GENERATION AND ASSEMBLY OF CELL-LADEN HYDROGELS ON A DIGITAL MICROFLUIDIC PLATFORM

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ABSTRACT

We demonstrate an integrated platform combining (1) digital microfluidic and (2) photo-patterning techniques to manipulate and fabricate cell-laden hydrogels for the tissue engineering application. Different hydrogels cured from individually-driven polymer droplets provide three-dimensional (3D) microenvironments for in vitro cell culture. With the ability to actively drive the polymer droplets, assemble the hydrogels, and arrange the cells inside the hydrogel, this work provides an essential approach to tissue engineering, cell-cell interaction, stem cell differentiation studies on a highly programmable digital microfluidic device.

KEYWORDS: Digital microfluidic, Photo-pattering technique, Cell-laden hydrogel

INTRODUCTION

The arrangements of cells and extracellular matrices (ECM) in different functional unit of various tissues are entirely different and significant, such as the radial arrangement of hepatocytes in hexagonal hepatic lobule. Mimicking the in vivo microenvironment can be beneficial for a variety of applications such as tissue engineering [1], cell-based assays [2], and stem cell differentiation [3]. Hydrogels, 3D crosslinked networks of hydrophilic polymers, resemble the physical characteristics of extracellular matrices [4] and are often used to encapsulate cells. They can be tailored to exhibit high permeability to oxygen, nutrients, and other water-soluble metabolites [5]. Cell encapsulating hydrogels can be used for the generation of 3D tissue engineering structures.

In order to generate biomimic cell-laden hydrogels, the regeneration of structural features is of importance in enabling the resulting function. These cell-laden hydrogels could be made to generate macroscale tissue from microgel units made of cell-seeded or cell-laden hydrogels. Typically, the microscale functional units were fabricated by emulsification, micro-molding, photo-patterning or microfluidic. Here, we propose a novel method to combine electrowetting-on-dielectric (EWOD) and photo-patterning techniques on a platform to complete multiple steps in a series.

THEORY

An EWOD-based digital microfluidic platform (Fig. 1) is studied to manipulate curable droplets containing cells for the preparation of cell-laden hydrogel of tissue engineering after curing. Photosensitive hydrogel, poly(ethylene glycol) diacrylate (PEG-DA), droplets were driven on-chip by EWOD with adjustable shapes and sizes. By using EWOD, basic microfluidic functions including creation, transportation, cutting, and merging hydrogel droplets can be implemented. In addition, multiple hydrogel droplets can be created simultaneously on a single device. Recently, many EWOD studies showed that the wettability of liquids on a dielectric surface is controllable with a high reversibility. When an electric voltage V was applied between the aqueous droplet and the electrode, the droplet spontaneously spreads out on the dielectric surface (Fig. 1(b)). The contact angle θ_V is modulated by the applied voltage according to the Lippmann-Young equation:

$$\cos\theta_V = \cos\theta_0 + \frac{\varepsilon_0 \varepsilon_r}{2\gamma_{LG} t} V^2 . \quad (1)$$

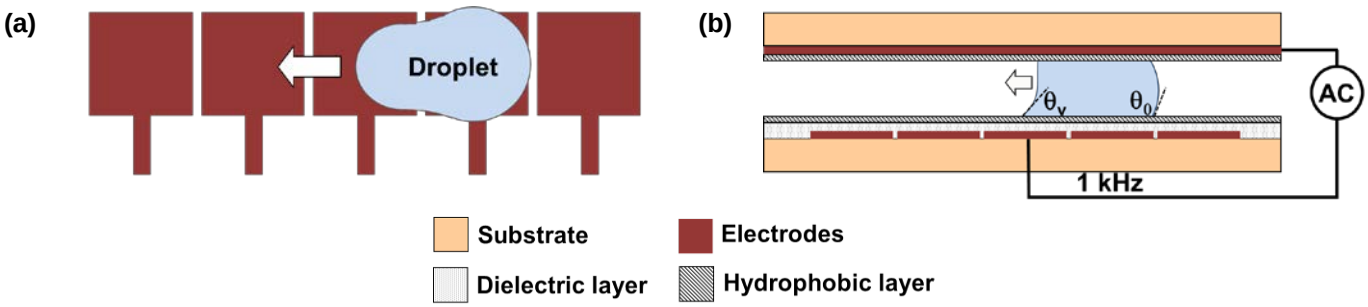

Figure 1. Conventional EWOD device. Droplet pumped to the left when applying a voltage. (a) Top view, (b) cross-sectional view.

In equation (1), ε_0 denotes the permittivity of the vacuum, ε_r the dielectric constant of the dielectric layer, γ_{LG} the gas-to-liquid interfacial tension and t the thickness of the dielectric layer. When the electric potential V was applied, the contact angle of liquids would be changed. When the electric potential V is removed, the changed contact angle returns to the initial contact angle θ_0.

EXPERIMENTAL AND RESULTS

In this paper, the geometry of cell-laden hydrogels were determined by the pattern designs of the electrodes. The configuration of the device is illustrated in Fig. 2. The device structure is shown in Fig. 1(b). A layer of ITO was on the top glass substrate and covered by a low surface energy material, Teflon (55 nm-thick Teflon). The bottom glass plate contained patterned electrodes (ITO), dielectric (1.6 μm-thick SU-8), and hydrophobic layers (55 nm-thick Teflon). By applying a voltage V on the electrodes between two plates, the hydrogel droplets were actuated because of the contact angle change.

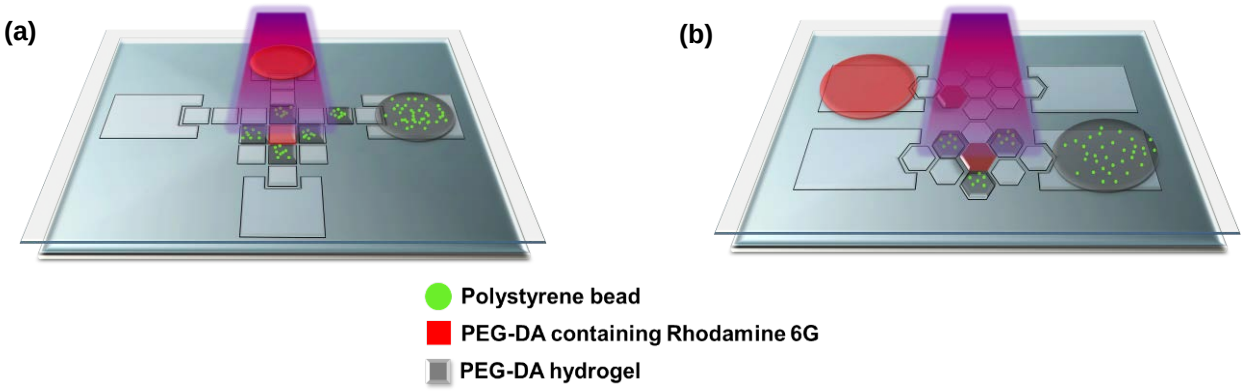

Figure 2. Fabrication of hydrogels with different shapes, (a) squares and (b) hexagons determined by the electrode design.

The fabrication and assembly of hydrogels containing fluorescent dye and polystyrene beads with different shapes were demonstrated in the parallel-plate devices. Two 0.7 μl hydrogel droplet containing 5 μm polystyrene beads and fluorescent dye (0.1 mM Rhodamine 6G) were assembled by the square-shaped electrodes as shown in Fig. 3. And the procedures of hexagonal hydrogels preparation and arrangement are shown in Fig. 4. By sequentially applying a voltage (60-100 V_{RMS} and 1 kHz) on the electrodes, the hydrogel droplets were actuated onto designed positions. Finally, hydrogel droplets were crosslinked to form microgels by UV light (365 nm) exposure.

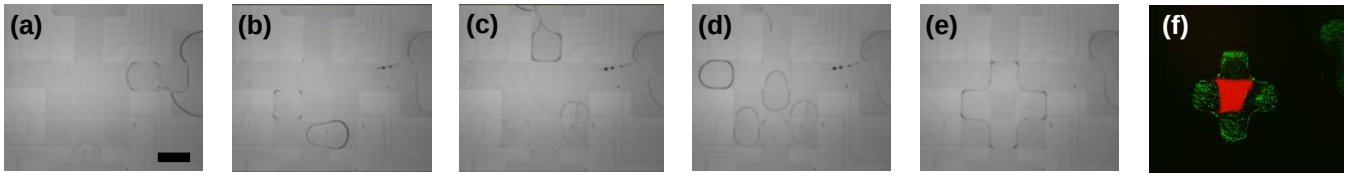

Figure 3. Procedures of square hydrogel preparation. (a)-(e) Creating and moving hydrogels by applying 60 -100 V_{RMS} and 1 kHz AC signals. (f) Fluorescent image of PEG-DA after UV light crosslinking. The scale bar is 1 mm.

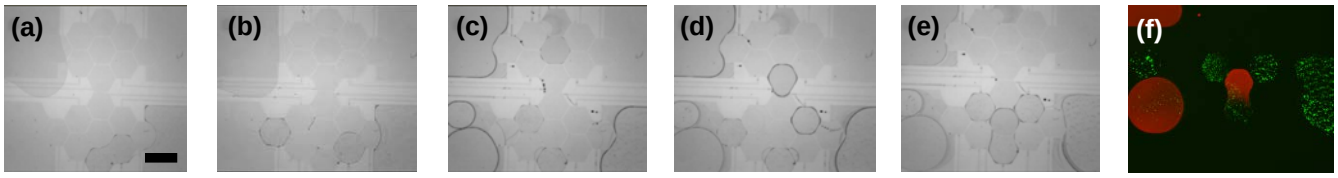

Figure 4. Hexagonal hydrogel preparation. (a)-(e) Creating, moving, and assembling hydrogels. (f) Fluorescent image of assembled and cured PEG-DA hydrogel. The scale bar is 1 mm.

To demonstrate the cell-laden hydrogels fabrication on the same parallel-plate device with same manipulated conditions, the precursor of cell-laden hydrogel was prepared using pluronic F127 (0.04 w/v%), 2×10^7 NIH-3T3 cells/ml, 0.25 w/v% photoinitiator and 15 w/v% 4 arms-PEG acrylate (Mw 20,000) in the phosphate buffer solution. After the manipulation of precursor of cell-laden hydrogel, the cell viability was analyzed by cell live/dead assay (LIVE/DEAD® Viability/Cytotoxicity Kit). From the cell viability test (Fig. 5), cells encapsulated in 4 arms-PEG acrylate aggregated, and most cells in hydrogel

maintained their viability. Moreover, we found that dead cells and live cells are at different heights in the cell-laden hydrogel. Cell viability was higher for the cells closer to the upper surface of the hydrogel, which requires further investigations.

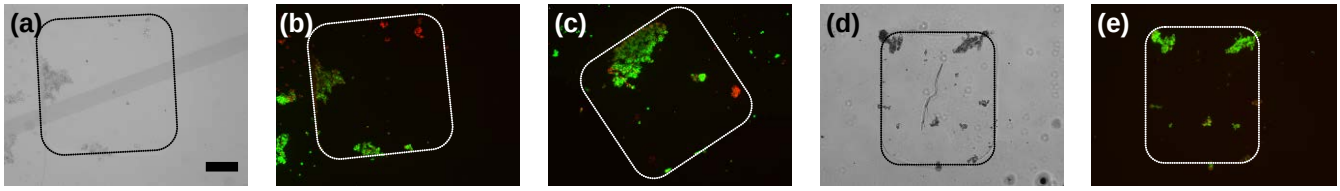

Figure 5. Cell viability analysis. (a), (d) Bright-field images. (b), (c) and (e) Fluorescent images. (a)-(c) Cell live/dead images on day 0. (d)-(e) Images on day 5. The live and dead cells were stained with Cacein AM (green) and Ethidium Homodimer-1 (EthD-1, red), respectively. The scale bar is 200 μm.

CONCLUSION

In this study, we demonstrated hydrogels in the square and hexagonal shapes on the parallel-plate devices utilizing EWOD and photo-curing methods. In this integrated platform, arrangements of hydrogels with different shapes were implemented by applying 60-100 V_{RMS} and 1 kHz signals. Manipulations of the hydrogels and the cell-laden hydrogels were also demonstrated.

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