Precision Micro-fabrication by Gelatin Patterning with Electrostatically-Injected Droplet (ELID) Method

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Abstract

In this paper, a precise line of gelatin was fabricated utilizing electrostatically-injected droplet (ELID) method. The goal of this study is to fabricate 3D structures of cells utilizing the ELID method. It is preferable to perform laboratory experiments with 3D structures of cells in tissue engineering and artificial organ. Inkjet technology was applied to fabricate 3D structures of cells. However, commercial inkjet technology was not enough to pattern liquid with biomaterials precisely because the viscosity of the liquid was high. Small droplets were ejected from a tip of a tube that was filled with liquid when the strong electrostatic field was applied to the water pin electrode. This phenomenon was named as Electrostatically-Injected Droplet (ELID) method. The ELID method had two good merits. Those were high resolution to print and ability to eject highly viscous liquid. These two merits were suitable to fabricate precise 3D structures of cells. It was demonstrated that living cells were not killed when the cells were ejected with the ELID method in spite of high voltage application. Current did not flow inside the cells but around the cells because the resistance value of the cell wall was a little higher than that of the liquid. It was required to pattern scaffolds between cells precisely to fabricate 3D structures of cells because the own weight of cells were above the bonding force between cells. Gelatin and collagen were typical examples of the scaffolds. The purpose of this paper was to pattern precise line of gelatin utilizing the ELID method. The experimental set-up consisted of the water pin electrode that was filled with the liquid with gelatin and a sheet of paper that was set on a plate electrode. Voltage was applied between these electrodes by a high voltage amplifier and a function generator. Gelatin was patterned when the plate electrode was moved in x and y directions with two linear stages. The viscosity of the liquid with gelatin was

low when the temperature of the liquid was high. When the temperature was over 40 degrees Celsius, cells were killed by heat. In consideration of these two matters, the experimental set-up was set in the condition that the temperature was 38 degrees Celsius. The fundamental characteristics to pattern liquid with gelatin utilizing the ELID method were investigated. The precise line of gelatin was patterned with this experimental set-up. The width of the line was about 6 micron meters. It was fine enough to be used as scaffolds because the diameter of cells was about several 10 micron meters.

1 Cell ejection utilizing the ELID method

The goal of this study is to fabricate 3-Dimensional cell structures [1-4] utilizing the ELID method. To achieve this goal, cells and scaffolds should be printed precisely.

Print of living cells was demonstrated by the ELID method.[5-7] Figure 1 and 2 are pictures of printed cells when one day has passed after cells were ejected utilizing the ELID method. From 45% to 70% of cells were living in spite of high voltage application because current did not flow inside cells but around cells. Initially from 20% to 40% of cells were died while rewarming after chilling treatment. Cells were living after the ELID method in case that the diameter of the tube was over 100 micron meters. In case that the diameter of the tube was less than 50 micron meters, it is difficult to eject continuously because cell attachment took place inside the tube. These results indicated that it was possible to print cells utilizing the ELID method.

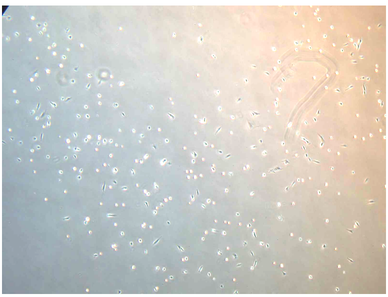

Figure 1: Big picture view of printed cells. Figure 2: Enlarged view of printed cells. (applied voltage: 2.0 kV)

2 Gelatin patterning utilizing the ELID method

In this study, gelatin was used as scaffolds between cells to fabricate 3D cell structures. An experimental set-up illustrated in Fig. 3 was constructed to investigate fundamental characteristics to pattern gelatin. A tube filled with liquid that contained gelatin was mounted perpendicular to a plate electrode made of stainless steel. DC voltage was applied by a function generator (Iwatsu, Tokyo, SG-4105) and a high voltage amplifier (Matsusada Precision Inc, HEOP-10B2). Paper was set on the plate electrode for easily viewable. The formation of the droplet was observed with a high-speed microscope camera (Photron Inc., Japan, FAST-CAM-MAX 120K model 1) with a light (Sanei Electric Inc., Japan, XEF-501S). The temperature around the experimental set-up was controlled at 38 degrees C because viscosity of gelatin was high in case of low temperature and cells were died over 40 degrees C.

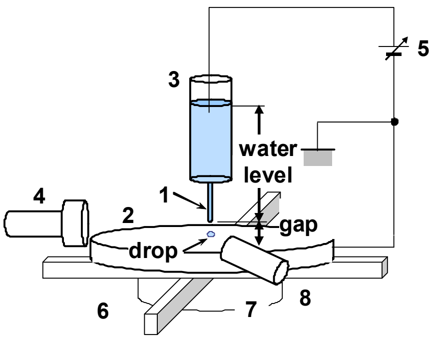

Figure 3: Experimental set-up of the ELID method. (1: water pin electrode, insulative capillary tube filled with ink, 2: metal plate electrode, 3: tank, 4: high speed camera, 5: high voltage amplifier and function generator, 6: linear stages that moves in x and y directions, 7: mechanical z-stage, 8: light)

Table 1 Specifications of gelatin.

Type	Details	Viscosity mPa・s	Strength g
1017	Porcine skin (acid processed)	3.5	235

Table 1 shows the specifications of the gelatin that was used in this experiment. Other kinds of gelatin were used in the experiment. The characteristics of ejected droplets and printed lines in case of other kinds of gelatin were investigated. The results were skipped because these characteristics were similar with the characteristics in case that the type 1017 was used.

Figure 4 shows the line width of patterned gelatin in case that the air gap was changed. This figure indicated that the narrow line of gelatin was patterned in the condition of low voltage and small air gap. When the applied voltage was increased, the line

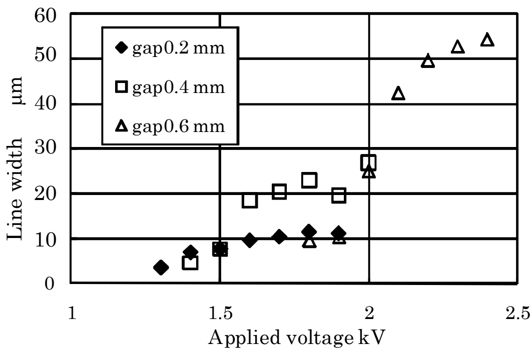

Figure 4 Line width of patterned gelatin in case that the air gap was changed.
(density: 1.0 %, Inner diameter of the tube: 0.1 mm)

width was wide. When the applied voltage was less than 1.5 kV, the width of the line was less than 10 micron meters. Because the size of cell was from 20 micron meters to 50 micron meters, the width of the printed line of gelatin was precise enough to be used as scaffolds of 3D cell structures.

3 Conclusions

The fundamental characteristics of patterning liquid with gelatin utilizing the ELID (Electrostatically-Injected Droplet) method were investigated. Precise line that width was less than 10 micron meters was patterned. Because the size of cell was several 10 micron meters, this precise patterned line will be useful to fabricate 3D cell structures.

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