A GUT-ON-A-CHIP STUDY: ENABLING ON-DEMAND MANIPULATION OF THE OUTER CELL MICROENVIRONMENT IN A MULTICOMPARTMENTAL 3D CULTURE ARRAY

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ABSTRACT

This paper reports a novel approach to build large arrays of cell-laden hydrogel microcompartments with well-controlled fluid flow to mimic the gut environment. Human intestinal epithelial cells (Caco-2) that were cultured in ~500 discontinuous compartments spontaneously grew into 3D folds on day 3. Mimicking interaction between intestinal eptithelial cells and intestinal bacteria was demonstrated in a long-term co-culture of *E. coli* adhered to Caco-2, the viability of which remained >70%. Also, different compartment geometries with large and small hydrogel interfaces were found to affect proliferation and cell spreading of Caco-2.

KEYWORDS: Gut-on-chip, microfluidics, 3D culture array

INTRODUCTION

3D spheroid and organoid tissue culture models are becoming increasingly popular in biomedical science and drug screening [1-2]. One of the most high-profile examples is *in vitro* culture of gut organoid cultures from single adult stem cells [3]. However, gut organoid cultures have typical shortcomings: displaying size heterogeneity, limited overall tissue shape (folded spheres), and short co-culturing time with bacteria [4]. The introduction of the parallelized microculture platform and the proof-of-concept data provided here demonstrate an alternative approach that can address the aforementioned shortcomings in future studies.

EXPERIMENTAL

Microchips were fabricated from polydimethylsiloxane (PDMS) using standard soft lithography techniques. The microchips contain pillars, capillary barriers, microchannels, and reservoirs. Microchannel and capillary barrier heights were 75 and 7.5 µm, respectively. Fig.1 shows an assembled microchip. A mixture of collagen (0.3%) and Caco-2 ($7 \cdot 10^6$ cells/ml) was patterned in the microchips by capillary pinning [5]. DMEM Glutamax supplemented with 20%(v/v) FBS, and 100 units/ml of pen/strep was pumped into the microchips at a constant flow rate (300 µl/h). To study cell-bacteria interactions, a culture medium–*E. coli* ($1.9 \cdot 10^7$cells/ml) mixture was injected into the microchips starting on the 8th day of culture for 36 h. Both fluid flow and static conditions were compared. Also, Caco-2 were cultured in trapezoid and rectangular compartments for 6 days under continuous perfusion.

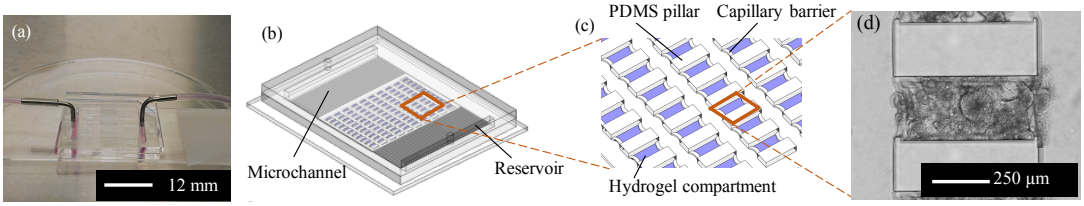

Figure 1: Overview of microfluidic chip design and the method of patterning hydrogels by capillary pinning. (a) A photograph of the microchip with attached tubing. (b) Schematic isometric view of the microchip with hydrogel patterns. (c) A zoomed-in schematic illustration of the capillary barriers, PDMS pillars, and hydrogel compartments. The height of capillary barriers is 1/4 of the microchannel height. The compartments are 200x500 µm. (d) Caco-2 cells grown under 300 µl/h flow rate, on day 8.

RESULTS AND DISCUSSION

Caco-2 started to spontaneously grow into 3D folds on day 2-3 of culture (Fig.2a-h). After day 8, cells filled the compartments completely and started migrating towards microchannels (Fig.1d). Based on observations using confocal microscope in Fig. 2i-j, after 6 days of culturing Caco-2 formed tubular structures with 3D folds inside,

resembling a lumen structure. Different compartment geometries with large and small hydrogel interfaces led to differences in proliferation and in cell spreading (Fig.2). Microfluidic perfusability prevented unrestrained over-proliferation of bacteria in the microchip while Caco-2 remained accessible by the bacterial cells in the fluidic culture. Caco-2 showed no indication of cell death at 300 µl/h flow rate with a suspension of *E. coli* (Fig. 3b). Similar viability was observed when *E. coli* was absent (Fig. 3a, c). When Caco-2 were co-cultured with *E.coli* under static conditions, approximately 30% of Caco-2 had died after 36 h (Fig. 3d).

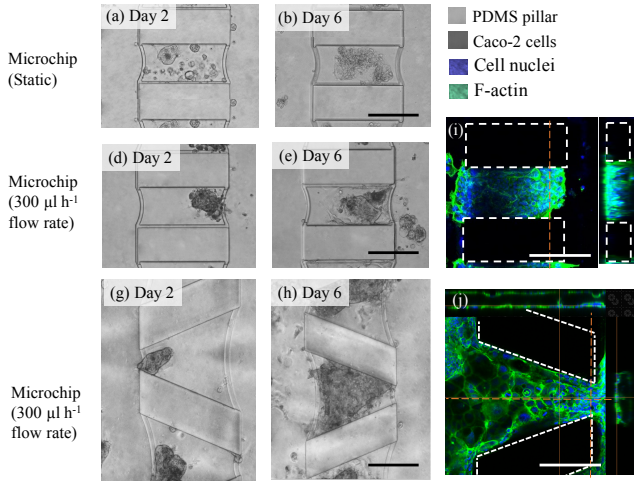

Figure 2: Top-view phase contrast images of Caco-2 cells in the microchip in different days of cell culture. The results are shown for the microchips operated (a-b) under static conditions and (d-h) under 300 µl/h flow rate, where (d-e) in rectangular, (g-h) in trapezoid shaped compartments. Confocal microscopy image of the Caco-2 cells grown under 300 µl/h flow rate on day 8 in (i) rectangular shaped (j) trapezoid shaped compartments. Dashed lines denote pillar boundaries, red dashed line shows location of y-z cross-section (inset right). Scale bars are 250 µm.

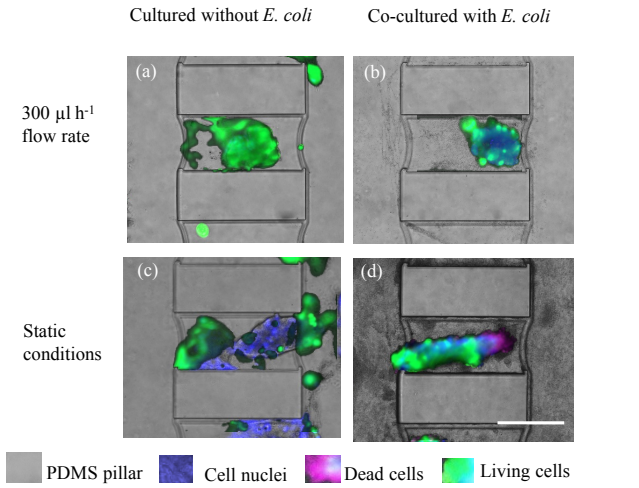

Figure 3: Top-view phase contrast microscopy images of live/dead assay bacteria co-culture operated (a) under 300 µh/l flow rate without E. coli cells, (b) under 300 µl/h flow rate with E. coli cells, (c) without fluid flow and without E. coli cells (d) without fluid flow with E. coli cells. The nuclei of Caco-2 cells were stained with DAPI (blue). Alive Caco-2 cells are shown in green and dead Caco-2 cells are shown in red colors. In Fig.s b and d, the dark cloudy appearance in the microchannels and the compartments is caused by E. coli colonies. Scale bar is 250 µm.

CONCLUSION

Enabling high-throughput culturing in a microfluidic environment, our approach has the potential to be used for building next-generation organotypic *in vitro* platforms, and creating separate 3D microenvironments, where a gradient of different metabolites can be applied to study tissue functions, drug screening, and perhaps *organ-on-chip assemblies*.

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