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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Enhanced Utilization of Induced Pluripotent Stem Cell–Derived Human Intestinal Organoids Using Microengineered Chips

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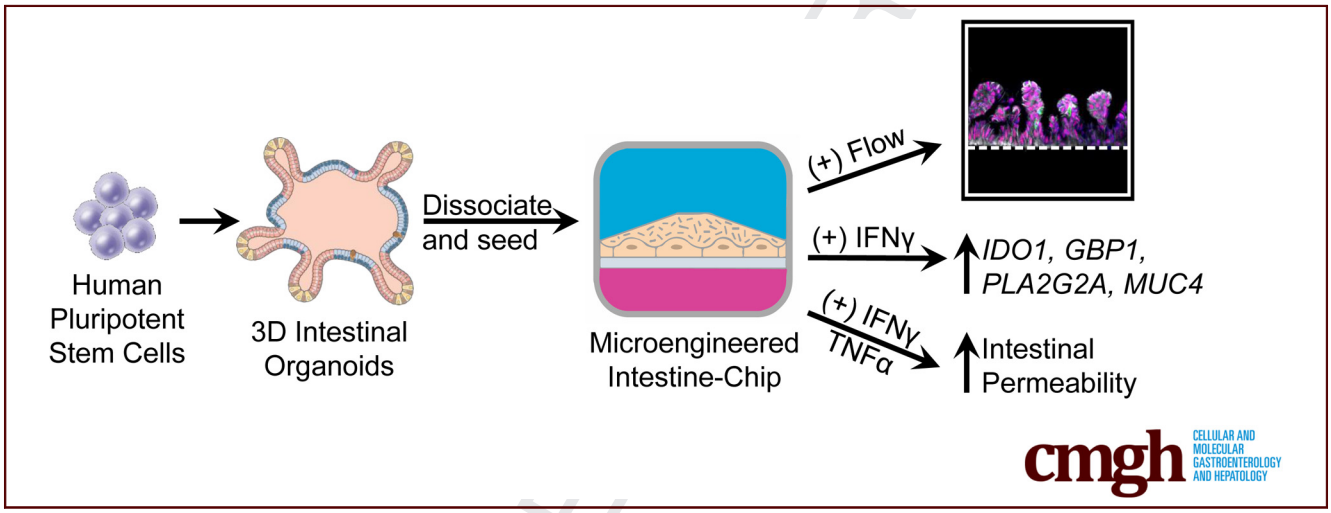

SUMMARY

The 3-dimensional structure of human intestinal organoids makes them challenging to use. Here we describe how organoids, derived from induced pluripotent stem cells, can be incorporated into small microengineered Chips making them more amenable for study.

BACKGROUND AND AIMS: Human intestinal organoids derived from induced pluripotent stem cells have tremendous potential to elucidate the intestinal epithelium's role in health and disease, but it is difficult to directly assay these complex structures. This study sought to make this technology more amenable for study by obtaining epithelial cells from induced pluripotent stem cell–derived human intestinal organoids and incorporating them into small microengineered Chips. We then investigated if these cells within the Chip were polarized, had the 4 major intestinal epithelial subtypes, and were biologically responsive to exogenous stimuli.

METHODS: Epithelial cells were positively selected from human intestinal organoids and were incorporated into the Chip. The effect of continuous media flow was examined. Immunocytochemistry and *in situ* hybridization were used to demonstrate that the epithelial cells were polarized and possessed the major intestinal epithelial subtypes. To assess if the incorporated cells were biologically responsive, Western blot analysis and quantitative polymerase chain reaction were used to assess the effects of interferon (IFN)-γ, and fluorescein isothiocyanate–dextran 4 kDa permeation was used to assess the effects of IFN-γ and tumor necrosis factor-α on barrier function.

RESULTS: The optimal cell seeding density and flow rate were established. The continuous administration of flow resulted in the formation of polarized intestinal folds that contained Paneth cells, goblet cells, enterocytes, and enteroendocrine cells along with transit-amplifying and $LGR5^{+}$ stem cells. Administration of IFN-γ for 1 hour resulted in the phosphorylation of STAT1, whereas exposure for 3 days resulted in a significant upregulation of IFN-γ related genes. Administration of IFN-γ and tumor necrosis factor-α for 3 days resulted in an increase in intestinal permeability.

CONCLUSIONS: We demonstrate that the Intestine-Chip is polarized, contains all the intestinal epithelial subtypes, and is biologically responsive to exogenous stimuli. This represents a more amenable platform to use organoid technology and will be highly applicable to personalized medicine

and a wide range of gastrointestinal conditions. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2017.12.008)*

Keywords: Human Intestinal Organoids; Induced Pluripotent Stem Cells; Small Microengineered Chips.

Studies examining human intestinal epithelial cell function have been severely hampered because primary intestinal epithelial cells rapidly undergo apoptosis when cultured *ex vivo*.[1,2] Although adenocarcinoma lines, such as Caco-2 cells, recapitulate some aspects of intestinal function, namely barrier function, a substantial breakthrough in the intestinal epithelial field occurred when it was reported that 3-dimensional human intestinal "organoids" (HIO) could be generated from either human biopsy samples[3,4] or induced pluripotent stem cells (iPSCs).[5] Irrespective of how these organoids are derived, they contain all the intestinal epithelial subtypes, are polarized toward the lumen, and can be maintained for prolonged periods of time in a tightly controlled milieu. However, there are substantial technical challenges associated with this technology. Organoids are heterogeneous both in shape and size, which may lead to inconsistent findings. Access to the lumen, which is crucial for assessing intestinal permeability, microbial-epithelial interactions, and drug absorption are technically challenging. Coculture with other cell types, such as immune cell subtypes or endothelial cells, is also difficult given that organoids are typically embedded in a 3-dimensional matrix.

One potential way to overcome such challenges is to combine intestinal organoid culture with microengineering technology. Small microengineered Chips are integrated systems that place living human cells in precisely microengineered environments that can more accurately recapitulate human physiology and disease states. They allow unprecedented control over key physiological aspects, such as interactions between tissues, mechanical forces, blood and immune components, and the biochemical milieu.[6] This engineering also allows the tuning and control of the microenvironment. Indeed, lung[7,8] and Caco-2 cells[9–11] have previously been incorporated into such Chips and studies examining epithelial-immune cell interactions, prolonged epithelial-microbial interactions, and permeability experiments have all successfully been performed.

Given that iPSCs can be generated from any individual,[12] iPSC-derived HIOs were chosen for incorporation into this Chip system, thereby permitting the study of intestinal epithelial cells from virtually any patient or nondiseased control. It also allows for the generation of other patient-specific cell types, such as macrophages,[13] dendritic cells,[14] and neutrophils,[15] that can also be incorporated into the Chip and used to study multicellular interactions. Furthermore, we have previously reported that lymphoblastoid cell lines can be reliably reprogrammed to form iPSCs.[16] Given there are numerous lymphoblastoid cell lines available in well-characterized worldwide repositories that are linked to patient clinical history and long-term genotype-phenotype data,[17] studies into epithelial cells and their interactions with other cell types from well-characterized patient cohorts are now possible.

Materials and Methods

Ethics Statement

All the cell lines and protocols in the present study were carried out in accordance with the guidelines approved by the stem cell research oversight committee and institutional review board at the Cedars-Sinai Medical Center under the auspice of the institutional review board stem cell research oversight committee protocols Pro00027264 (Derivation of Intestinal Stem Cells). All authors had access to the study data and have reviewed and approved the final manuscript.

Cell Lines and Culturing Conditions

Two iPSC lines (CS83iCTR-33n1 and CS688iCTR-n5) were obtained from the iPSC Core at Cedars-Sinai. Both lines were fully characterized and were confirmed to be karyotypically normal. All iPSC lines were maintained in an undifferentiated state on Matrigel-coated plates in mTeSR1 media (Stem Cell Technologies) under feeder-free conditions. All iPSC cultures were tested monthly for mycoplasma contamination. Caco-2 (ATCC HTB-37) cells were cultured in EMEM supplemented with 10% fetal bovine serum (Gibco).

Small Microengineering Chip Microfabrication

Chips were fabricated using modified methods for Chip microfabrication as previously described.[18] Briefly, poly(dimethylsiloxane) (PDMS) prepolymer was mixed at a 10:1 ratio of PDMS base to curing agent, wt/wt using a planetary mixer (Thinky ARE-310). PDMS prepolymer was then cast onto molds forming the microchannels of the upper layer (1000 μm wide $\times$ 1000 μm high) and lower layer (1000 μm wide $\times$ 200 μm high). The membrane was cast onto a silicon mold that was fabricated using photolithography and deep reactive ion etching, resulting in 7-μm pores. The components were cured overnight and removed from the mold. The upper layer, membrane, and lower layer were permanently bonded via plasma bonding to form the complete Chip.

Generation of Human Intestinal Organoids From Induced Pluripotent Stem Cells

The generation of HIOs from iPSCs involves a multistep technique whereby iPSCs were directed to form definitive endoderm, epithelial structures, and ultimately organoids.

Abbreviations used in this paper: **GBP1, guanylate binding protein 1; HIOs, human intestinal organoids; IDO1, indolamine 2,3-dioxygenase 1; IFN-γ, interferon-γ; iPSCs, induced pluripotent stem cells; PDMS, poly(dimethylsiloxane); TNF-α, tumor necrosis factor-α.**

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