DIGITAL MICROFLUIDIC PLATFORM FOR THE CREATION, MAINTENANCE AND ASSAY OF LIVER-LIKE ORGANOIDS

Sam H. Au[1], Shruthi Mahesh[1] and Aaron R. Wheeler[1,2]
[1]Institute for Biomaterials and Biomedical Engineering, University of Toronto, CANADA
[2]Department of Chemistry, University of Toronto, CANADA

ABSTRACT

The liver is a vital organ responsible for metabolism, protein synthesis, bile production, detoxification and drug clearance. We present a platform for creating and assaying artificial liver models consisting of three dimensional co-cultures of liver-like organoids *in vitro* using digital microfluidics to provide precise control over the organoid sizes and local microenviroments. Findings include: (a) cultured fibroblasts actively contracted the hydrogel matrix in a collagen-concentration dependent manner, and (b) hepatocytes secrete more albumin in three dimensional organoids relative to their two dimensional counterparts.

KEYWORDS: Digital microfluidics, liver model, organoids, hepatocytes, albumin

INTRODUCTION

Despite the fact that three-dimensional (3D) cell cultures are known to support cell densities [1] and phenotypes [2] that are more similar to *in vivo* tissues than analogous two-dimensional (2D) culture systems, the vast majority of *in vitro* cells are cultured in the latter format because they are far easier to culture and assay. Building on recent work in which single cell-types were encapsulated in hydrogels and assayed using digital microfluidics (DMF) [3-4], we have developed a system to co-culture cells in liver-like "organoids" – microscale collagen constructs seeded with NIH-3T3 fibroblasts and HepG2 hepatocytes. As far as we are aware, this is the first digital microfluidic system for the co-culture of cells in three dimensions. In contrast to previous hepatocyte-stromal microfluidic models [5-6] which are limited in their abilities to specifically control the microenvironment of individual tissue constructs, DMF facilitates the straightforward addressing of experimental conditions to individual constructs in real time. Furthermore, the platform enables the creation, maintenance, and visualization of organoids over days-weeks.

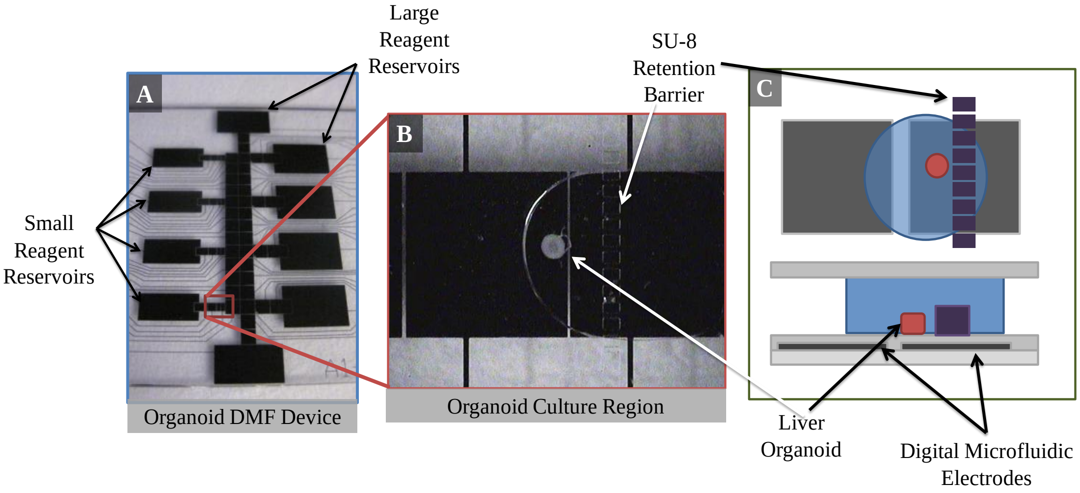

Figure 1: A) Picture of DMF device used for 3D co-culture. B) Close-up of culture region containing liver organoid and SU-8 retention barrier. C) Top- and side-view schematics of culture region

EXPERIMENTAL

DMF Devices featuring multiple reagent reservoirs capable of dispensing 10 different reagents (Figure 1A) were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) fabrication facility using photolithography and wet etching. To confine cells to an organoid culture region, 70 micron high SU-8 retention barriers were fabricated on top of parylene dielectric and

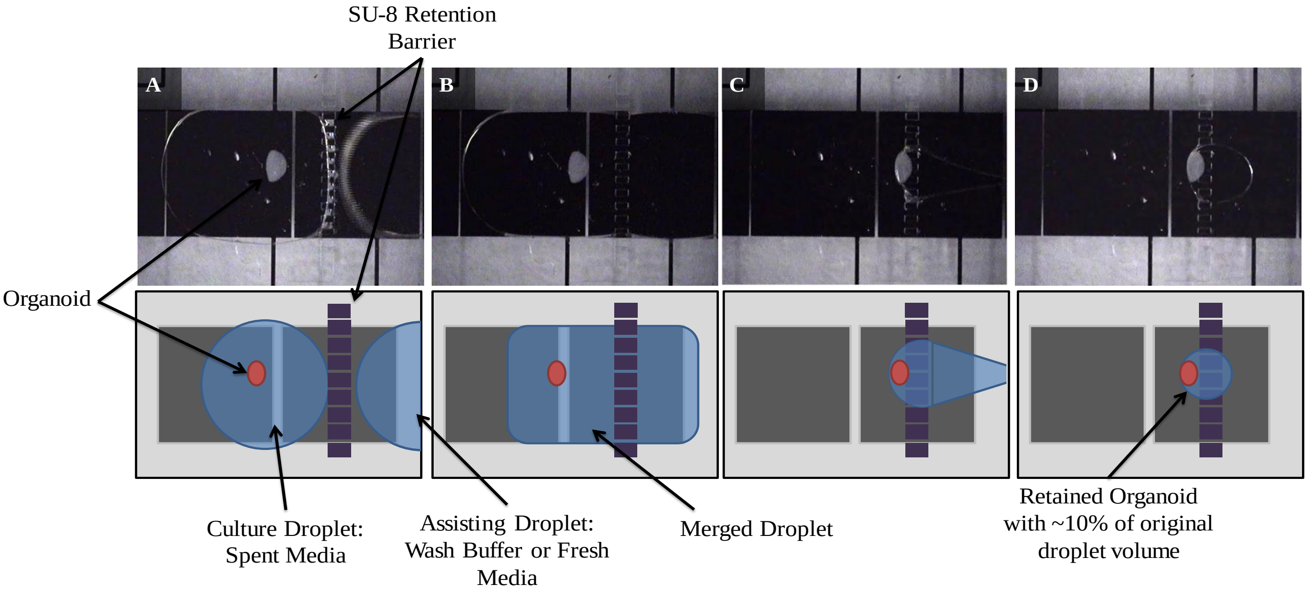

Figure 2: Pictures (top) and schematics (bottom) of organoid feeding procedure on digital microfluidic device. A) An assisting droplet containing fresh media or wash buffer is brought to the barrier. B) The assisting droplet is merged with the droplet containing the organoid. C) The merged droplet is driven away for disposal while the organoid is unable to cross the barrier. D) A small volume ~10% of residual liquid is retained with the organoid. Fresh media or buffer is then brought to the organoid as required.

subsequently coated with Teflon-AF® to render the barriers hydrophobic (Figure 1B/C). Devices were assembled with an ITO–glass top plate separated by a spacer formed from two pieces of double-sided tape (total spacer thickness 140 μm). Liver organoids were generated by mixing neutralized rat tail collagen I at collagen concentrations of 2.9, 2.0, 1.5 or 0.9 mg/mL with 2 x 10^6 cell/mL of HepG2 hepatocytes and 2 x 10^6 cell/mL of NIH-3t3 fibroblasts for albumin secretion studies or with 2 x 10^6 cell/mL of fibroblasts for contraction studies and incubated in a humidified chamber at 37°C for 45-60 minutes to allow the collagen to gel. For comparison, two-dimensional (2D) cultures were generated by allowing neutralized collagen I to air dry on devices for 30 minutes before seeding with cells. Organoids were fed daily with DMEM/F12 (50:50) containing 8% fetal bovine serum and 2% calf serum supplemented with 0.6% (wt/v) pluronic F88 by driving an assisting droplet to the retention barrier where it is merged with the original droplet bearing the organoid (Figure 2A), after which the merged droplet was driven away (Figure 2B/C). Approximately 10% of original droplet volume was retained (Figure 2D), ensuring that organoids remained hydrated. This process (i.e., using the assisting droplet) was developed to overcome the challenge inherent in moving an unassisted droplet through the hydrophobic retention barrier. For imaging, organoids were gently transferred to an electrode-free portion of the DMF devices using a micropipette, live-dead stained with 4 µM calcein-AM and 2 µM ethidium homodimer-1 in phosphate buffered saline for 30 minutes at room temperature, and then imaged with a Leica DM2000 upright microscope. Albumin secretion was quantified using a Human Albumin Elisa Kit (Abnova Corporation).

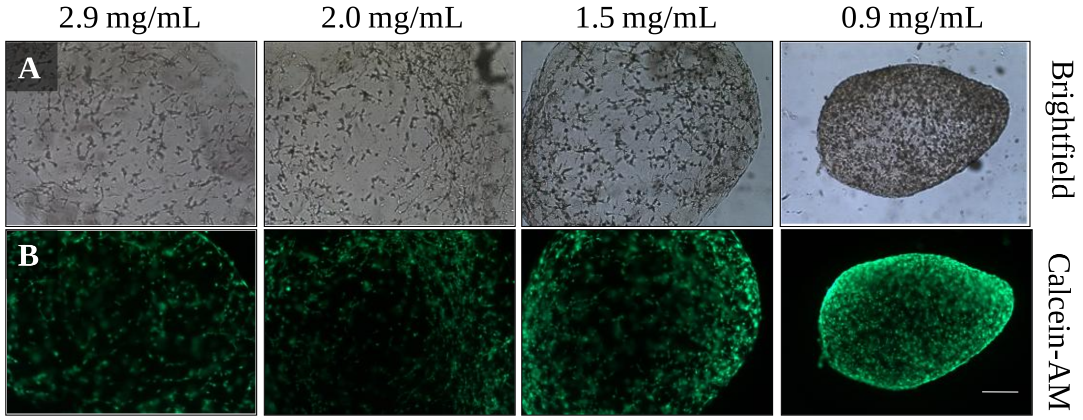

Figure 3: Organoids containing NIH-3T3 fibroblasts after 7 days in brightfield (A) and live-cell stained with calcein-AM (B). Cells were seeded at a density of 2 x 10^6 cell/mL in constructs of 2.9, 2.0, 1.5 or 0.9 mg/mL collagen with identical starting volumes. Scale bar represents 50 microns.

RESULTS AND DISCUSSION

To demonstrate the capacity to generate constructs *in situ*, cells in droplets of neutralized collagen I were seeded and then gelled. Because the stiffness of extracellular matrix is known to affect cellular processes such as growth, spreading, and morphology [7], we evaluated the effects of changing the stiffness of the extracellular matrix by varying the concentration of collagen in solution from 0.9-2.9 mg/mL (Figure 3). The organoids actively contracted and remodeled in a collagen-concentration-dependent manner. The least concentrated gel (0.9 mg/mL) contracted to less than half the diameter of the most concentrated (2.9 mg/mL) gel. The vast majority of cells remained viable after a week as determined by calcein-AM staining.

Albumin secretion (an important function in liver tissue) was monitored as a test for *in vivo*-like phenotype. As shown in Figure 4, 1:1 HepG2:NIH-3T3 organoids generated and cultured on the DMF system secreted nearly twice as much albumin per cell per day than cells cultured in two dimensions on collagen-coated substrates after 2 days.

CONCLUSION

We have developed a microscale system for the creation, manipulation, and addressing of individual co-cultured 3-D tissue constructs capable of supporting high cell densities and *in*-*vivo*-like phenotypes. We propose that similar systems may be eventually make 3D culture an attractive alternative to traditional 2D techniques.

ACKNOWLEDGEMENTS

We thank Dean Chamberlain (University of Toronto) for helpful discussions. We thank the Canadian Institutes for Health Research (CIHR) and the Natural Sciences and Engineering Research Council (NSERC) for financial support. SHA thanks NSERC for a graduate fellowship and ARW thanks the Canada Research Chair (CRC) program for a CRC.

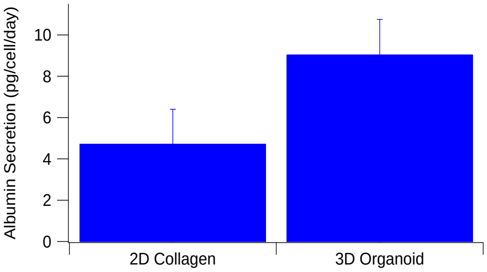

Figure 4: Albumin secreted per cell per day after 2 days for HepG2 hepatocytes and NIH-3t3 fibroblasts seeded at a density of 2 x 10^6 cell/mL each in 2 dimensions on collagen versus in 3 dimensions in organoids. Error bars represent ± 1 S.D.

REFERENCES

[1] McGuigan, A., Bruzewicz, D., Glavan, A., Butte, M., and Whitesides, G. *PLOS One* 2008, 3, 1905- 1911.
[2] Kim, K., Ohashi, K., Utoh, R., Kano, K., and Okano, T., *Biomaterials* 2012, 33, 1406-1413.
[3] Fiddes, L., Luk, V., Au, S., Ng, A., Kumacheva, E. and Wheeler, A. *Biomicrofluidics*, 2012, 6, 014112
[4] Eydelnant, I., Li, B. and Wheeler, A. *IEEE Conf. Microelectromech. Sys., Institute of Electrical and Electronics Engineering*, Piscataway, NJ, 2012, pp. 898-901.
[5] Hwang, C., Sant, S., Masaeli, M., Kachouie, N., Zamanian, B., Lee, S. and Khademhosseini, Ali. *Biofabrication*, 2010, 2, Article No.: 035003.
[6] Tsang, V., Chen, A., Cho, L., Jadin, K., Sah, R., DeLong, S., West, J. and Bhatia, S. *FASEB Journal* 2007, 21, 790-801.
[7] Wells, R. *Hepatology* 2008, 47, 1394-1400

CONTACT

***** Sam Au, tel +1-416-946-5702, sam.au@utoronto.ca