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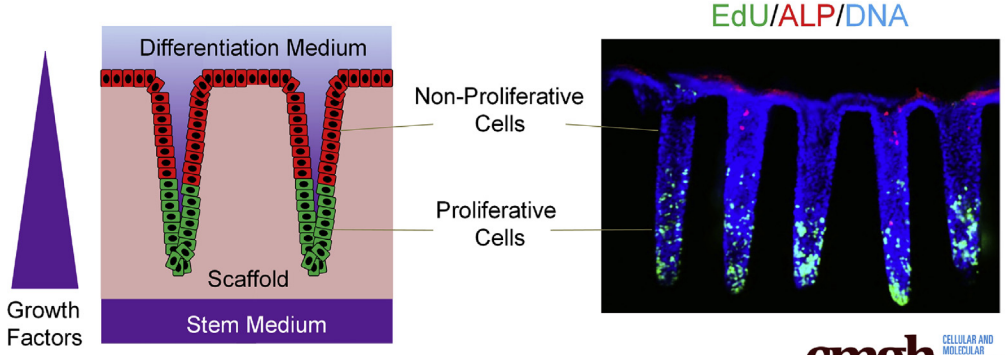
ORIGINAL RESEARCH

Formation of Human Colonic Crypt Array by Application of Chemical Gradients Across a Shaped Epithelial Monolayer

Yuli Wang,[1] Raehyun Kim,[2] Dulan B. Gunasekara,[1] Mark I. Reed,[1] Matthew DiSalvo,[2] Daniel L. Nguyen,[1] Scott J. Bultman,[3] Christopher E. Sims,[1] Scott T. Magness,[2] and Nancy L. Allbritton[1,2]

[1]Department of Chemistry, [3]Department of Genetics, University of North Carolina, Chapel Hill, North Carolina; [2]Joint Department of Biomedical Engineering, University of North Carolina, Chapel Hill, and North Carolina State University, Raleigh, North Carolina



SUMMARY

Human colonic epithelia were cultured on a microfabricated scaffold under a growth factor gradient to generate crypt structures. Responses to cytokines or bacterial metabolite gradients were assessed from altered stem and differentiated cell numbers and locations.

BACKGROUND & AIMS: The successful culture of intestinal organoids has greatly enhanced our understanding of intestinal stem cell physiology and enabled the generation of novel intestinal disease models. Although of tremendous value, intestinal organoid culture systems have not yet fully recapitulated the anatomy or physiology of the in vivo intestinal epithelium. The aim of this work was to re-create an intestinal epithelium with a high density of polarized crypts that respond in a physiologic manner to addition of growth factors, metabolites, or cytokines to the basal or luminal tissue surface as occurs in vivo.

METHODS: A self-renewing monolayer of human intestinal epithelium was cultured on a collagen scaffold microfabricated with an array of crypt-like invaginations. Placement of chemical factors in either the fluid reservoir below or above the cell-covered scaffolding created a gradient of that chemical across the growing epithelial tissue possessing the in vitro crypt structures. Crypt polarization (size of the stem/proliferative and differentiated cell zones) was assessed in response to gradients of growth factors, cytokines, and bacterial metabolites.

RESULTS: Chemical gradients applied to the shaped human epithelium re-created the stem/proliferative and differentiated cell zones of the in vivo intestine. Short-chain fatty acids applied as a gradient from the luminal side confirmed long-standing hypotheses that butyrate diminished stem/progenitor cell proliferation and promoted differentiation into absorptive colonocytes. A gradient of interferon-γ and tumor necrosis factor-α significantly suppressed the stem/progenitor cell proliferation, altering crypt formation.

CONCLUSIONS: The in vitro human colon crypt array accurately mimicked the architecture, luminal accessibility, tissue polarity, cell migration, and cellular responses of in vivo intestinal crypts. *(Cell Mol Gastroenterol Hepatol 2018;5:113–130; https://doi.org/10.1016/j.jcmgh.2017.10.007)*

Keywords: Intestinal Epithelial Cells; Intestine-On-A-Chip; Stem Cell Niche; Polarized Crypt.

Organ-on-chip technology is a rapidly advancing field that is expected to usher in completely new approaches to drug testing and biological study.[1] Organ-on-chip devices strive to combine microengineered environments with living cells to produce physiologic systems. Devices are being developed to recapitulate the structure and function of a variety of organs, including liver, heart, and lung.[2] The expectation is that these devices, particularly when incorporating human cells, will create a revolution in the study of human biology and drug development.[3,4] Among these systems, the large intestine especially represents an important organ for in vitro study of intestinal physiology and the evaluation of the effects of pharmaceutical agents and microbial metabolites. For example, pharma and food companies are intensely interested in screening the gut microbiome and the effects of prebiotics and probiotics by virtue of their roles in metabolism and their influences on the human body.[5,6] Despite this importance, major challenges exist in creating an in vitro intestinal epithelium because the intestinal lining is a highly polarized tissue and primary gut epithelium rapidly dies in standard culture. Current gold standard methods for intestinal assays use tumor cell lines, such as Caco-2, and animals.[7–10] This is problematic because tumor cells lack many of the features of normal intestinal tissue and animal models are expensive and increasingly fraught by ethical concerns.

Recently developed intestinal stem-cell culture methods are expected to dramatically improve this situation.[11,12] It now is possible to create multicellular structures known as organoids or mini-guts from primary animal and human stem cells.[13–15] These structures possess self-renewing stem cells and their differentiated progeny to reproduce intestinal epithelium in a culture dish. The potential for the organoid technology is enormous; nevertheless, the enclosed cystic, spherical architecture of organoids presents severe limitations.[16–20] Because of the bulk properties of the matrix and lack of spatial control of growth factors (eg, biochemical gradients), intestinal organoids form embedded within a paddy of Matrigel (Corning, Tewksbury, MA) with an enclosed, inaccessible lumen and random buds lacking distinct stem/transit-amplifying and differentiated cell compartments.[21,22] These characteristics preclude the use of the organoid system in numerous applications and a true living construct suitable for assay of dietary metabolites, cytokines, microorganisms, and drug interactions has not been available.[23,24] What is needed is the ability to recreate the epithelium of the organoid system in an open-faced geometry while maintaining its cellular composition, polarity, and physiology. Our group has been striving to achieve such an intestine-on-chip system for both small and large intestines.[25–32]

To address the limitation of the enclosed lumen of organoids, we surveyed a variety of biomaterials, and identified a collagen hydrogel scaffold that enabled a self-renewing planar monolayer culture of colonic epithelial cells with properties similar to those of organoids, but whose luminal surface was readily accessible.[25] Nevertheless, these monolayers lacked the 3-dimensional architecture and tissue polarity of in vivo colon crypts. To overcome this shortcoming, in the current study we incorporated microfabrication of the hydrogel scaffold and spatial control of growth factors in a simple-to-use open format. Primary cells from a colonoscopic biopsy were first expanded in the aforementioned monolayer system. The collagen hydrogel scaffold was microfabricated in a microwell architecture possessing the shape of human colonic crypts. A gradient of growth factors along the crypts' z-axis was used to induce the polarization of the crypts such that stem/progenitor cells were confined to the basal region, while nonproliferative cells were situated along the upper and luminal aspects of the crypts. The in vitro human colonic crypts were compared with native in vivo colon crypts in terms of architecture, luminal patency, tissue polarity, and cell migration. To show the utility of this organ-on-a-chip system, the platform was used to study the impact of metabolites and cytokines on cellular proliferation and location within the tissue.

Materials and Methods

Cell Culture Media

The media compositions are listed in Tables 1 and 2. The culture media (expansion medium [EM], stem medium [SM], and differentiation medium [DM]) for human colonic crypts and epithelial cells were prepared from a mixture of advanced Dulbecco's modified Eagle medium/F12 medium (12634010; ThermoFisher, Waltham, MA) and Wnt-3A, R-spondin 3, noggin (WRN) conditioned medium (see later) at a volumetric ratio of 1:1, and supplemented with 1× GlutaMAX (35050061; ThermoFisher), 1× B27 supplement (12587010; ThermoFisher), 10 mmol/L HEPES (15630-080; ThermoFisher), 1.25 mmol/L N-acetyl cysteine (194603; MP Bio, Santa Ana, CA), 10 mmol/L nicotinamide (N0636; Sigma-Aldrich, St. Louis, MO), 50 ng/mL epidermal growth factor (315-09; Peprotech, Rocky Hill, NJ), 10 nmol/L gastrin (AS-64149; Anaspec, Fremont, CA), 10 nmol/L prostaglandin E2 (14010; Cayman Chemicals, Ann Arbor, MI), 3 μmol/L SB202190 (S1077; Selleckchem, Houston, TX), 100 U/mL penicillin-streptomycin (15140122; ThermoFisher), and 50 μg/mL primocin (ant-pm-1; InvivoGen, San Diego, CA).[33] A total of 10 μmol/L Y27632 (A3008-200; ApexBio, Houston, TX) was used in the first 48 hours after cell plating to prevent dissociation-induced cell apoptosis. WRN conditioned medium was prepared from L-WRN cells (CRL-3276; ATCC, Manassas, VA) following a published protocol.[14] This cell line produces Wnt-3A, R-spondin 3, and

Abbreviations used in this paper: **ALP, alkaline phosphatase; BSA, bovine serum albumin; DM, differentiation medium; DM-B, differentiation medium plus 5 mmol/L butyrate; DM-D, DM plus 10 μmol/L DAPT; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EdU, 5-ethynyl-20-deoxyuridine; ELISA, enzyme-linked immunosorbent assay; EM, expansion medium; IFN-γ, interferon-γ; KRT20, cytokeratin 20; Muc2, mucin 2; NHS, N-hydroxysuccinimide; Olfm4, olfactomedin-4; P, passage; PBS, phosphate-buffered saline; PDMS, polydimethylsiloxane; PTFE, polytetrafluoroethylene; SCFA, short-chain fatty acid; SEM, scanning electron microscope; SM, stem medium; TNF-α, tumor necrosis factor-α; ZO-1, zonula occludens-1.**

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