ABSTRACT
A microdevice was developed to linearly array colon crypts to assay the impact of Wnt-3a on colonic cells. Crypts were isolated from a transgenic mouse and then cultured in the device under a Wnt-3a gradient. 69 ± 8% of total stem-cell activity as measured by Sox9eGFP+ fluorescence was present in colonoid regions nearest the source. Under homogenous Wnt-3a growth conditions, only 46± 8% of total stem-cell activity existed in regions nearest the source. Under a Wnt-3a gradient, colonoids were displayed polarized growth with high stem-cell activity near the source and low stem-cell activity near the sink.

KEYWORDS: Colon, Organoids, Intestinal Stem-Cell, Gradient, Microdevice

INTRODUCTION
The colonic stem cell niche reside at the base of crypts with differentiated cell types located near the luminal end of the colon crypt (Fig. 1). The stem cells divide daily giving rise to progenitor cells, which differentiate and migrate up the crypt in response to factors believed to be present as a gradient along the crypt axis [1]. Recent advances in culture techniques now enable expansion of organoids derived from crypts, termed colonoids, which contain colonic stem cells and their differentiated lineages [2]. Despite the potential of these culture systems, there are two major limitations: i) the colonoids grow into large cysts that only vaguely resemble the intestinal epithelium in vivo and ii) the tissue fails to polarize as stem- and differentiated-cells are randomly distributed within the colonoids. This research looks to address both of these limitations.

THEORY
Colonoids embedded in Matrigel-filled polystyrene tissue culture dishes have been the gold-standard for intestinal crypt culture. Additionally growth in a dish does not provide architectural guidance and is incompatible with gradient generation. Microfabrication techniques are well-suited
to create 3-D structures with defined, micrometer-scale geometries. These structures can influence cell morphology and function by positioning cells in a structured culture environment that mimics the tissue-specific architecture, mechanical properties and biochemical signaling [3]. The device described in the current work utilized an innovative microfabricated design to introduce crypts to individual growth regions of physiologically-relevant dimensions. Additionally, crypts were exposed to a Wnt-3a gradient enabling culture of colonoids under heterogeneous growth conditions.

**EXPERIMENTAL**

*Fabrication of the device:* The PDMS device was designed to have 50 individual crypt capture sites. Capture sites were located between 2 large pentagons (Fig. 3C). The device was prepared using 3 microfabrication steps. A master mold was made by spreading a 250 µm thick layer of 1002F over a glass slide followed by baking and UV exposure. After a post-exposure bake, the master mold was silanized and used as a template to rapidly form the PDMS device. The PDMS device and the glass slide were plasma treated for 2 min, aligned and brought into conformal contact to form a permanent bond. The assembled device was baked at 95 °C overnight to enhance the bond strength (Fig. 3B).

*Loading of crypts, selective placement of Matrigel and on-chip culture:* The device was sterilized with 70% ethanol and rinsed with phosphate buffered saline (PBS) ×5. Intact crypts were isolated from the distal colon of a mouse and were pelleted by centrifugation at 150×g for 90 seconds. The supernatant was removed, the crypts were mixed with cold PBS, added to the top ports, and allowed to flow onto the array where they were captured in micron-scale traps (Fig. 3A). The PBS was aspirated and Matrigel was quickly added to the device. Excess Matrigel was gently aspirated leaving crypts in each of the individual capture sites encased in interconnected Matrigel pockets (Figure 3C-D). Excess gel entering the reservoirs was removed and the gel was solidified at 37 °C for 15 min. Appropriate media (with or without Wnt-3a) was added into the source and sink reservoirs to set up the gradient across the Matrigel plug. Media was replenished daily to ensure a stable gradient (Fig. 3E).

**RESULTS AND DISCUSSION**

Individual ‘culture pockets’ of Matrigel were formed on the device in which multiple crypts were cultured into colonoids. 95 ± 4% of traps successfully captured crypts (Fig. 4A,B). Crypts obtained from CAG-DsRed/Sox9eGFP transgenic mice were cultured in the device enabling stem and transit-amplifying cells (marked by eGFP expression) to be visualized in real-time [4]. 66 ± 7% of the crypts cultured in the microchannel under standard growth conditions formed colonoids compared to 73 ± 5% of crypts cultured in a standard 24-well plate. After 5 days in culture, colonoids grew throughout the entire culture region. Under these conditions, 46± 8% of
the eGFP fluorescence intensity was present in the upper half of the colonoid (Fig. 4D). The device itself did not negatively impact colonoid development from crypts nor did it result in colonoid polarization with respect to the location of stem and transit amplifying cells (Fig. 4C). When colonoids were exposed to a Wnt-3a gradient by placing Wnt-3a (100 ng/mL) only in the source region, 69 ± 8% of the eGFP fluorescence of the colonoid was present in the upper half of the colonoid suggesting that the colonoids were now polarized with respect to the location of stem and transit amplifying cells.

CONCLUSION

Individual, isolated colonic crypts possessing stem-cells were captured on a microdevice and exposed to a concentration gradient of Wnt-3a. The colonoids were polarized with high stem-cell activity near the source and low stem-cell activity near the sink. This work represents our lab’s continuous improvement in the building of a “colon-on-a-chip” using primary cells/tissues with the goal of producing organ-level function of colonic epithelium for controlled experiments.

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