

Efficient Establishment and Growth of Human Intestinal Organoid-Derived Monolayer Cultures

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INTRODUCTION

Organoid culture systems are a cutting-edge tool for regenerative medicine, disease modeling, and drug screening. STEMdiff™ Intestinal Organoid Kit and IntestiCult™ Organoid Growth Medium (OGM; Human) are used for the derivation and expansion of human intestinal organoids from human pluripotent stem cells (hPSCs) and primary tissues, respectively. However, 3D Matrigel®-embedded organoids can be difficult to apply to certain experimental or high-throughput formats, which are more amenable to the traditional 2D monolayer culture typically used with the cancer cell line Caco-2, or other intestinal cell lines. We have developed a method that uses IntestiCult™ OGM to culture human, mouse, or hPSC-derived organoids as a 2D monolayer culture that continues to recapitulate unique features of the intestinal epithelium. The focus of the current study is to characterize the established 2D monolayers derived from organoids, and to compare the physiological properties of these intestinal epithelial monolayers to the Caco-2 cell line. To do this, primary human intestinal organoids were seeded in IntestiCult™ OGM onto Corning® Matrigel®-coated tissue culture and Transwell® plates. After 7 days, the cultures were analyzed by immunohistochemistry, transepithelial electrical resistance (TEER) measurements to measure barrier integrity, and by Ussing chamber analysis to measure ion transport.

MATERIALS AND METHODS

Organoid-Derived Monolayer Culture

Intestinal organoids derived from hPSC cultures, human biopsies, or mouse intestinal samples were cultured and passaged in 50% Corning® Matrigel® domes according to manufacturer protocols supplied with the STEMdiff™ Intestinal Organoid and IntestiCult™ OGM Kits. To establish the monolayer cultures, mature, 7- to 10-day-old organoid cultures were harvested from the Matrigel® domes using Gentle Cell Dissociation Reagent (GCDR) and then pooled. Organoids were washed once with DMEM/F-12 and incubated at 37°C for 10 minutes in 0.05% trypsin-EDTA to dissociate the organoids into small clumps and single cells. The cells were washed again in DMEM/F-12, then resuspended in IntestiCult™ OGM containing 10 µM rho-kinase inhibitor Y-27632. The cell suspension was then seeded into Transwell® inserts or tissue culture wells pre-coated with a 2% Matrigel® solution.

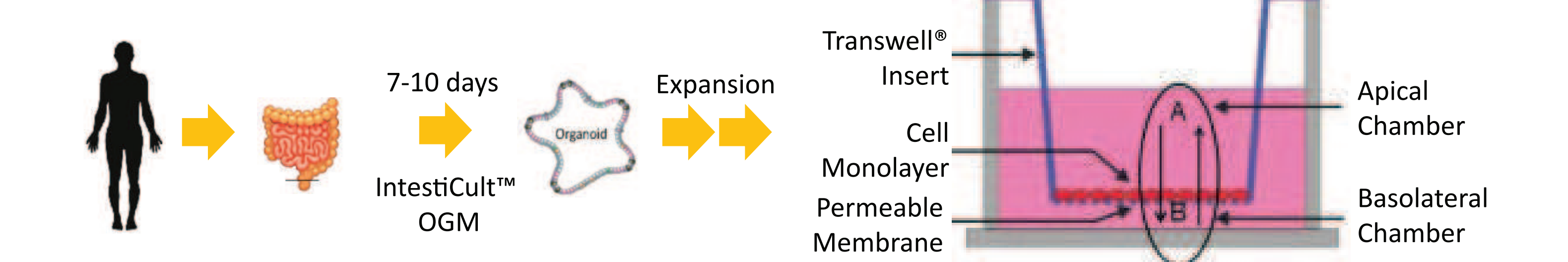


FIGURE 1. Workflow for the Establishment of Monolayer Cultures in a Transwell® Insert

Human PSC-derived intestinal and biopsies collected from the intestines of donor patients were cultivated as 3D intestinal organoid cultures using IntestiCult™ OGM, to maintain and expand the stem cell population. Organoids were then collected and seeded into a Transwell® culture insert with IntestiCult™ OGM + 10 µM Y-27632 to establish a monolayer culture.

RESULTS

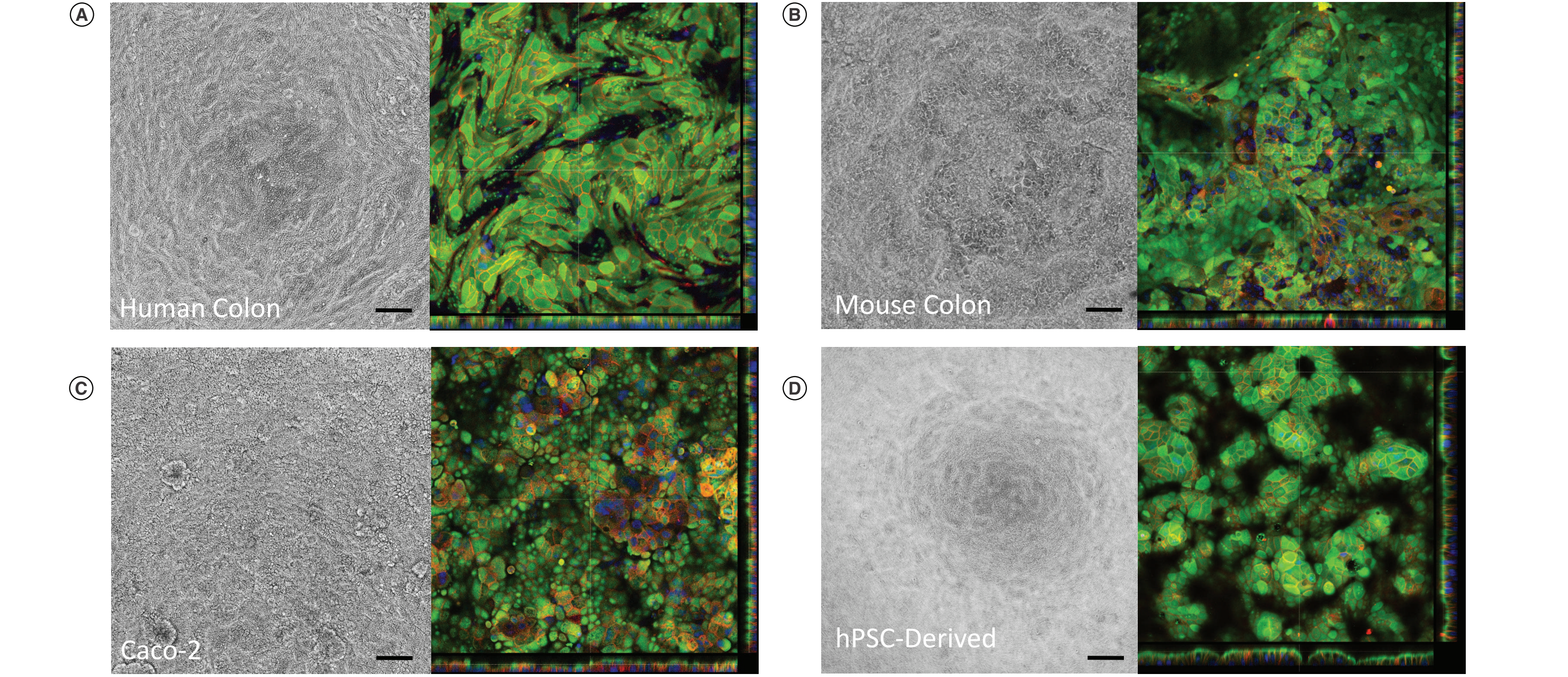


FIGURE 2. Organoid-Derived Monolayer Cultures

Representative brightfield (left) and immunofluorescent images (right) of monolayers derived from (A) human colon, (B) mouse colon, (C) Caco-2 cells (P25, D21), and (D) hPSC-derived organoids (H9 embryonic stem cell line). Immunofluorescent staining for villin (green), E-cadherin (red), and DAPI stain for cell nuclei (blue). Villin staining along the apical edge of the cells indicates cell polarization, and E-cadherin staining indicates the presence of adherens junctions. DAPI stain indicates the presence of the nuclei near the basolateral pole of the epithelial cells. Scale bars = 500 µm.

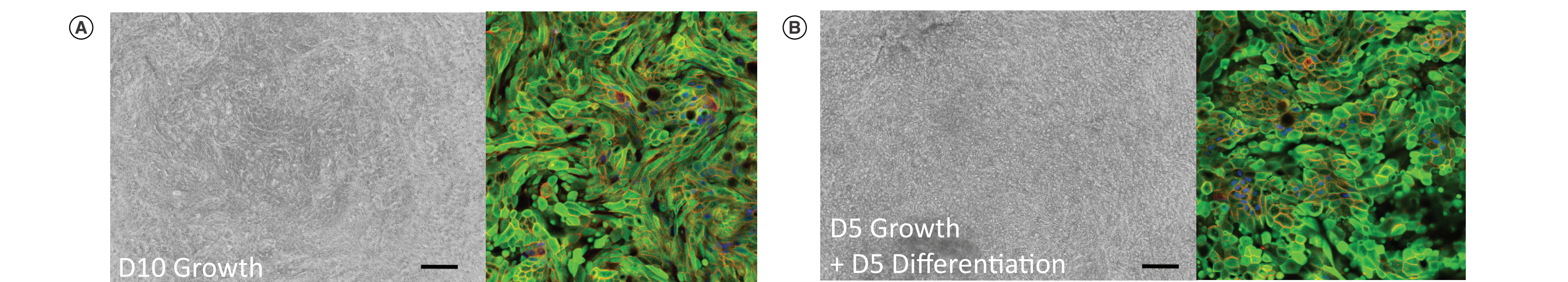


FIGURE 3. Differentiation of Human Colonic Monolayers

To enhance differentiation of the primary intestinal monolayer culture, Component B from IntestiCult™ OGM can be removed and replaced with DMEM/F-12, which removes the factors supporting the stem cell niche and transit amplifying cells, and allows further differentiation of the epithelium. Compared to the IntestiCult™ OGM control (A), the differentiated culture (B) increased maturation and polarization of enterocytes as illustrated by the increased “cobblestone” appearance of the monolayer in the increase of villin staining along the apical border of the epithelial cells. Scale bars = 500 µm.

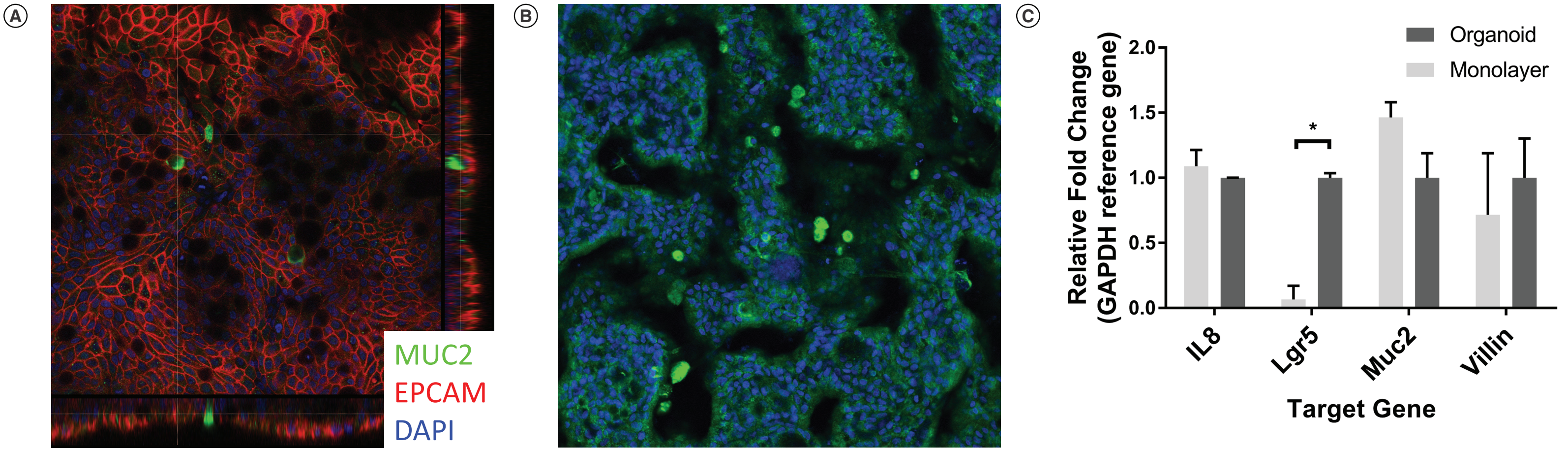


FIGURE 4. Organoid-Derived Monolayers Express Markers for Different Cell Types

Anti-MUC2 antibody (green) was used to stain for the presence of goblet cells within the intestinal monolayer cultures. Positive staining for the presence of goblet cells was detected in both (A) human colon organoids and (B) hPSC-derived (H9 cell line) monolayers. (C) The 3D organoid cultures maintained in IntestiCult™ OGM maintain the stem cell niche. The monolayer format, even without added differentiation steps, supports a more differentiated state, as exemplified by a relative drop in Lgr5 expression and an increase in MUC2 expression (n = 3, *p < 0.01).

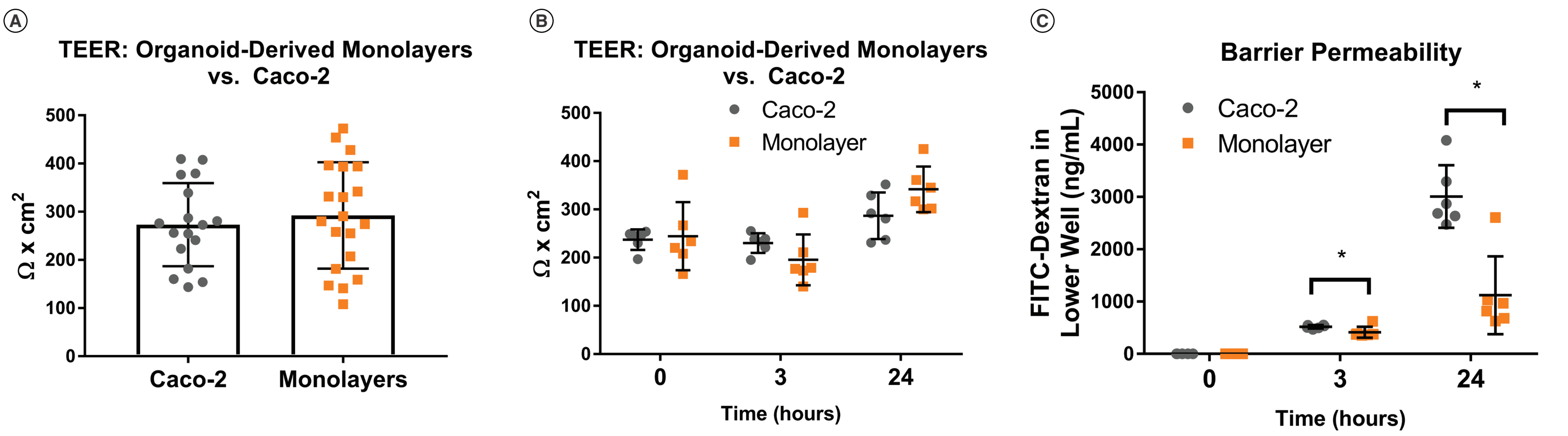


FIGURE 5. Organoid-Derived Monolayers Have High TEER Values and Low Permeability to FITC-Dextran

A common measure for the thickness of a cell monolayer and the quality of the cell-cell tight junctions is a measurement of trans-epithelial electrical resistance (TEER). (A) A direct comparison of organoid-derived monolayers grown for 7 days with IntestiCult™ OGM and Caco-2 cells grown for 21 days with DMEM/F-12 + 10% fetal bovine serum (FBS) indicates that under those conditions, they each yield comparable TEER values. Organoid-derived monolayers have TEER values averaging 358.5 ± 22.6 Ω*cm² (SEM; n = 12), compared to an average of 301.1 ± 22.3 Ω*cm² (SEM; n = 10) for comparable Caco-2 monolayers, demonstrating that these cells have equivalent or greater barrier function. (B) Equally, when the medium in both cultures is replaced with DMEM/F-12 + 2% FBS, TEER readings remain consistent in both cultures over a 24-hour period. (C) However, under the same experimental conditions, when FITC-dextran is loaded into the apical well, the Caco-2 cells prove to be much more permeable over the course of 24 hours, while the organoid-derived monolayer maintains superior barrier function over time (n = 6, *P < 0.05).

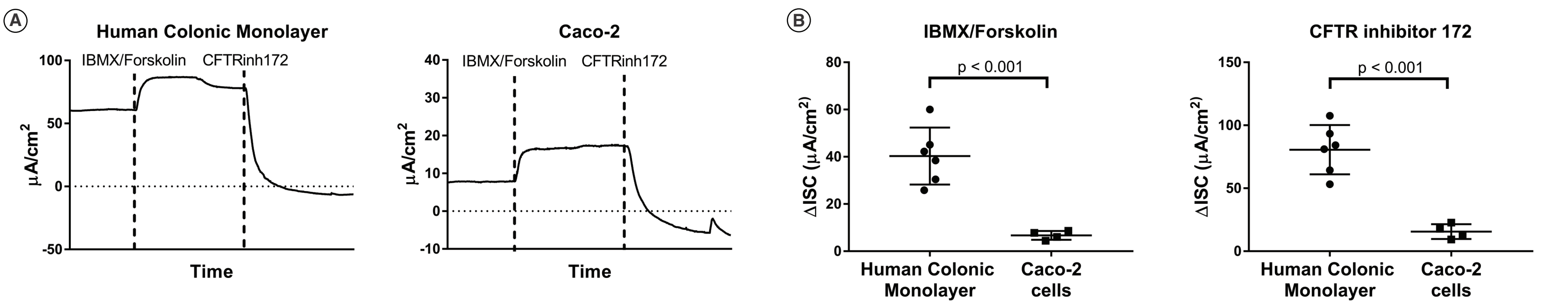


FIGURE 6. Superior CFTR Activity in Organoid-Derived Monolayers Compared to Caco-2 Cell Culture

CFTR is one of the key ion transporters involved in regulating fluid secretion in the intestinal epithelium, and when defective in cystic fibrosis it can cause significant health complications in both the lungs and intestine. (A) Here, we used an Ussing chamber to compare the activity of CFTR upon activation by IBMX/forskolin and inhibition by CFTR inhibitor 172 (CFTRinh172) in human colonic monolayers and Caco-2 cells. (B) Treatment with IBMX and forskolin increases the activity by a change in short-circuit current (ΔISC) of 40.3 ± 7.62 µA/cm² (SEM; n = 6) in human colonic monolayers, significantly more than 6.7 ± 0.61 µA/cm² (SEM; n = 4; p = 0.04) for Caco-2 cell cultures. Treatment with CFTRinh172 reduced CFTR activity by a ΔISC of 80.6 ± 5.96 µA/cm² (SEM; n = 6) in human colonic monolayers compared to 15.5 ± 1.98 µA/cm² (SEM; n = 4; p < 0.0001) in Caco-2 cell cultures, demonstrating increased sensitivity in colonic monolayers. Similar experiments were conducted on hPSC-derived organoids, however, they demonstrated no responsiveness to CFTR activation or inhibition.

Summary

- IntestiCult™ OGM Human can be used to establish a primary cell monolayer culture directly from a 3D organoid culture
- These organoid-derived monolayers are confluent, polarized, and contain tight junctions
- Diverse cell types are present, including stem cells, enterocytes and functional goblet cells in organoid-derived monolayers.
- Organoid-derived monolayers are superior to Caco-2 cell cultures at maintaining barrier integrity
- CFTR activity in human colonic monolayers is superior to Caco-2 cell cultures and is more responsive to both activation and inhibition molecules.