Cell-manipulation techniques have been developed for single-cell arrays that can be applied to high-throughput single-cell analysis for genomics and proteomics. Rapid and precise cell-positioning techniques are important to estimate the characteristics of a substantial number of cells. Numerous cell-manipulation techniques have been developed based on optical, acoustic, magnetic, microfluidic, and electrokinetic mechanisms, as well as cell adhesive properties.

Concerning electrokinetic methods, dielectrophoresis (DEP) has been widely used as a manipulation tool for the patterning and positioning of both particles and cells. DEP is one of the available cell-manipulation techniques and has been widely used for separating, concentrating, and aligning cells. DEP methods are attractive due to their rapidity, massiveness and necessity scheme of labeling. However, the DEP force is temporary, and the disappearance of DEP regulation occurs upon switching off the voltage application, which leads to a redispersion of cells accumulated for cell patterning. Thus, immobilization techniques, such as covalent bonding via cross-linking agents, microwell arrays, cell-adhesive proteins, and the encapsulation into hydrogels, have been incorporated into the DEP manipulation technique to maintain patterned cells at their directed positions. The encapsulation of patterned cells into photopolymerizable hydrogel is among suitable methods to obtain a cell array with precise positioning. The gelation of patterned cells is driven by the negative dielectrophoretic (n-DEP) field of the polymerizing medium. The behaviors of the cells were observed with an optical microscope (IX72, Olympus Co. Ltd.) for photochemical polymerization, UV light (4.0 mW cm⁻²) was irradiated to the suspension in the device through the upper grid electrode. The upper and bottom substrates were removed from the device to obtain polymerized gel structures. The gel structures with patterned cells were observed and characterized by using an optical microscope.

In this study, we used a DEP device consisting of a grid electrode to form a cell array. The cells suspended in a prepolymer solution of hydrogel are directed to the targeted position to form an island organization of cells. The gelation of prepolymer solution in the DEP device with cellular patterns was induced by irradiating ultraviolet (UV) light; hence, patterned cells embedded in the hydrogel sheets can be obtained after removing the grid electrode. Furthermore, control of the optical transparency of the grid electrode allows one to fabricate cubes with single cells and cell aggregation.

A grid electrode was fabricated on an indium-tin oxide (ITO) substrate by photolithography. Figure 1A shows a schematic design of the grid electrode used as an upper substrate for the cell patterning device. An array of 10000 (100 × 100) panels with a 90-μm square was fabricated by a negative photoresist (5 μm thick, SU-8 3025, MicroChem Corp., Newton, MO) to define the grid electrode with a 10-μm width exposed to the solution. The substrate with the grid electrode was mounted on the flat ITO substrate via polyester film with a microfluidic channel (11 mm wide, 20 mm long and 60 μm thick).

Huh-7 hepatoma cell lines (Huh-7 cells) were dispersed in a prepolymer solution consisting of a 20(v/v)% of poly(ethylene glycol) diacrylate (Aldrich), 1(v/v)% of 2-hydroxy-2-methylpropiophenone (Tokyo Kasei Kogyo Co. Ltd.) used as a photoinitiator, 75(v/v)% of 200 mM sucrose and 4(v/v)% of a DMEM medium. Figure 1B shows a cross-sectional view of the patterning device, which was filled with the cell suspension. AC voltages (40 Vpp, 50 kHz) with the opposite phases were then applied to the upper grid electrode and lower ITO electrode, respectively, from a function generator (7075, Hioki EE Co., Japan) so as to manipulate cells with n-DEP by generating an ununiform electric field in the channel. The behaviors of the cells were observed with an optical microscope (IX72, Olympus Co. Ltd.). For photochemical polymerization, UV light (4.0 mW cm⁻²) was irradiated to the suspension in the device through the upper grid electrode. The upper and bottom substrates were removed from the device to obtain polymerized gel structures. The gel structures with patterned cells were observed and characterized by using an optical microscope.

Figures 2A and 2B show images of cells in the device before and after applying an AC voltage to the upper grid electrode and the lower ITO electrode. Cell suspensions prepared in a 190 mM sucrose solution containing a 5(v/v)% DMEM medium with a concentration of 1.4 × 10⁷ cells mL⁻¹ were injected into
When we applied an AC voltage, those cells dispersed in the channel (Fig. 2A) were directed to aggregate at the positions under the panel of a SU-8 layer within 30 s, resulting in the formation of an island-like organization by cells (Fig. 2B and Movie 1 in Supporting Information). A relatively weak electric field appeared at the upper parts under panels, because strong electric fields were formed between the grid on the upper substrate and the ITO on the lower substrate. Thus, the cells were moved under panels with weak electric field regions by n-DEP at this frequency.

We estimated the distributions of the number of cells aggregated under each panel. Figure 3 shows a histogram of the number of aggregated cells. When a cell suspension with a concentration of $3.5 \times 10^7$ cells mL$^{-1}$ was injected into the channel, the average number of cells under the single panel was found to be 17.2 cells. The average number decreased with decreasing the initial concentration of cells. The average numbers of the aggregated cells for initial concentrations of $1.4 \times 10^7$, $7.8 \times 10^6$ and $1.6 \times 10^6$ cells mL$^{-1}$ were investigated and found to be 7.6, 3.8 and 0.9 cells, respectively. The variances obtained from the histogram were calculated and found to be 2.8, 1.9, 2.1 and 0.6 in descending order of the cell concentrations. This result suggests that the number of cells aggregated at the panels can roughly be controlled by preparing different concentrations of cell suspensions. Especially, we can arrange single cells at panels of 50 – 60%, when a suspension with an initial concentration of $1.6 \times 10^6$ cells mL$^{-1}$ was injected. The obtained average numbers of cells were...
approximately 20% lower than that calculated from the initial concentrations and volume of the channel. This decrease could be caused by the plugging of cells at the inlet.

The island organization of cells can be simply formed by n-DEP with regulating the number of cells included in the cell aggregations. However, cell aggregations easily redisperse after switching off the AC voltage due to depletion of the n-DEP force. We used a photoreactive hydrogel polymer to encapsulate the cells patterned by n-DEP and removed the aggregated cells embedded in the hydrogels. The cells (1.4×10⁷ cells mL⁻¹) were dispersed in a prepolymer solution containing a photoinitiator, and injected into the channel. An AC voltage was applied to form a cell pattern by n-DEP. In the prepolymer solution used in the present work, Huh-7 cells accepted p-DEP over 500 kHz and n-DEP below 100 kHz, respectively. By applying an AC voltage with 50 kHz, cells moved toward the region under the panels, and were accumulated to form an island-like organization (Movie 2 replayed at 8-fold speed in Supporting Information). However, the time required to form the island organization in a prepolymer solution (approximately 6 - 7 min) was longer than that in a sucrose solution. This is most likely due to the high viscosity and high conductivity of the prepolymer solution compared to those of a sucrose solution.

The entire device was then exposed to UV light (2.4 mW cm⁻²) for 90 s from above to induce photopolymerization. Figure 4 shows images of hydrogels containing the patterned cells (approximately 60 μm thickness). The hydrogels were peeled by using a cell scraper after the upper grid electrode was removed from the device. When UV light was irradiated, the patterned cells were embedded in the flexible hydrogel sheet (Fig. 4A). The embedded cells remained in the position arranged by n-DEP after the sheet was transferred. Next, we used grid electrodes colored with black as a template for patterning with cells. The ITO layers were colored with black by applying −1.3 V for 30 s electrochemically, but the details of the mechanism for this discoloration are unclear. Again, cells were accumulated at regions under the panels by applying n-DEP; subsequently, UV light was irradiated from the upper side of the device with a colored grid. Figure 4B shows an image of the obtained hydrogel cubes with cell aggregation. The grid electrodes colored with black acted not only as a template for the cell pattern, but also as a photomask due to the nontransparent function of the coloring. Thus, the prepolymer solution under the panels was polymerized by irradiating UV light, and thereby stimulating the formation of the hydrogel cubes with cell aggregation. Furthermore, hydrogel sheets with a single cell array and hydrogel cubes with a single cell can be easily formed by regulating the concentration of cell suspension (Figs. 4C and 4D). In addition, single cells embedded in the hydrogel cubes were arranged in the center when viewed from any direction. This may be due to the balance between gravity and the n-DEP force along the vertical direction.

In conclusion, flexible hydrogel sheets embedded an array of aggregations formed with different numbers of cells and cubes encapsulated cell aggregations can be easily and rapidly fabricated by combining cell patterning based on n-DEP and immobilization by hydrogel gelation. Sheets and cubes with cells were fabricated in a simple manner for switching on the voltage to manipulate cells to the desired position and rapid manner for switching on UV light to immobilize cells at some position. A variety of gel structures with cells could be fabricated by using various template electrodes.

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Supporting Information

Supporting Information includes Movie 1 for forming the cell pattern by n-DEP in sucrose solution and Movie 2 in prepolymer solution. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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