CONSTRUCTION OF 3D HYDROGEL-BASED CELL CULTURE WITH MULTIPLE PATTERNS AND HETEROGENEOUS BIOMATERIALS USING ELECTRO-MICROFLUIDIC TECHNIQUES

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ABSTRACT

Simultaneous patterning of different bio-molecule-laden hydrogel pre-polymers, including poly(ethylene glycol) diacrylate (PEGDA) and gelatin methacrylate (GelMA), was achieved using electro-microfluidic techniques. Three dimensional microstructures were constructed with designed dimensions and material properties after hydrogel polymerization. Fluorescent-particle-embedded hydrogels were adopted as proof of concept of this research, while different cell-laden hydrogels, including mice fibroblast cells (NIH-3T3) and human hepatocytes (HepG2) were employed to perform 3D cell mono- and co-culture. With this technique, 3D hydrogel-based cell culture in heterogeneous microstructures with desired material and geometrical properties is accomplished.

KEYWORDS: 3D cell mono-/co-culture, hydrogel-based microstructure, electro-microfluidics

INTRODUCTION

To reach the ultimate goal of tissue engineering, development of three-dimensional microstructures mimicking the in-vivo physiological situation is of a high demand. Recently, hydrogel-based biomaterials were widely adopted and investigated with a variety of techniques, due to their tunable material properties and liquid-to-gel cross-linking mechanisms. Among these techniques, electro-microfluidic technology has advantages of (1) simultaneous manipulations of different liquid biomaterials with the volume of nanoliters and of (2) programmabilities among various fluids.

The demonstrated electro-microfluidic platform has the capability of achieving the featured advantages integrated with the two main fluid driving mechanisms, electrowetting-on-dielectric (EWOD) and liquid dielectrophoresis (DEP). By applying voltage onto the device, precise manipulations of liquid biomaterials is accomplished and further applied to cell and tissue engineering. Photo-cross-linkable hydrogels, including poly(ethylene glycol) diacrylate (PEGDA) and gelatin methacrylate (GelMA), were manipulated at the pre-polymer state and sequentially polymerized by UV light exposure to form a firm 3D microenvironment. Mice fibroblast cells (NIH-3T3) and human hepatocytes (HepG2) were investigated for cell mono- and co-culture. 3D heterogeneous microenvironments are precisely built by realizing simultaneous patterning of multiple hydrogel pre-polymers.

THEORY

Electrowetting-on-dielectric (EWOD) is one of the driving forces behind the electro-microfluidic technology. This phenomenon can be physically described by Young-Lippmann equation:

$$\cos \theta(V) = \cos \theta_0 + \frac{\varepsilon_\text{d} \varepsilon_\text{f} V^2}{2 \gamma_{\text{la}}}$$

(1)

By applying voltage $V$ across a substrate with dielectric layer, the contact angle of the liquid droplet and the substrate underneath alters from $\theta_0$ to $\theta(V)$. This phenomenon is affected by the permittivity of vacuum and the dielectric, $\varepsilon_\text{d}$ and $\varepsilon_\text{f}$, respectively, the thickness of the dielectric, $t$, and the surface tension at the liquid-ambience interface, $\gamma_{\text{la}}$.

Alongside EWOD, DEP is another basic fluid driving mechanism of electro-microfluidics. By voltage application, the liquid between the two plates of the electro-microfluidic platform is actuated by the DEP force $F_{\text{DEP}}$:

$$F_{\text{DEP}} = \frac{\varepsilon_\text{d} (\varepsilon_\text{f} - \varepsilon_\text{a}) W}{2h} V^2.$$  

(2)
where $V$ is the applied voltage, $h$ the gap between the two plates, $w$ the width of the electrodes, and $\varepsilon_f$ and $\varepsilon_a$ the permittivity of the fluid and the ambience, respectively.

**EXPERIMENTAL**

As shown in Figure 1, our electro-microfluidic platform consists of two plates. The top ITO glass plate is simply spin-coated with a hydrophobic layer. The bottom plate contains patterned ITO electrodes covered by dielectric and hydrophobic layers. Liquids are manipulated between the two plates with appropriate voltage applications.

![Figure 1](image1.png)

*Figure 1: Cross sectional view of the electro-microfluidic platform.*

As for the manipulated fluid, PEGDA (Sigma-Aldrich) was added with different colors of fluorescent particles before being put onto this platform. To construct 3D scaffold, HepG2 and NIH-3T3 ($2 \times 10^7$ cell/mL) were put into GelMA pre-polymers (5% w/v, diluted by PBS solution) as the adopted biomaterials. After 7 to 9 days of cell culture of the induced scaffolds, DAPI/f-actin dye were applied to the induced hydrogel-based scaffolds to verify the results. All hydrogel pre-polymers in this research were prepared by implementing photo-initiator Irgacure 2959 (0.5% w/v, Pufong Enterprise CO; Ltd.).

**RESULTS AND DISCUSSION**

By applying voltage onto the electrodes, we can successfully manipulate diverse liquid biomaterials simultaneously into desired arrangements, including fluorescent-particle-laden and cell-laden hydrogel pre-polymers. As shown in Figure 2, various patterns of fluorescent-particle-imbedded hydrogel-based matrices were built after hydrogel manipulation and polymerization.

![Figure 2](image2.png)

*Figure 2. Multiple pre-polymer liquid manipulation and cross-linking on the electro-microfluidic platform. (a)-(c) Bright field pictures of multiple patterns of fluorescent-particle-laden hydrogels. (d)-(f) Fluorescent images of corresponding patterns of (a)-(c), respectively. (scale bar: 1mm)*
3D hydrogel-based microstructures were constructed and further applied to perform cell mono- and co-culture, as shown in Figure 3. 3D cell distribution, cell migration and cell alignment were observed after 7 to 9 days of cell culture in the hydrogel microstructures.

![Figure 3. Microscopic images of cell culture. (a)-(d) Fluorescent (DAPI/f-actin dye) images of the patterned hydrogel-based microstructures after 9 days of cell culture. (a), (b) By changing the depth of focus of these two images sharing the same vision, 3D cell distribution can be observed by comparing the cells on focus and out of focus. (c) NIH-3T3 cells aligned along the direction of the microstructure boundaries. (d) HepG2 cells, as the dashed red curves marked, migrated into territories originally occupied by NIH-3T3 cells, as the red arrow indicated. (e), (f) Bright field images of the induced microenvironment after 7 days of cell culture. HepG2 cells, the darker parts in the pictures, as the red dashed lines indicated, and NIH-3T3 cell co-culture in interdigitating patterns.](image)

**CONCLUSION**

By using the electro-microfluidic platform, simultaneous patterning of various liquid biomaterials with the volume of nanoliters was accomplished. Hydrogels were adopted as the manipulated fluid, in order to construct 3D microstructures after cross-linking. Various fluorescent particles and cells were dispersed in hydrogel pre-polymers to construct 3D microstructure for cell mono- and co-culture. Heterogeneous hydrogel microstructures were established for future applications to 3D cell culture and tissue engineering. Other hydrogels of different cross-linking mechanisms as well as various biomolecule-laden hydrogel pre-polymers are potential candidates of the manipulated biomaterials.

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**REFERENCES**


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