DIGITAL MICROFLUIDICS FOR ON-DEMAND 3D MICROGEL FORMATION AND FUNCTIONAL MYOCARDIAL TISSUE ASSAYS

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ABSTRACT

Three-dimensional (3D) cell culture allows for *in vitro* biological systems that more representative of *in vivo* phenotypes than traditional two-dimensional culture. Combining these systems with relevant cell types (e.g., cardiomyocytes) is of interest for drug screening to facilitate the identification of targets with biological relevance. However, 3D cell culture reagents are costly and the fragility of matrix materials makes them difficult to manipulate using conventional tools. Here we report the first DMF method capable of on-demand formation of complex hydrogel geometries. We then demonstrate the utility of this method by generating the first functional myocardial tissue constructs in sub-microliter volumes. Moreover we demonstrate the ability to form microgels from multiple hydrogel types, characterize reagent transport, use the device to automate cell seeding in 3D, and perform functional assays on beating cardiomyocyte microgels.

KEYWORDS: Digital microfluidics, hydrogel, 3D cell culture, cardiomyocytes, primary cells

INTRODUCTION

Hydrogel based three-dimensional (3D) cell culture has increased in prominence because of the more *in vivo*-like phenotypic response of cells cultured in this way. For this reason 3D cell culture is of interest for tissue engineering applications and improving the output of *in vitro* biological assays, in particular for drug screening [1]. Unfortunately, hydrogel culture methods remain under-utilized in part because of associated reagent costs and challenges in the manipulation and manual handling of delicate hydrogel material.

A number of microfluidic strategies have been proposed to address these issues. Microfluidics provides the ability to manipulate sub-microlitre volumes of liquid thereby reducing reagent consumption. Furthermore, the associated low Reynolds number flow through microfluidic channels allows for gentle hydrogel handling and reduces subsequent damage to gels during reagent exchange. Two significant challenges of microchannel 3D culture remain: (1) diffusion limits within hydrogels, and (2) channel clogging. Motivated by these challenges, we sought to exploit recent developments in reagent dispensing and solids handling on digital microfluidic (DMF) devices. In particular we hypothesized that the open geometry and individual addressability of electrodes would provide some relief from the challenges associated with microchannel-based methods.

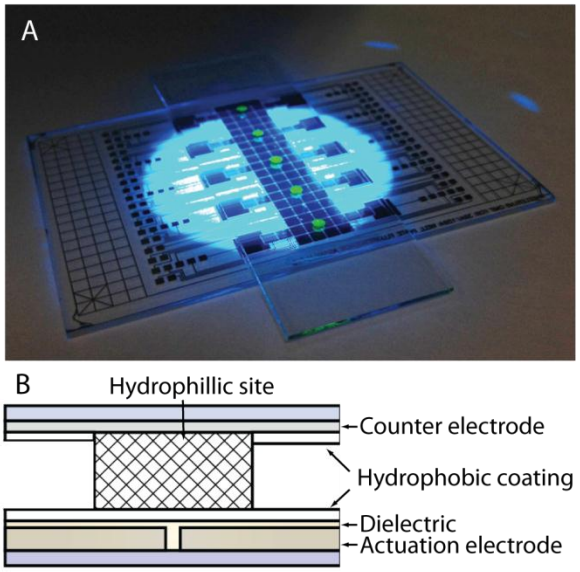

Figure 1: Digital microfluidic device. (A) Photograph of device on stereomicroscope with five independently addressable fluorescently labelled hydrogel pillars. (B) Cross-sectional schematic of DMF device geometry.

DMF technology facilitates electrostatic manipulation of discrete nano- and micro-litre droplets across open electrode arrays providing the advantages of single sample addressability, automation, and parallelization. The DMF geometry supports complex liquid handling functions including the translation, merging, and splitting of droplets. Recently, interest has increased in DMF handling and addressing of three-dimensional solids: paper discs for blood screening, porous polymer monoliths for solid-phase extractions, and agarose disc based assays [2]. The robust and multifunctional nature of DMF provides for a viable method to address implementation obstacles to hydrogel based microfluidic cell culture.

Here we report the first microfluidic platform for on-demand formation of precise hydrogel microstructures of a range of geometries in virtual microwells. We use this method to automate cell seeding in three-dimensions, media exchange at regular intervals, and fixing and staining of cells for on-device confocal imaging. We use this method for the formation of functional and responsive myocardial tissue in submicrolitre droplets. Finally, when compared to automated robotic liquid handling systems, we find that DMF provides a 100-fold reduction in reagent consumption and improved handling of hydrogels.

EXPERIMENTAL

DMF fabrication

Electrode bearing bottom-plates were fabricated as described previously [3]. Hydrophillic sites were generated on the ITO coated top-plate by a fluorocarbon lift-off technique [3]. Briefly, photoresist patterns were formed by photolithography, followed by spin-coating with Teflon-AF (1% wt/wt in FC40, 3000 RPM, 30 sec), baking on a hot-plate (10 min, 165 °C), then immersion in acetone until liftoff occurred (~ 5-10 sec).

Cell culture and preparation

Primary cardiomyocytes (CM) were isolated from neonatal (1–2 day old) Sprague–Dawley rat hearts. Prior to experiments cells were resuspended in CM media at 20×10^6 cells/mL. This suspension was diluted in Geltrex, a commercially available extracellular matrix hydrogel, to a final cell density of 10×10^6 cells/mL. All reagents were supplemented with either 0.02% Pluronics F68 or 0.02% Pluronics F127.

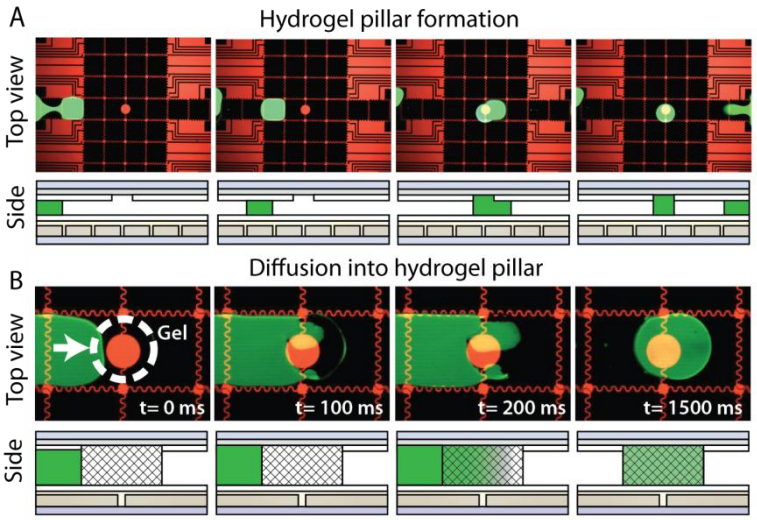

Figure 2: Digital microfluidics for hydrogel microgel formation. (A) Top view (stereomicroscopy) and side view (schematic) of passive dispensing mechanism in the formation of microgels. (B) Top and side views of FITC-Dextran (4 kDa) into microgel.

Hydrogel pillar formation and characterization

Aliquots of 50% Geltrex in media supplemented with 0.02% Pluronics F127 at 4 ºC or 1% low melting point agarose in deionized water supplemented with 0.02% Pluronics F127 at 30 ºC were loaded onto a device by simultaneous reservoir electrode actuation and pipetting. Droplets of hydrogel solution were electrostatically manipulated across hydrophilic sites patterned on device top-plates thereby forming subdroplets that were subsequently cross-linked at 37 ºC or 25 ºC to form the hydrogel pillars. Diffusion experiments were performed by manipulating droplets of fluorescein or FITC-Dextran (4-400 MW) across preformed hydrogel pillars. Images were recorded by fluorescent stereomicroscopy.

Cardiomyocyte assay

CMs in Geltrex were cultured for four days with media exchange at 4, 24, 48, and 72 hours. At 96 hours culture media was replaced with either CM media or CM media supplemented with epinephrine. CM beating was imaged on an inverted microscope. Individual cell contractions were analyzed for frequency, duration, and magnitude (displaced distance).

RESULTS AND DISCUSSION

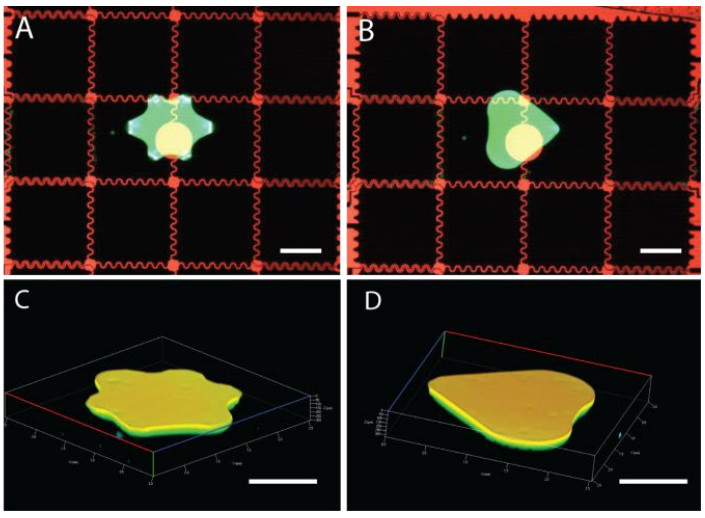

Figure 3: DMF hydrogel geometries. Multiple geometries can be formed by modifying the design of the patterned region on the device top-plate. (A,B) Stereomicroscope images And (C,D) confocal microscope images of star-shaped and heart-shaped hydrogel pillars. Scale bars = 1 mm.

We recently developed a method for forming hydrophilic cell culture sites relying on a Teflon lift-off procedure [3]. Here we report the application of this method for the on-demand rapid generation of individually addressable hydrogel structures (Figure 1). As droplets of non-crosslinked agarose or Geltrex were translated across the patterned hydrophilic sites sub droplets were generated that conformed to the shape of the hydrophilic site (Figure 2). After gelling, the apparent diffusion coefficients of fluorescently labeled dextrans across a range of molecular weights was determined by imaging reagent exchange across microgels by stereomicroscopy (Figure 2B). Diffusion coefficients determined from these experiments validate the efficiency of reagent exchange for 3D cell culture and agreed with known values. By varying the design of the hydrophilic patches it is possible to form a wide variety of microgel geometries in agarose and Geltrex (Figure 3). Stereomicroscopy demonstrates fidelity of the hydrogels with the designs (Figure 3A,B) and confocal microscopy demonstrates that the gel is conformal throughout the z-axis (Figure 3C,D).

We tested the ability to seed and maintain functional primary rat cardiomyocytes in gels formed and maintained by DMF. Cells were suspended at high density in Geltrex and then were electrostatically manipulated across hydrophilic sites. Incubation (37 ºC) resulted in polymer cross-linking and distribution of cells throughout the matrix. Media was exchanged daily by electrostatic translation of droplets across the hydrogel pillars. After four days of culture, cell morphology associated with matrix remodeling was observed by brightfield and fluorescent imaging of on-device fixed and stained cells. Further, spontaneous beating of cardiomyocytes commenced at a physiologically relevant 48 beats per minute (BPM). With epinephrine stimulation the beat rate increased to 96 BPM in conjunction with increases of contraction duration (1.8-fold) and contraction length (2.8-fold).

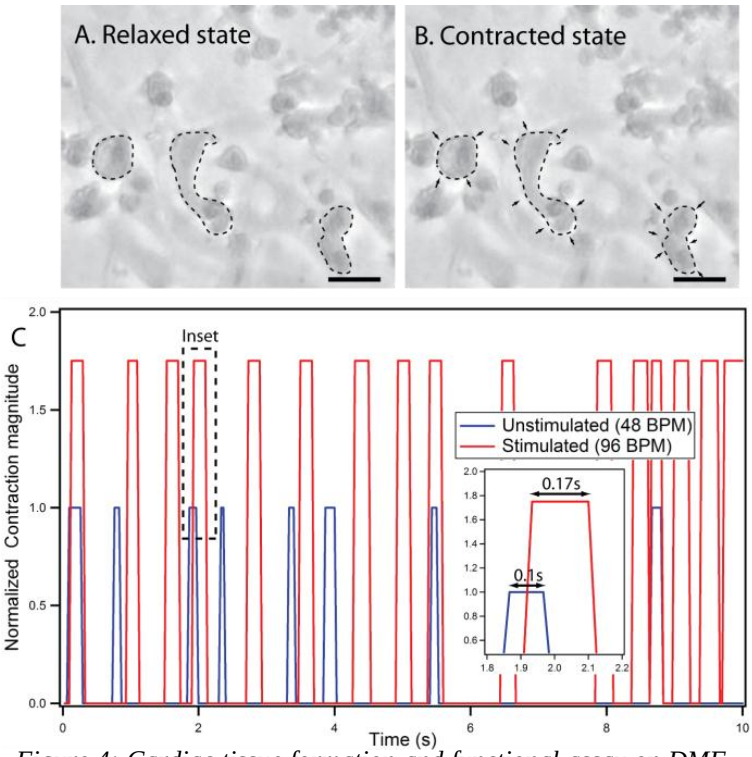

Figure 4: Cardiac tissue formation and functional assay on DMF. (A,B) After three days morphology associated with matrix remodelling was observed. Cells presented as elongated and spontaneous beating commenced. Tissue contraction was observed by brightfield microscopy. Scale bar = 20 µm. (C) Representative contraction data from single cells over a 10s interval.

CONCLUSION.

This work represents a novel method for the formation of a range of hydrogel geometries that are readily and independently accessible for reagent exchange. Further we create the first functional and individually addressable sub-microlitre myocardial tissue that is responsive to external stimuli. We anticipate these methods will be useful in a range of disciplines from materials science to tissue engineering.

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REFERENCES

[1] Tibbitt, M.W. & Anseth, K.S. Biotechnol Bioeng 103, 655–663 (2009).
[2] Choi et al. Annu Rev Anal Chem, 5(1):413-40. (2012).
[3] Eydelnant, I.A., et al. Lab on a Chip 12, no. 4 (2012): 750-757.

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