Gelatin Patterning Utilizing ELectrostatically-Injected Droplet (ELID) Method

Shinjiro Umezu (1, 2), Motoya Kawanishi (1), Yuta Katsumi (1), Takashi Kitajima (2), Makoto Sakuragi (2), Hitoshi Ohmori (2), and Yoshihito Ito (2)
1: Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259-1292, JAPAN
2 RIKEN, The Institute of Physical and Chemical Research, 2-1 Hirosawa, Wako, Saitama 351-0198, JAPAN

Abstract

Electrostatically-Injected Droplet (ELID) method took place when high voltage was applied between a capillary tube filled with ion conductive liquid and a metal plate electrode. At the last DF conference, we reported that living cells were printed and demonstrated fabrication of simple 3D structures which contained living cells and scaffolds by the ELID method. However the stiffness of the simple 3D structures were low, it was necessary to print not only line-shaped scaffolds but also sheet-shaped scaffolds. In this paper, we developed new printing machine "Bio-Cell On". We patterned a precise line and a thin sheet of gelatin with this machine. The width of the line was about several micron meters. It was narrow enough to be used as scaffolds because the diameter of cells was about several 10 micron meters. The thickness of the sheet was controlled by the time and amplitude of voltage application.

Introduction

The goal of this study is to fabricate precision 3-Dimensional cell structures utilizing ELID (ELectrostatically-Injected Droplet) method. It is preferable to perform laboratory experiments with 3D cell structures in tissue engineering and artificial organ. However it is difficult to fabricate 3D cell structures because own weight of cell is above the bonding force between cells. Commercial piezo inkjet technology was applied for 3D positioning of alginate capsule which contained living cells [1, 2]. Alginate capsule was used as scaffolds instead of gelatin liquid or collagen liquid because these were difficult to eject due to high viscosity. Because alginate capsules were easy to stick each other, 3D positioning of cells was succeeded. However cells could not contact each other by the wall of the alginate capsule. To clear this problem, inkjet technology should be more powerful to eject highly viscous liquid. Our inkjet technology, ELID (ELectrostatically-Injected Droplet) method, had two merits; those were high resolution and ability to eject highly viscous liquid. These merits were suitable to print cells precisely and eject highly viscous scaffolds. At the last DF conference, we reported that living cells were printed and 3D structures which contained living cells were fabricated utilizing the ELID (ELectrostatically-Injected Droplet) method [3]. To fabricate precision 3D cell structures, scaffolds should be printed precisely and fabricated 3D structure . when high voltage was applied between electrodes, small droplets were dispersed like spray from the tip of the nozzle. This spray mode was suitable for fabricating sheet-shape of gelatin.

Experimental Set-up

In this study, gelatin was used as scaffolds between cells to fabricate 3D cell structures. An experimental set-up illustrated in Fig. 1 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

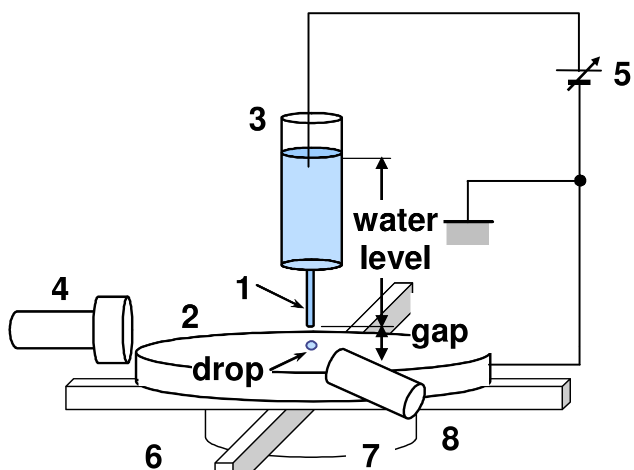

Figure 1: Experimental set-up of ELID. (1: water pin electrode, insulative capillary tube filled with gelatin, 2: metal plate electrode, 3: tank, 4: high speed camera, 5: high voltage amplifier and function generator, 6: linear stages, x and y directions, 7: mechanical z-stage, 8: light)

Table 1 Specifications of gelatin.

Type	Details	Viscosity mPa・s	Strength g
1013	Cow bone gelatin (alkali processed)	3.5	292
1014	Cow bone gelatin (alkali processed)	2.8	107
1015	Porcine skin (acid processed)	4.6	293
1016	Porcine skin (acid processed)	1.9	101
1017	Porcine skin (acid processed)	3.5	235
1018	Porcine skin (acid processed)	2.1	130

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, FASTCAM-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. Six types of gelatin that specifications were listed in table 1 were used in this experiment.

Results

Precision Line

Line structure which contained gelatin was fabricated utilizing the ELID method. Figure 2 shows the width of the line in case that the kinds of gelatin were changed. Small droplets that size were from several micron meters to several ten micron meters were ejected by the ELID method. This result matched the former results because the viscosity of the liquid was almost the same described in the papers [4-6]. Figure 3 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 less than 1.5 kV, the width of the line was less than 10 micron meters, shown in Fig. 4. Because the size of cell was from 20 micron meters to 50 micron meters, the printed gelatin line was precise enough to be used as scaffolds.

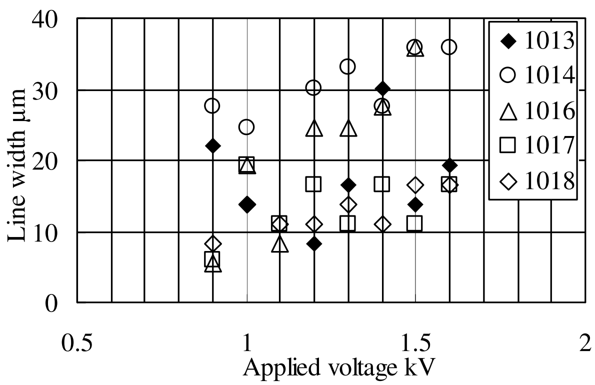

Figure 2: Line width of printed gelatin. (density: 1.0%)

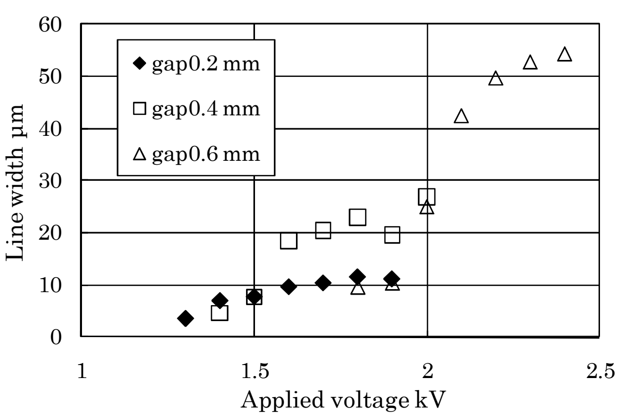

Figure 3: Line width of printed gelatin. (air gap: 0.1 mm, inner diameter of the tube: 0.1 mm, density:1.0 %)

Figure 4: Printed narrow gelatin line.

Thin Sheet

The stiffness of printed 3D structure which contained cells and gelatin was not high. Stiffness of the structure will be increased when the gelatin line and gelatin sheet were printed repeatedly. We fabricated gelatin sheet utilizing spray mode of the ELID method. When high voltage was applied to the water pin electrode, small droplets were dispersed like spray from the tip of the nozzle. This spray mode was suitable for fabricating gelatin sheet. Figure 5 showed the modes of the ELID method. When the applied voltage increased, spray was took place. When the air gap was increased, the threshold voltage of spray mode was increased because the threshold electrostatic field of spray mode was constant. Figure 6 to 8 showed the pictures of spray coated gelatin. When the liquid level was high, sometimes relatively large droplets were ejected while spraying because pressure of the liquid was high. Because uniformly coated gelatin sheet was suitable to use as sheet-shaped scaffolds, liquid level should be

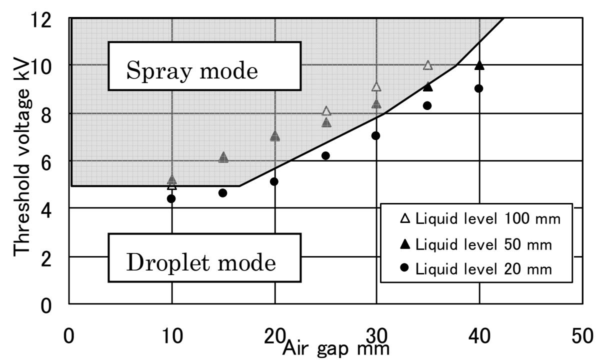

Figure 5: Mode of the ELID method.

Figure 6: Picture of spray coated gelatin.
(liquid leverl: 2.0 mm, applied voltage: 9.0 kV)

Figure 7: Picture of spray coated gelatin.
(liquid leverl: 5.0 mm, applied voltage: 9.0 kV)

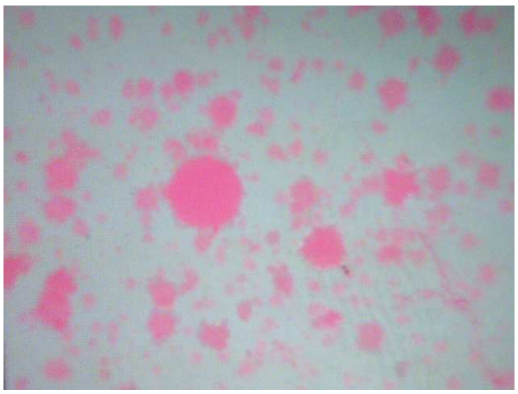

Figure 8: Picture of spray coated gelatin.
(liquid leverl: 10.0 mm, applied voltage: 9.0 kV)

low to prevent the ejection of the relatively large droplets. Figure 9 shows the width and thickness of spray coated gelatin sheet. Liquid with gelatin was high viscosity when the temperature was low. When the target was cooled with peltier unit, gelatin sheet will be formed rapidly. In this experiment, the shape of the gelatin sheet was not affected by the peltier unit. However, we recognize the effect of peltier unit in case of thick gelatin sheet.

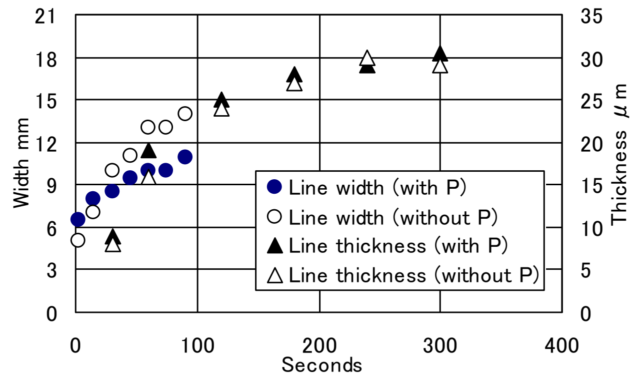

Figure 9: Width and thickness of spray coated gelatin. (with P: target was cooled with peltier unit, without P: target was not cooled)

Conclusions

We investigated fundamental characteristics of patterning liquid with gelatin. Precise line that width was less than 10 micron meters was patterned. Because the size of cell was several 10 micron meters, the precise patterned line is suitable to be used as scaffolds. When the applied voltage was high, small droplets were dispersed like spray. This spray mode was used to fabricate gelatin sheet. The stiffness of 3D cell structure will be high in case that the gelatin lines and gelatin sheet were patterned repeatedly.

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Author Biography

UMEZU, Shinjiro received the BE (2001), MS (2003) and Ph.D (2006) degrees in Mechanical Engineering from Waseda University. He was a research associate at Waseda University since 2003 to 2007. He was a special postdoctoral researcher at Riken since 2007 to 2009. He is now an Assistant Professor at Tokai University. He was awarded Best Presentation Award twice. His research interests include imaging technology and bio-mechanical fabrication utilizing micro drop injection and ultra precision mechanical fabrication (ELID grinding).