CELL-LADEN MICROGELS ASSEMBLY BY DIELECTROPHORESIS

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ABSTRACT

Microgels with different sizes and shapes are prepared by the photolithography process and manipulated by dielectrophoresis (DEP) to control the position of the cell-laden microgels, construct bio-mimicking cellular microenvironments, and help cells to perform specific biofunctions. Manipulations of 3D cell-laden microgels by the presented DEP-based platform are rapid, precise, and highly programmable. Microgels with various shapes patterned by the photolithography processes provides geometry cues for cells, leading to, for example, different differentiation ways of stem cells. Combining with the manipulation and patterning technique, delicate and diverse cellular microenvironments for cells are possible, which achieves the purpose of programmable differentiation and spatially regulation of microtissues into macrotissues.

KEYWORDS

Cell-laden microgel, DEP, gelatin methacrylate (GelMA), polyethylene glycol (PEG), polyethylene glycol diacrylate (PEG-DA)

INTRODUCTION

Manipulating patterned cell-laden microgels [1] would control the cellular microenvironment and improve the function of microtissue. Various microgel structures have been studied to provide geometry cues for stem cell differentiation. [2] We use photolithography process and photo-sensitive hydrogels to fabricate the microgels with various shapes determined by photomask patterns. After transferring these 3D cell-laden microgels onto a microfluidic device, dielectrophoresis (DEP) [3] is applied to spatially regulate the cell-laden microgels, as shown in Figure 1. So, in this study, we desired to fabricate 3D cell-laden microgels in various geometries, in order to stimulate cells obtaining the specific biofunction spontaneously. We utilized the DEP-based strip-shaped electrodes to assemble microgels into corresponding locations, forming specific organization macrogels and cellular microenvironment. The presented device is an ideal platform for cell-laden microgels assembly and remodeling, which can be applied in cell-cell cocultures and tissue engineering.

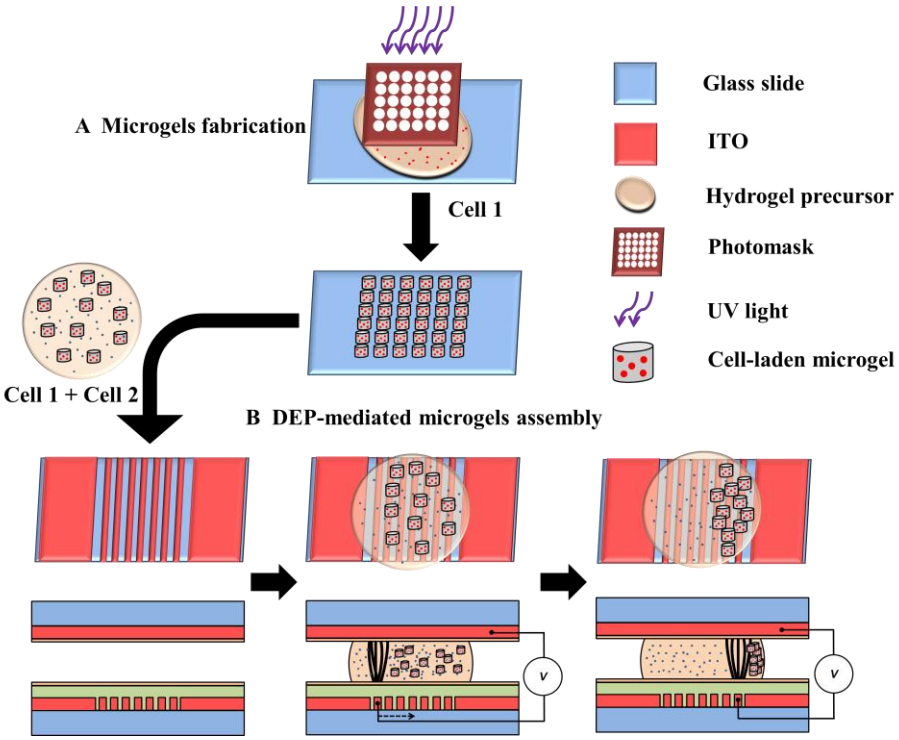

Figure 1: Preparation and manipulation of 3D cell-laden microgels.

PRINCIPLE

As shown in Figure 2. When applying an electric signal between two electrodes having different sizes, the non-uniform electric field would polarize the particles in the field and actuate them. Positive dielectrophoresis (pDEP) attracts the particles towards high electric field strength regions, while negative dielectrophoresis (nDEP) repels the particles from high electric field strength regions.

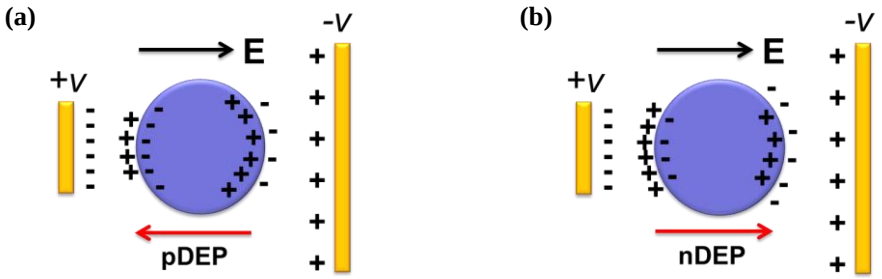

Figure 2: Non-uniform electric field between parallel electrodes having different sizes would polarize particles and induce DEP forces. (a) pDEP would attract particles to the strong electric field region. (b) nDEP would repel particles from the strong electric field region.

EXPERIMENT

In the cell encapsulation study, we embedded NIH-3T3 fibroblasts in 5%, 10%, 15% gelatin methacrylate (GelMA) and polyethylene glycol (PEG) (MW 20,000) prepolymer solutions. Following by UV light crosslinking (360-480 nm, 576 W/cm^2) using photomask patterns, microgels in cubic structures (1 mm × 1 mm × 200 μm) were obtained as shown in Figure 3.

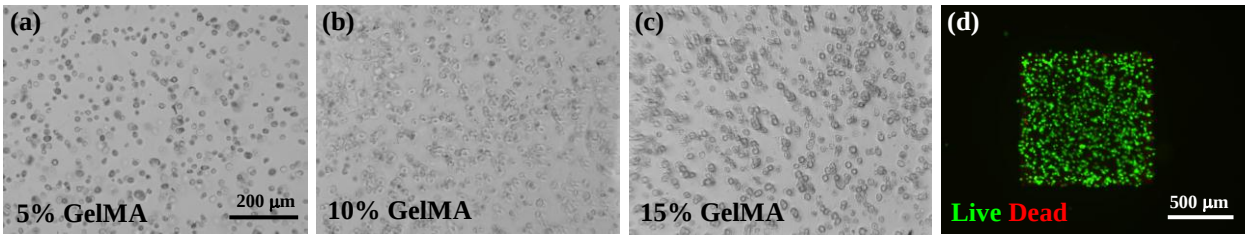

Figure 3: Characterizing the viability of NIH-3T3 cells in GelMA with different concentrations. (a)-(c) The phase contrast image of NIH-3T3 cells encapsulated in GelMA cubic microgels. (d) Live/dead assay of 15% GelMA cubic microgel after photolithography process.

The cell viability in the GelMA and PEG microgels was tested by the calcein-AM/Ethd-1 dyes and analyzed by the ImageJ software, as shown in Figure 4 and Figure 5. GelMA microgels with different prepolymer percentages held good biocompatibility above 70.17%, while the cell viability of PEG was lower than GelMA and in the range between 14.28% and 83.25%. However, the stiffness of GelMA was not high enough to detach the microgels from a glass substrate. Therefore, we used PEG microgels in the following manipulating experiments. As shown in Figure 5, on day 0, the 15% PEG offered the highest cell viability at 83.25% than other two groups, i.e., 51.05% and 46.21% for the 5% and 10% PEG, respectively. For the 5% PEG, the cell viability maintained 54.82% until day 3, and dropped to 14.28% in day 5. The viability of the 10% PEG was the most stable from day 0 to day 5 among the three groups. Hence, we chose the 10% PEG as the encapsulation biomaterial in the following experiments.

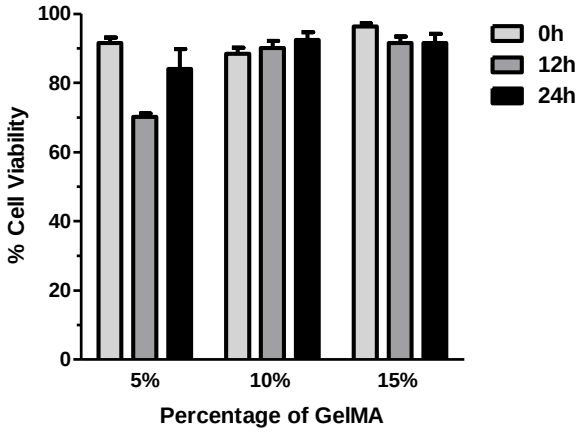

Figure 4: The cell viability analyses for GelMA with different percentages at 0, 12, and 24 hours.

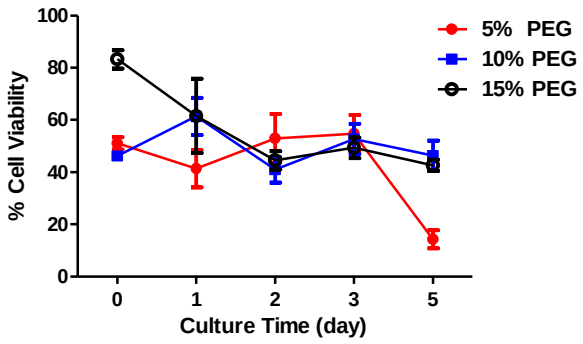

Figure 5: The cell viability analyses for PEG with different percentages on different days.

Furthermore, we fabricated 100% PEG-DA (MW 575) cubic structures (100 μm × 100 μm × 100 μm) by UV light crosslinking (360-480 nm, 188.8 W/cm^2) and photomask patterning. The microgels were transferred onto DEP-based devices by a pipette. When we applied a 40 V_{RMS}, 170 kHz signal on the strip-shaped electrodes sequentially from left to right, the microgels were driven in a pure PEG-DA prepolymer solution droplet (0.25 μL) by the nDEP forces as shown in Figure 6(a)-(d). Various microgels and biomimicking electrodes pattern can be designed for different applications, inducing study of different cell differentiation ways in a biomimicking macrotissue.

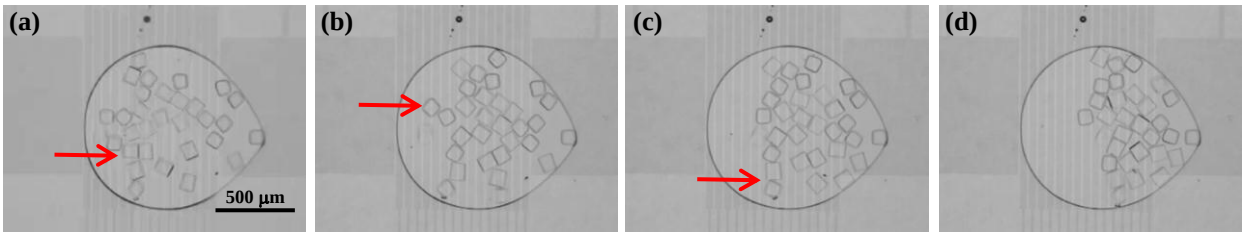

Figure 6: PEG-DA microgels driven by negative DEP force when applying 40 V_{RMS}, 170 kHz to the strip-shaped electrodes sequentially from left to right.

During assembling the dispersed microgels into an accumulated state by nDEP, we used the ImageJ software to analyze the microgels velocity. We further tested the effects of anti-sticking surfactant, pluronic F127. The microgels velocity with pluronic F127 at the concentration of 0%, 0.04%, 0.1%, and 0.5% was 17.59±0.71, 40.14±2.61, 68.04±7.7, and 193.21±24.74 μm/min, respectively, as shown in Figure 7. The microgels velocity was improved with the increased pluronic F127 concentration. Moreover, the 0.5% pluronic F127 reduced the required manipulating time from 20 minutes to 2 minutes compared with 0% pluronic F127.

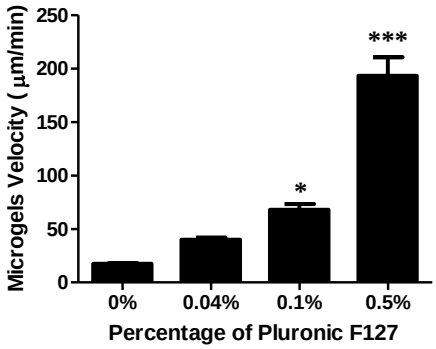

Figure 7: The effects of pluronic F127 concentrations to the microgels velocity.

CONCLUSION

In this study, we fabricated cubic cell-laden microgels by the photolithograph process. According to the cell encapsulation study, GelMA microgels with different prepolymer percentages performed good biocompatibility, while the cell viability of PEG microgels was lower than GelMA. However, considering the proper stiffness of microgels that were easier to handle, we chose the 10% PEG as the encapsulation biomaterial in the DEP-manipulating experiments. We transferred PEG-DA microgels onto DEP-based device by a pipette, and drove them in a pure PEG-DA prepolymer solution droplet. Various microgels and biomimicking electrodes pattern can be designed for different applications, inducing the study of different cell differentiation ways in a biomimicking macrotissue.

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