CELL COCULTURE WITHIN ELECTRICALLY PATTERNED CELLS AND HYDROGEL STRUCTURES

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ABSTRACT

We report a new method patterning cells and hydrogels on two different scales to form a biomimetic *in vitro* liver tissue by electromicrofluidic (EMF) techniques. Compared to our previous work [1-2], here we show (1) a variety of hydrogels with varied stiffness and corresponding encapsulated cell types, (2) the arrangement of multiple cell types to recapitulate hepatic lobules, and (3) manipulations of photo- and thermally-polymerizable hydrogels into heterogeneous hydrogel microstructures. In this study, EMF, capable of simultaneous control of multiple fluids and particles with appropriate electrical signals, is used to construct heterogeneous hydrogel microstructure and pattern multiple cells on various scales into an *in vitro* hepatic-lobule-like arrangement in one step on a single device.

INTRODUCTION

Recently, techniques have been reported to develop organs-on-chips and *in vitro* tissue that imitates the *in vivo* anatomical microenvironment and preserves the physiological functions. To create functional *in vitro* tissues, it is essential to recapitulate the *in vivo* tissue in the aspects of its geometry, scale, and stiffness [3-4]. However, establishment of three-dimensional (3D) microstructures with multiple cell types in biomimetic arrangements on a wide range of scales remains technically challenging. Here, electromicrofluidic (EMF) is adopted as a powerful tool and platform to construct biomimetic and heterogeneous microstructures for cell culture and coculture. Human hepatic lobule is chosen as the *in vivo* model of this research, as human umbilical vein endothelial cell (HUVEC), human lung fibroblast (HFL1), and human hepatocellular carcinoma (HepG2) are encapsulated into hydrogels to perform cell coculture. We successfully arranged multiple cell types in patterned hydrogels that held dissimilar stiffness. In addition, we integrated two crosslinking strategies on a single EMF device.

PRINCIPLE

Electrowetting-on-dielectric (EWOD)

EWOD is the phenomenon of contact angle change on a dielectric surface with an applied electric signal, described by Young-Lippmann equation:

$$\cos\theta(V) = \cos\theta_0 + \frac{\varepsilon_0\varepsilon_d}{2\gamma_{la}t}V^2, \quad (1)$$

where ε_0 is the permittivity of vacuum, ε_d and t are the relative permittivity and thickness of the dielectric layer, respectively, and γ_{la} is the liquid–ambience interfacial tension. When voltage V is applied across the dielectric layer, the contact angle of a conductive droplet on the dielectric changes from θ_0 to $\theta(V)$. EWOD is used here to manipulate hydrogels through the contact angle change.

Liquid dielectrophoresis (LDEP)

In contrast, LDEP is the main force to drive dielectric liquids with none or small contact angle change with the applied electric signals.

$$F_{LDEP} = \frac{\varepsilon_0(\varepsilon_f-\varepsilon_a)w}{2d}V^2, \quad (2)$$

where ε_f and ε_a are the relative permittivity of the fluid and the ambience, respectively, V is the applied voltage between the plates, d is the gap between two parallel plates, w is the width of the electrodes [5]. By integrating EWOD and LDEP forces, conductive and dielectric hydrogels can be driven on the EMF platform.

Dielectrophoresis (DEP)

In this study, we generate non-uniform electric fields in the hydrogel by applying a high frequency electric signal, and the suspended particles and cells are moved by DEP. The DEP force can be described as:

$$F_{DEP} = 2\pi a^3\varepsilon_f Re(f_{CM})\nabla E^2, \quad (3)$$

where a is the radius of particle, ε_f is the permittivity of suspension fluid, f_{CM} is Clausius-Mossotti factor and ∇E^2 is the gradient of electric field. When we apply an electric signal with appropriate frequency and amplitude, particles and cells can be moved by negative DEP force.

DESIGN

The EMF device consisted of a top plate and a bottom plate as shown in Fig. 1. For observation purposes, we choose ITO glass plates as the substrate. Predesigned biomimetic electrodes (Fig. 2) corresponding to human hepatic lobule was patterned on the bottom plate and coated by a 1.7-μm-thick SU-8 as the dielectric layer and a 55-nm-thick Teflon as the hydrophobic layer. The top plate contained a blank electrode covered by a hydrophobic layer. 100 μm-thick spacers were used to determine the gap between the plates and the height of the hydrogel structures.

The manipulation of multiple crosslinkable fluids and particles on cross-scales was demonstrated with an EMF device. Multiple fluids were manipulated concurrently between two parallel plates by applying appropriate electrical signals (Fig. 1). We patterned hydrogel prepolymer solutions with varied properties to form *in vitro* hepatic lobule by predesigned electrodes (Fig. 2) using EWOD and LDEP mechanisms. Cells and particles were encapsulated in their corresponding hydrogel prepolymers with suitable stiffness to form an *in vivo*-like arrangement on linear-shaped electrodes by DEP on cells and particles (Fig. 3). After UV exposure, we refilled the gap between the cell-laden hydrogels with a thermally crosslinkable hydrogel to accomplish the preparation of the *in vitro* hepatic-lobule-like microstructure (Fig. 4).

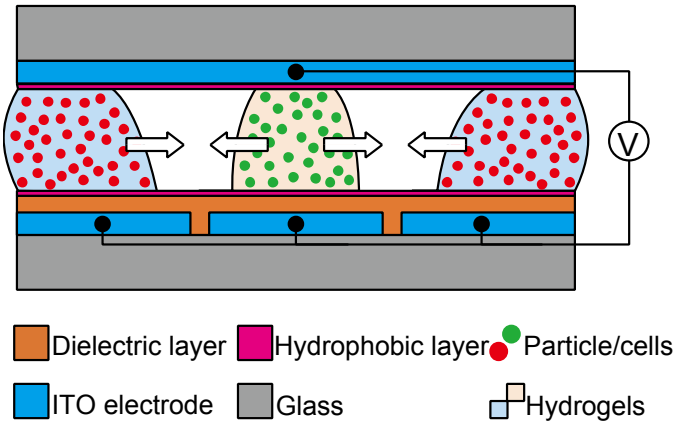

Figure 1: Schematic diagram of the electromicrofluidic (EMF) device in a cross sectional view showing multiple fluids manipulated concurrently on a single device.

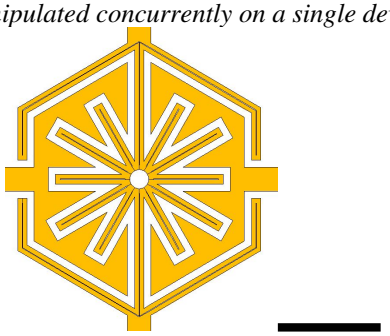

Figure 2: Biomimetic electrode patterns recapitulating hepatic lobules. (Scale bar: 1 mm)

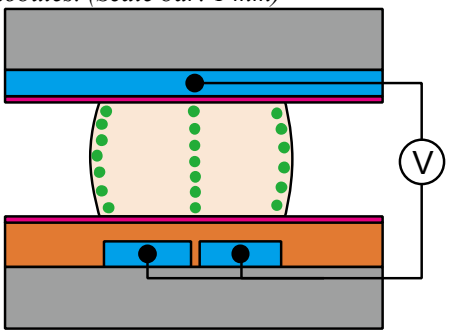

Figure 3: The cross sectional view of linear-shaped electrode with a slit at the center to manipulate particles and cells by negative DEP.

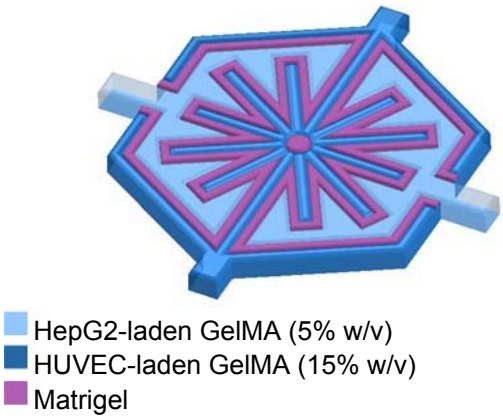

Figure 4: A heterogeneous hepatic-lobule-like microstructure prepared with EMF.

EXPERIMENT

Patterned particle-encapsulated microstructure

Two poly (ethylene glycol) diacrylate (PEGDA) prepolymer solutions of varied average molecular weights *Mn*, 250 and 575, were indicated with added fluorescent particles of different colors. As shown in Fig. 5, two PEGDA solutions were simultaneously deformed into hepatic lobule patterns, where the green fluorescent particles were further rearranged by the slit pattern at the center of the linear electrodes to recapitulate the vascular structure (Fig. 5).

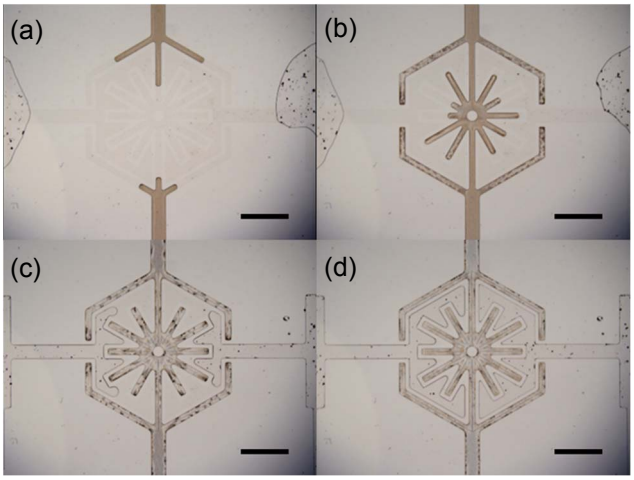

Figure 5: Hepatic lobule like hydrogel microstructure established by simultaneous manipulations of two varied PEGDA prepolymer solutions containing different fluorescent particles. The experimental process is shown in a sequence from (a) to (d). (Scale bar: 1 mm)

After photocrosslinking, as shown in Fig. 6, multiple hydrogels and particles were concurrently arranged and settled to predesigned patterns. The green fluorescent particles encapsulated in the hydrogel were patterned on the linear-shaped electrodes.

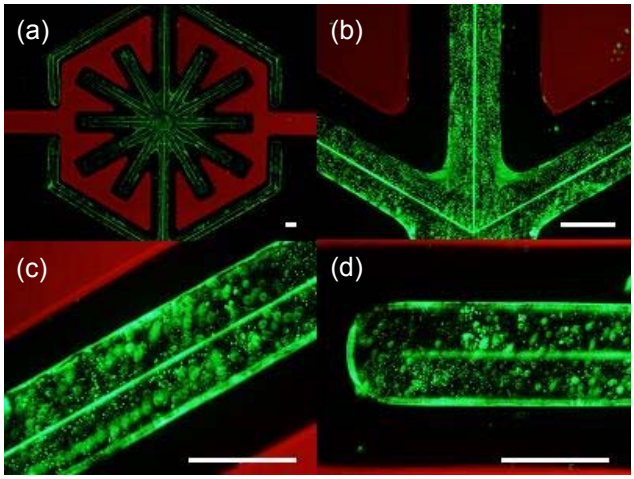

Figure 6: Fluorescent images of the PEGDA microstructures after photocrosslinking. Images were taken under various magnification, including (a) 2X, (b) 10X, and (c) and (d) 20X objective lens. (Scale bar: 200 μm)

Biomimetic hepatic lobule tissue

To construct *in vitro* hepatic-lobule-like microstructures, HepG2 and HUVEC (both concentration 1×10^7 cell/mL) were dispersed in two gelatin methacryloyl (GelMA) prepolymers (concentration 5% and 15% w/v, diluted by low conductivity buffer) with different stiffness. To improve the cell proliferation of HUVEC, we added HFL1 (concentration 5×10^6 cell/mL) into the hydrogel prepolymers containing HepG2. Stiffer and softer GelMA prepolymer solutions were patterned into hepatic lobule geometries with appropriate cells encapsulated. Fig. 7 shows the manipulation of HUVEC- and HepG2-laden GelMA prepolymer solutions and the arrangement of cells to form hepatic-lobule-like microstructures.

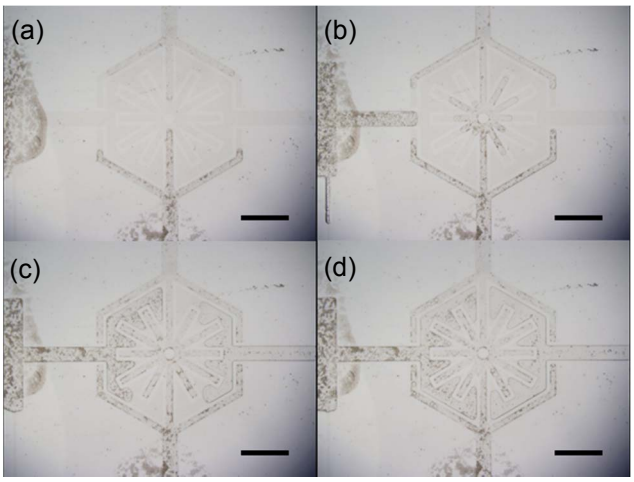

Figure 7: Hepatic-lobule-like in vitro tissue constructed on the EMF device. (a) to (d) The HUVEC-laden GelMA prepolymer solutions (15% w/v) were driven from the upper and lower parts of the images, while HepG2-laden GelMA prepolymer solutions (5% w/v) were patterned from the left and right parts of the images to the center. (Scale bar: 1 mm)

After UV exposure, we disassembled the parallel plates and obtained the cell-laden GelMA hydrogels firmly attached on the top plate with proper surface treatments. We subsequently refilled the gap between the GelMA hydrogel structures with thermally crosslinkable Matrigel. 3D *in vitro* hepatic-lobule-like cell-laden microstructures were thus constructed after thermal crosslinking. Cell coculture and proliferation were observed inside the microstructures as shown in Fig 8.

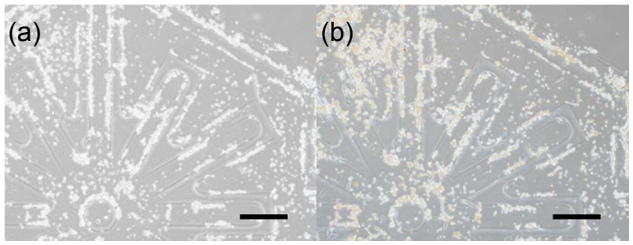

Figure 8: HepG2 and HUVEC proliferation inside the patterned GelMA- and Matrigel-based matrices. (a) Before incubation and (b) after 2 days in culture. (Scale bar: 500 μm)

CONCLUSIONS

In this study, EMF platform was used to pattern different cell-laden hydrogel pre-polymers and different cell types into *in-vivo*-like arrangements in one step. The *in vitro* biomimetic liver tissue was fabricated via biocompatible hydrogel with different stiffness and crosslinking strategies to encapsulate corresponding cell types. The hydrogel-based biomimetic cell culture scaffold was constructed to culture cells and to form *in vitro* liver tissue with appropriate cell morphologies and phenotype functions.

By using different hydrogels and electrodes of biomimetic patterns, *in-vivo*-like 3D cell-laden microstructures were constructed after hydrogel crosslinking. Expecting the influence of patterned morphologies and cell multi-culture towards cell behaviors, the cell-laden microstructures will ultimately form living tissues with certain level of physiological functions.

In the future, the induced tissue can serve as an ideal *in vitro* model for basic medical research, translational studies, and drug screening. This platform is also a potential candidate for personalized medicine as drug testing and development tools, to reach the goal of regenerative medicine and organs-on-chips.

REFERENCES

[1] M.-Y. Chiang, Y.-W. Hsu, H.-Y. Hsieh, S.-Y. Chen, and S.-K. Fan, "Constructing 3D heterogeneous hydrogels from electrically manipulated prepolymer droplets and crosslinked microgels", *Science Advances*, vol. 2, no. 10, 2016, e1600964.

[2] Y.-T. Lo, J.-H. Lee and S.-K. Fan, "Biomimetic and heterogeneous microstructure with multiple hydrogels using electromicrofluidic techniques," *MicroTAS'20 Conference*, Dublin, Oct 9-13, 2016, pp. 397-398.

[3] C. T. Ho, R. Z. Lin, W. Y. Chang, H. Y. Chang and C. H. Liu, "Rapid heterogeneous liver-cell on-chip patterning via the enhanced field-induced dielectrophoresis trap." *Lab on a Chip*, vol. 6, 2006, pp. 724-734.

[4] M. Nikkhah, N. Eshak, P. Zorlutuna, N. Annabi, M. Castello, K. Kim, A. Dolatshahi-Pirouz, F. Edalat, H. Bae, Y. Yang and A. Khademhosseini, "Directed endothelial cell morphogenesis in micropatterned gelatin methacrylate hydrogels." *Biomaterials*, vol. 35, 2012, pp. 9009.

[5] S.-K. Fan, T.-H. Hsieh, and D.-Y. Lin, "General digital microfluidic platform manipulating dielectric and conductive droplets by dielectrophoresis and electrowetting," *Lab on a Chip*, vol. 9, 2009, pp. 1236-1242.

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