Fundamental Characteristics of Printed Cell Structures Utilizing Micro-Drop Injection

Shinjiro Umezu[,**], Takashi Kitajima[**], Hitoshi Ohmori[**] and Yoshihiro Ito[**] (*: Tokai University; Hiratsuka-shi, Kanagawa, Japan, **: RIKEN, The Institute of Physical and Chemical Research, Wako-shi, Saitama, Japan)*

Abstract

Micro-drop injection took place when high voltage was applied between a capillary tube filled with ion conductive liquid and a metal plate electrode. Micro-drop injection has two merits; those are high resolution to print and ability to eject highly viscous liquid. Recently many researchers applied commercial inkjet for bio-printing. Main problem of this application is difficulty to print relatively highly viscous liquid. I think the merits of micro-drop injection will clear this problem. So, I applied the micro-drop injection for printing living cells and highly viscous scaffolds to make 3D cell structures. Cells were not killed in spite that high voltage was applied by micro-drop injection. Because current did not flow inside cell but around cell. In this paper, we cleared the fundamental characteristics of patterning living cells and gelatin and fabricated 3D cell cylinder utilizing micro-drop injection.

Introduction

The object of this study is to fabricate 3-Dimensional cell structures utilizing micro-drop injection. 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.

A fabrication method was suggested [1]. When 3D scaffolds were put into liquid with cells, cells were attached to the surface of the scaffolds. However it was difficult to control density of the attached cells and there was much waste of cells. Another fabrication method [2] was carried out to clear these problems. Commercial piezo inkjet technology was applied for 3D positioning of alginate capsule which contained living cells. Alginate capsule was used as scaffolds instead of gelatin liquid or collagen liquid because these liquid 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.

We have investigated mechanism and fundamental characteristics of the micro-drop injection and now been applying for new printing technology of high image quality and 3D printing technology of glass paste [3]. The micro-drop injection have two merits, higher resolution than commercial printer and ability to eject highly viscous liquid [3]. We were able to eject glass paste that viscosity was 30000 mPas. In this paper, we applied the micro-drop injection for printing cell structures. We investigated fundamental characteristics of printing cells and printing gelatin as scaffolds. We fabricated 3D cell cylinder utilizing cells and gelatin.

Micro-Drop Injection

Experimental Set-up

An experimental set-up illustrated in Fig. 1 was constructed to investigate characteristics of micro-drop injection. A tube filled with ink 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). 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)

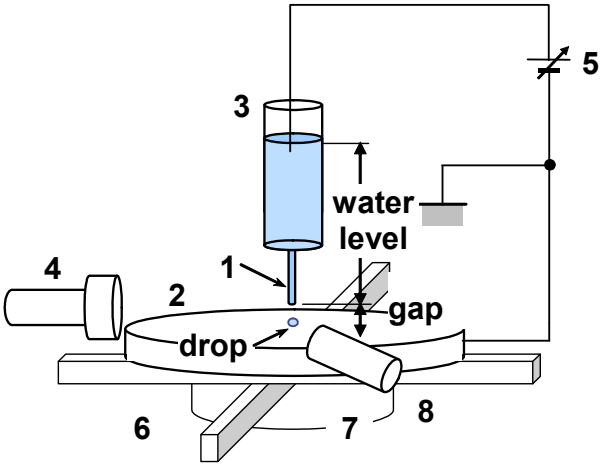

Figure 1: Experimental set-up of single nozzle inkjet system. (1: water pin electrode, insulative capillary tube filled with ink, 2: metal plate electrode, 3: ink tank, 4: high speed camera, 5: high voltage amplifier and function generator, 6: linear stages, x and y directions, 7: mechanical z-stage,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

Characteristics of Micro-Drop Injection

Fundamental characteristics of micro-drop injection were already reported [3]. We would like to introduce the especially important characteristics. In case that the voltage was about 2 to 4 kV, the Taylor cone [4] was formed at the tip of the tube. The tip of the cone was separated and very small droplet was formed. The print quality of printed samples utilizing the micro-drop injection is higher than that of printed samples utilizing a commercial inkjet printer shown in Fig. 2.

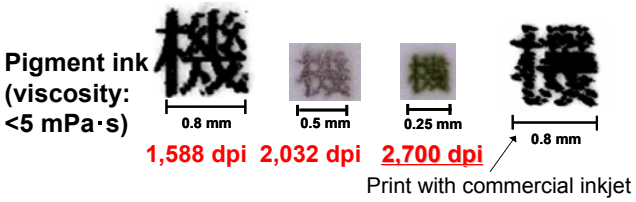

Figure 2: Print samples of Chinese character "mecha" by using the micro-drop injection and a commercial inkjet (liquid material: pigment ink)

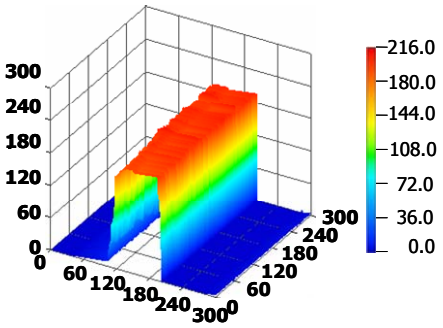

Figure 3: Print samples of 3D line. (material: glass paste)

The micro-drop injection had a potential to realize 3D printing with emulsified liquid that consisted largely of nano-particles, because highly viscous liquid, more than 30,000 mPa.s, were able to be ejected by the micro-drop injection. Compositions of liquid we have synthesizes for the 3D printing are 20 % alumina nano-particles (440 nm), 3 % binder (PVA), 0.2 % dispersing agent, and 77 % water. The viscosity was 12 mPa.s and the contact angle was 70 deg. Figure 3 shows a demonstrated 3D pattern samples.

Patterning Living Cell

Experimental Set-up

Merits of micro-drop injection were suitable to print living cells and to fabricate 3D cell structures because cell structures should be precise and liquid with scaffolds was relatively highly viscous. However, micro-drop injection required high voltage over 1kV. Sometimes people are killed by electric shock through consent. Household voltage is about several hundred V. However, voltage of the micro-drop injection is about several kV. We should investigate effect on the ejected cells by the micro-drop injection.

An experimental set-up shown in Fig. 4 was constructed to investigate characteristics to print living cells utilizing the micro-drop injection. Bone stem cells were used in this experiment. The tube filled with the liquid which contained the cells was hanged down perpendicular to a dish filled with medium. Voltage was applied between the syringes and the dish by a power supply (voltage range: -5kV ~ +5kV, Matsusada Precision Inc, Tokyo, HVR-10P). The air gap was adjusted by a z-stage and the dish was moved in x and y directions with two linear motors. Voltage application and motion of the linear stages were controlled by a PC.

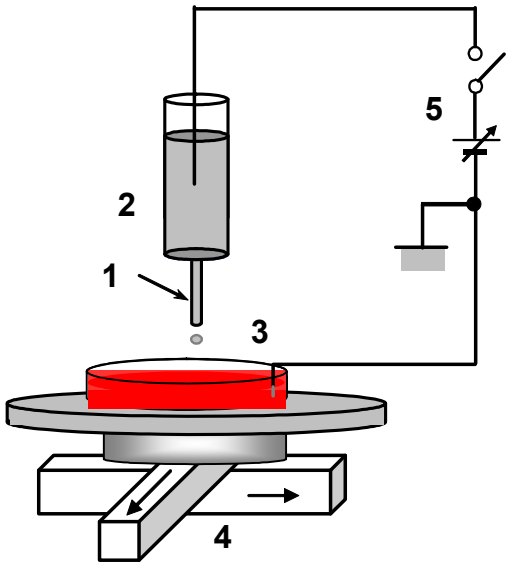

Figure 4: Experimental set-up of cell patterning utilizing electrostatic inkjet. (1: water pin electrode, insulative capillary tube was mounted at tip of syringe, 2: tank, filled with liquid of cells, 3: dish, filled with medium, 4: xyz linear stages, 5: DC high voltage power supply)

Result

Figure 5 and 6 show pictures of printed cells when one day has passed after cells were ejected utilizing micro-drop injection. Figure 7 and 8 show the enlarged view of living cells and dead cells. Living cells were attached to the dish and spread. Dead cells were not attached to the dish and floating in the medium. 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 micro-drop injection 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

Figure 5: Picture of printed cells. (applied voltage: 2.0 kV)

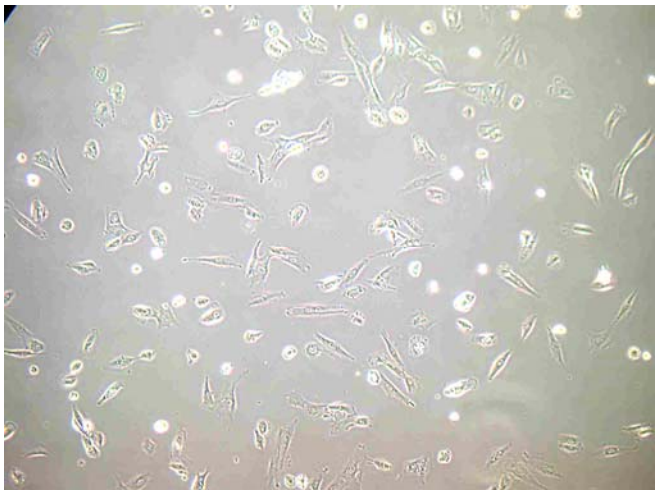

Figure 6: Picture of printed cells. (applied voltage: 3.2 kV)

Figure 7: Enlarged view of living cells.

Figure 8: Enlarged view of dead cells.

attachment took place inside the tube. These results indicated that it was possible to print cells utilizing the micro-drop injection.

Patterning Scaffolds and Fabrication of 3D Cell Structure

Experimental Set-up

An experimental set-up illustrated in Fig. 1 was used to investigate characteristics of the droplet formation of liquid with gelatin by the micro-drop injection. The temperature around the experimental set-up was controlled at 38 degree C because Gelatin became solidified under 36 degree C and cells were died over 40 degree C.

Results

Figure 9 shows continuous images of droplet formation and separation captured by the high speed camera. A Taylor cone [4] was formed at the tip of the tube because voltage in mode 2 [2] was applied between the water pin electrode and the plate electrode. The tip of the Taylor cone was separated and became a small droplet repeatedly. Figure 10 shows the diameter of the ejected droplets when the applied voltage was changed. The diameter of the droplets was about several 10 micron meters. The diameter became large when the applied voltage was increased. Figure 11 shows the diameter of the ejected droplets in case that

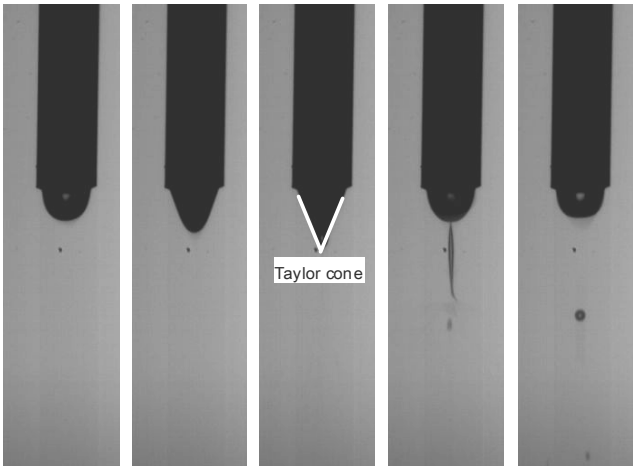

Fig. 9 Movie of droplet formation of liquid with gelatin. (density of gelatin: 1.0 %, applied voltage: 2.4 kV, air gap: 3.0 mm)

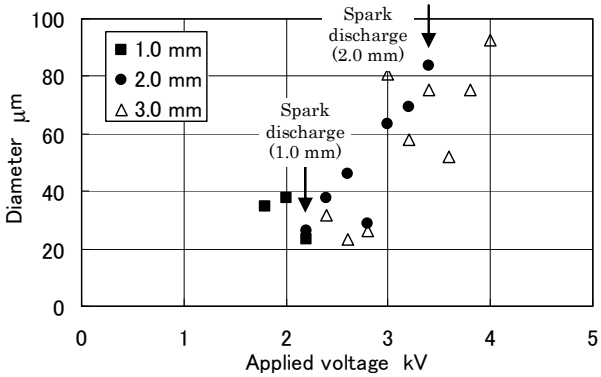

Fig. 10 Diameter of the ejected droplet when the applied voltage was changed. (density of gelatin: 1.0 %, parameter: air gap)

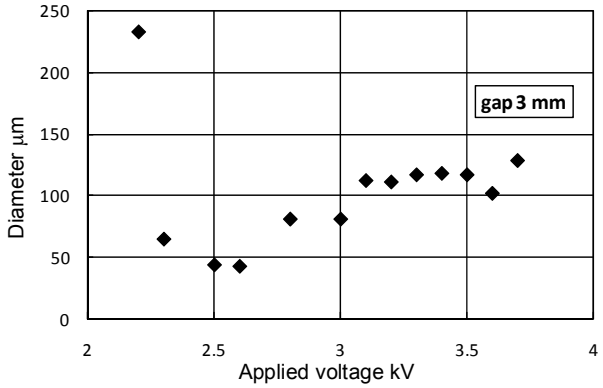

Fig. 11 Diameter of droplet of liquid with gelatin when the applied voltage was changed.(density of gelatin: 2.0%)

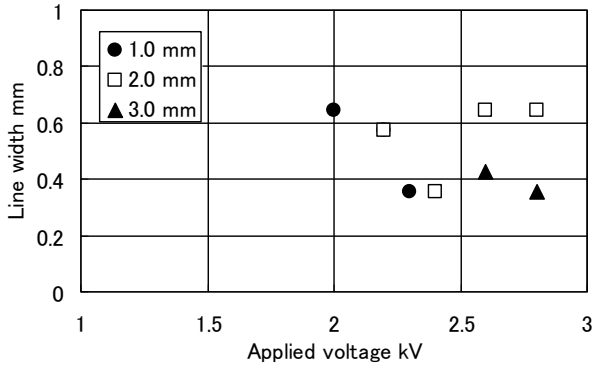

Fig. 12 Width of gelatin line when the applied voltage was changed.(density of gelatin: 1.0%)

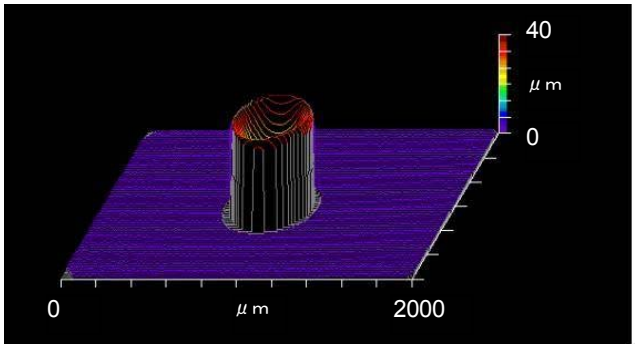

Figure 13: Picture of cell cylinder.

the density of gelatin was 2.0 %. The size of droplets and the trend of the size were almost the same in the condition that the density of gelatin was 1.0 %. Figure 12 shows the width of gelatin line when the applied voltage was changed in case that the density of gelatin was 1.0 %. The line width was not changed when the applied voltage and the air gap were changed. The line was wide in comparison with the ejected droplets because the attached droplet on the plate electrode was spread after landing. Figure 13 shows the 3D cell cylinder when liquid with cells and liquid with scaffolds were ejected alternately.

Conclusions

We investigated fundamental characteristics of patterning liquid with living cells and liquid with gelatin utilizing micro-drop injection. The diameter of ejected droplets was controlled by the amplitude of the applied voltage. Most of ejected cells were living in spite of high voltage application because current did not flow inside cells but around cells. 3D cell structure was fabricated by printing cells and gelatin alternately.

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References

[1] X. Yang, R. S. Tare, K. A. Partridge, H. I. Roach, N. M. Clarke, S. M. Howdle, K. M. Shakesheff and R. O. Oreffo, Induction of Human Osteoprogenitor Chemotaxis, Proliferation, Differentiation and Bone Formation by Osteoblrast Stimulating Factor-1/Pleiotrophin: Osteoconductive Biomimetic Scaffolds for Tissue Engineering, *J. Bone and Mineral Research*, 18, 1, pp. 47-57 (2003).

[2] C. Henmi, M. Nakamura, Y. Nishiyama, K. Yamaguchi, S. Mochizuki, K. Takiura and H. Nakagawa, Development of an effective three dimensional fabrication technique using inkjet technology for tissue model samples, *AATEX*, 14, Special Issue, pp. 689-692 (2007).

[3] S. Umezu, K. Katahira and H. Ohmori, New Micro Fabrication Techniques Utilizing Electrostatic Inkjet Phenomena, *Proc. 10th anniversary int'l conference EUSPEN 2008*, pp. 443-447 (2008).

[4] G. Taylor, Disintegration of water drops in an electric field, *Proc. Roy. Soc. London*, A 280, pp. 383–397 (1964).

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).