EFFICIENT ESTABLISHMENT AND LONG-TERM MAINTENANCE OF 3-DIMENSIONAL HUMAN SMALL INTESTINAL AND COLONIC ORGANOIDS USING A NOVEL INTESTICULT™ ORGANOID GROWTH MEDIUM (HUMAN)

Ryan K. Conder[1], Fisal Elstone[1], Marianne Lankhorst[1], Martin Stahl[1,2], Tyler Brown[1], Michael West[1], Michael J. Riedel[1], Bruce A. Vallance[2], Terry E. Thomas[1], Allen C. Eaves[1,3], and Sharon A. Louis[1]

[1]STEMCELL Technologies Inc., Vancouver, BC, Canada [2] BC Children's Hospital Research Institute, Vancouver, Canada [3] Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

Introduction

Intestinal organoids are a valuable model for studying epithelial stem cell biology as well as for investigating the structural and functional mechanisms of the mammalian intestine. Recently, we developed IntestiCult™ Organoid Growth Medium (OGM) Mouse, a novel medium for the establishment and long-term maintenance of mouse intestinal organoid cultures. This medium has helped eliminate much of the variability inherent to mouse intestinal organoid cultures and allowed for greater standardization. With the release of the counterpart medium to the mouse OGM for human intestinal cells, similar benefits are seen in human organoid cultures enhancing the research possibilities of this model system.

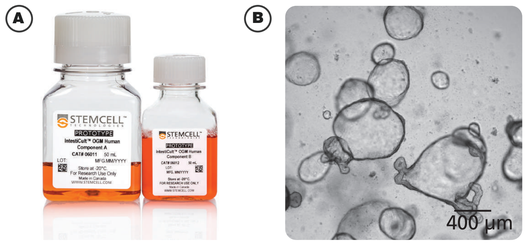


Figure 1. Human Colon-Derived Organoids can be Cultured using IntestiCult™ OGM (Human)
Colonic organoids can be established and maintained in vitro from individual patients using **(A)** IntestiCult™ OGM (Human). **(B)** The culture is representative of typical organoids at day 5 - 7 of each passage.

Methods

Crypt Culture

Intestinal crypts were isolated from both patient tissue resections and biopsies. The lamina propria and submucosal layers were removed from the tissue samples before processing. Biopsies or tissue samples were minced into fragments and collected in a 1.5 mL Eppendorf Tube®, washed with PBS and transferred to 15 mL conical vial. The human colon tissue samples were then incubated with Gentle Cell Dissociation Reagent (GCDR) for 30 minutes at room temperature with gentle agitation. The sample was pelleted and re-suspended in DMEM/F12 + 1% Bovine Serum Albumin where the fragments were vigorously pipetted approximately 20 times to release the crypts from the tissue. The contents of the vial were then passed through a 70 µM filter to remove debris from the crypt suspension. The liberated crypts were then washed again, pelleted and resuspended in an ice-cold mixture of 50% IntestiCult™ Organoid Growth Medium (Human) and Corning® Matrigel®. The mixture, containing the intestinal crypts was plated in a 24-well plate that had been previously incubated at 37˚C to create a dome structure and immediately incubated at 37˚C for 10 minutes. The wells were then filled with IntestiCult™ OGM (Human) and cultured at 37˚C with 3 medium changes per week. Organoids were passaged every 7 - 10 days by dissociation in GCDR for 10 minutes and plated in 50 µL Corning® Matrigel® domes at an approximate density of 200 organoids per well (5 patient samples were used in this study).

2-Dimensional Monolayer

Cultured human intestinal organoids were dissociated with 0.05% trypsin-EDTA for 5 minutes and the resulting single-cell suspensions were seeded in IntestiCult™ OGM onto Corning® Matrigel®-coated Transwell® inserts. IntestiCult™ OGM was exchanged every 2-3 days as the cells grew to confluence, approximately 5-8 days post seeding.

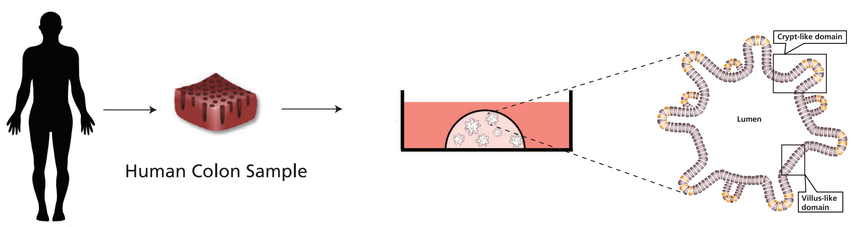


Figure 2. Biopsy Culture and Passaging

Growth Percentage

For each culture, a growth percentage of either crypts or organoid fragments was determined by calculating the number of organoids growing on day 5 as a fraction of the total number of crypts that had begun to form an epithelium approximately 4 hours after plating. The growth percentage as a representative metric for the efficiency of organoid establishment was tracked for each culture.

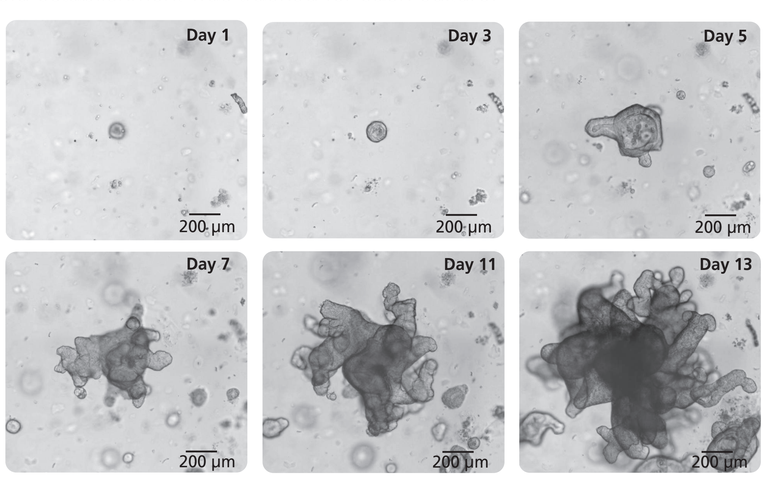


Figure 3. Human Colonic Crypts Establish Mature Organoids within 10 Days
Dissociated intestinal crypts form visible spheres within 1 day post culture. These increase in size and often begin to form "buds" by day 7. By day 11, multi-budded structures representing mature organoids can by passaged or collected and used in end-point analysis.

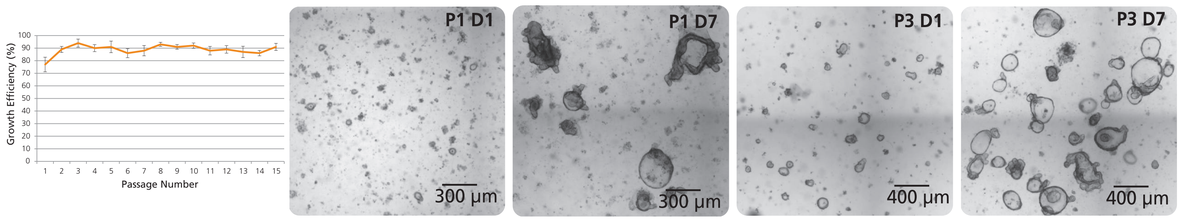


Figure 4. Human Colon-Derived Organoids can be Passaged Efficiently
Organoid cultures can be efficiently expanded over 5 passages. Data taken from 3 individual patient samples demonstrates that after primary culture, the growth percentage remained consistently greater than 75% and increased in later passages. From the second passage, organoid density and morphology also remained consistent throughout passaging (Mean ± SEM, n = 3, Technical Replicates). Images show organoid morphology at day 1 and day 7 after passaging.

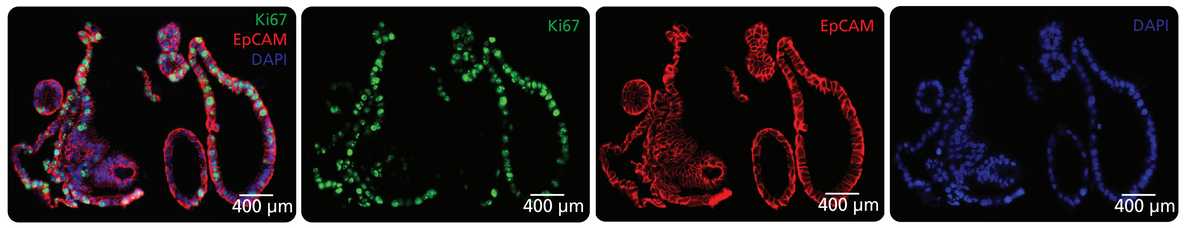
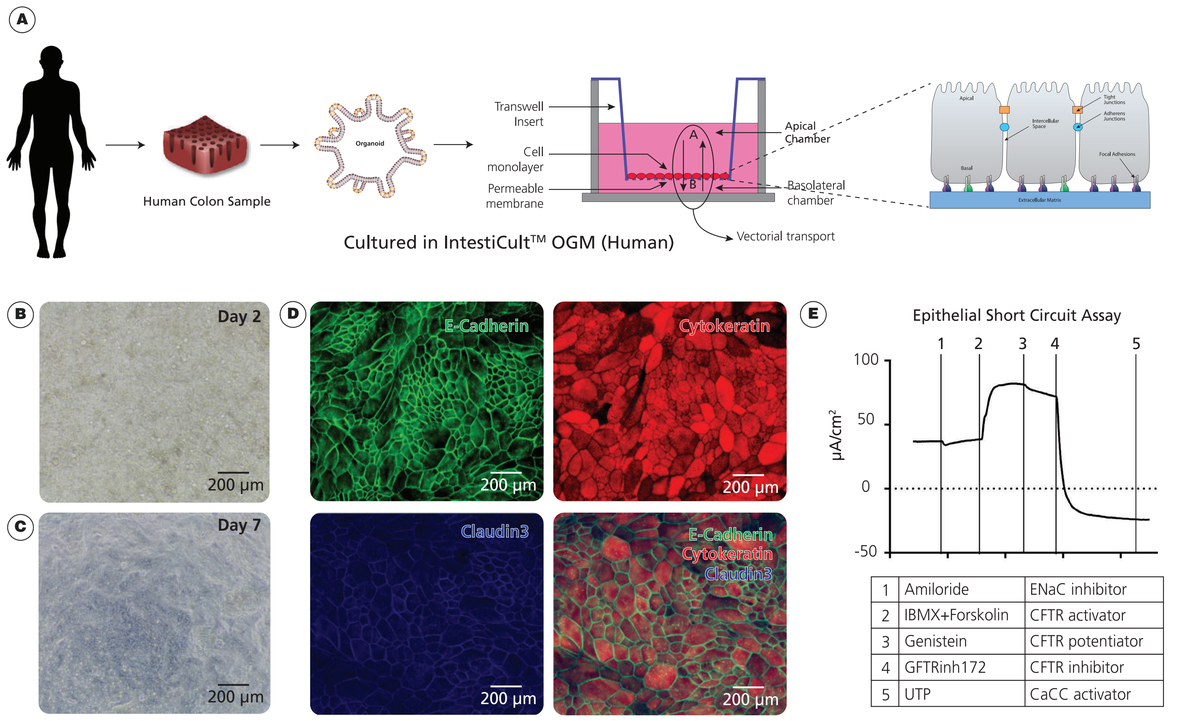


Figure 5. Human Colon-Derived Organoid Cultures Mimic the Human Intestinal Epithelium
IntestiCult™ OGM (Human) promotes robust growth of intestinal stem cells (ISC) and provides necessary conditions for the development of intestinal crypts. Ki67 (green) staining of proliferating cells shows high levels of ISC and transit amplifying cells primarily in the intestinal crypt. Further localization of EpCAM (red) defines the cellular junctions of the epithelia and DAPI (blue) marks the cell nuclei.



1	Amiloride	ENaC inhibitor
2	IBMX+Forskolin	CFTR activator
3	Genistein	CFTR potentiator
4	GFTRinh172	CFTR inhibitor
5	UTP	CaCC activator

Figure 6. 2-Dimensional Monolayers from Human Colon Derived Organoids
2-Dimensional monolayers, grown on transwell plates can be efficiently formed from human colon derived organoids grown in IntestiCult™ OGM (Human) **(A)**. Brightfield images of colonic epithelial monolayers at 2 days **(B)** and 7 days **(C)** post seeding. 3 day old monolayers display protein markers of E-cadherin (green), Cytokeratin (red) and Claudin3 (blue) **(D)**. Transepithelial resistance Using chamber readings **(E)** from cells treated with Amiloride (1), IBMX+Forskolin (2), Genistein (3), CFTRinh172 (4) and UTP (5) (n = 3).

Summary

Advantages of IntestiCult™ Organoid Growth Medium (Human):

- COMPLETE - A complete medium not requiring additional cytokines or growth factors
- RELEVANT - Enables efficient culture and expansion of intestinal stem cells and differentiated cell types within organoids
- SUPPORTS - Both 3D and 2D intestinal cultures
- ROBUST - Consistent generation of intestinal-derived organoids in one week from multiple patient samples
- EASY-TO-USE - Simple format and easy-to-follow, optimized protocol