the functionality of this transporter. The *in vivo* transplantation studies showed that the engrafted colonoids healed the lesion and regenerated the epithelial layer.

Conclusions: Our new colonoid model shows promise as a platform for drug screening, toxicity testing experiments, and colon tissue repair.

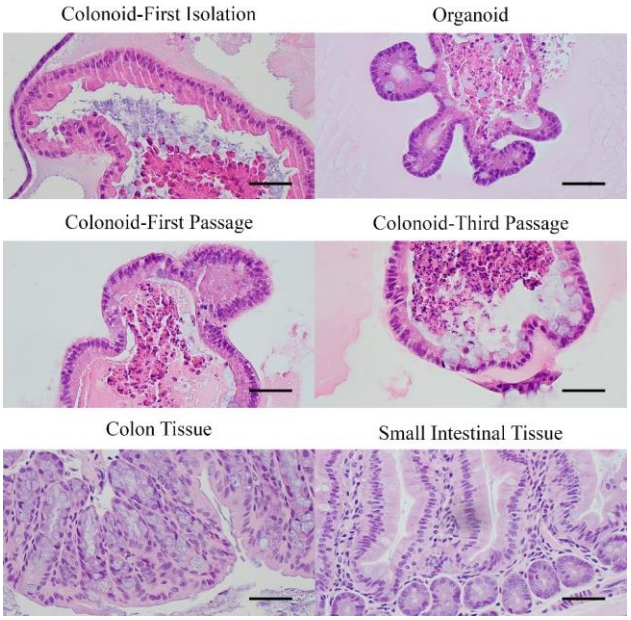

Figure 1. Representative hematoxylin and eosin staining of colonoids at first isolation, first and third passage, small intestinal organoids, and primary tissues of 129 ASF mice. Cultured colonoids simulate the normal colic epithelium structure while organoids more closely resemble normal colon. Scale bars represent 50 µm.

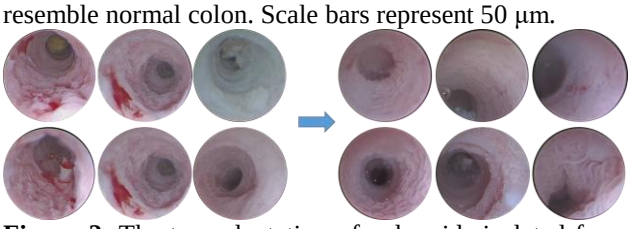

Figure 2. The transplantation of colonoids isolated from 129S ASF mice to 129S ASF IL-10 knock-out mice using colonoscopy. Colon images before (left) and after (right) the IL-10 colonoids transplant treatment showed that the engrafted colonoids healed the lesion and regenerated the epithelial layer.

References:

[1]Davoudi Z, Wang Q, et al. Marine Drugs. 2021;19: 282.
[2]Davoudi Z, Wang Q, et al. JBMR A. 2018;106: 876.