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Fundamental characteristics of printed cell structures utilizing electrostatic inkjet phenomena

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ABSTRACT

In this paper, we investigated the fundamental characteristics to print cells and cell structures utilizing electrostatic inkjet phenomena. When high voltage was applied to a tube filled with conductive liquid, a small droplet was formed at the end of the tube and separated repeatedly. These phenomena were the electrostatic inkjet. The electrostatic inkjet phenomena had two merits; those were high resolution and ability to eject highly viscous liquid. These merits were preferable to print liquid with cells and liquid with scaffolds because these liquids were relatively of high viscosity. There is concern about damage on cells by high voltage application. However, in spite of high voltage application, the ejected cells were living. Current was not flowing inside cells but around cells because the membrane resistance of cell was higher than the resistance of the medium. We demonstrated to print line shape, wall shape and cylinder shape that contained cells utilizing the electrostatic inkjet.

1. Introduction

The object of this study is to print three dimensional cell structures utilizing electrostatic inkjet phenomena. It is preferable to perform laboratory experiments with 3D cell structures in tissue engineering and artificial organ [1]. When the technology to fabricate 3D cell structures will be developed, myocardial actuator [2] will be powerful because the density of the myocardial cells will be controlled and cell sensors [3] which can detect chemical drug or poison from a target will be speedy and high accuracy. However it is difficult to fabricate 3D cell structures. When a cell was set on another cell, the upper cell moved down because the own weight of cell was above the bonding force between cells. Scaffolds between cells were required to keep the position of cells.

A fabrication method [4] was suggested to fabricate 3D cell structures. 3D shaped Scaffolds were fabricated. When the 3D scaffolds were put into liquid with cells, cells were attached to the surface of the scaffolds. However this method had some problems; those were difficult to control the density and the position of the attached cells and much waste of cells those were not attached to the scaffolds. Commercial inkjet technology was applied to clear these problems. The commercial piezo inkjet technology was developed and applied for 3D positioning of alginate capsule which contained cells [1]. Alginate capsule was used as scaffolds instead of gelatin or collagen because liquid with gelatin or collagen was difficult to eject due to high viscosity. They demonstrated the ejected cells were not crushed under the pressure that was generated by the deformation of piezo when the cells were ejected. Because alginate capsules were easy to stick each other, 3D positioning of cells was succeeded. However cells could not contact each other because the physical contact between cells was prevented by the wall of the alginate capsule.

Another method utilizing commercial thermal inkjet technology was suggested to clear the problem [5]. Clusters of cells were printed on a bio paper that was worked as scaffolds. They demonstrated that the ejected clusters of cells were not killed by heat of the thermal inkjet and 3D positioning of the clusters were succeeded. However, precise 3D cell structures were better to apply for the tissue engineering, the myocardial actuators and the cell sensors.

We have investigated the mechanism and fundamental characteristics of the electrostatic inkjet phenomena and now been applying for new printing technology of high image quality, 3D printing technology of glass paste [6] and mask-less etching technology [7]. The phenomena have two merits, higher resolution than commercial printer and ability to eject highly viscous liquid. We were able to eject glass paste that viscosity was 30,000 mPa s. In this paper, we applied the electrostatic inkjet for printing living cells. We investigated fundamental characteristics of printing cells utilizing the electrostatic inkjet. We demonstrated that cells were not killed by the high voltage application when the electrostatic

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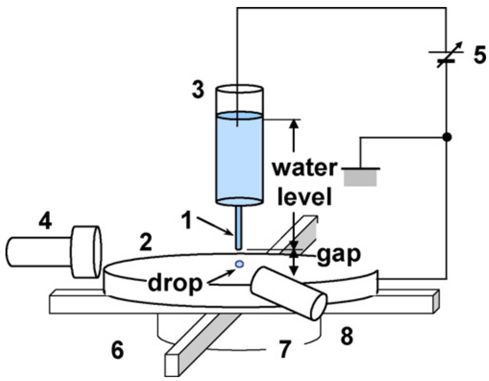

Fig. 1. Experimental set-up of electrostatic inkjet (1. water pin electrode, insulative capillary tube filled with ink; 2. metal plate electrode; 3. ink tank; 4. high-speed camera; 5. high voltage amplifier and function generator; 6. linear stages, *x* and *y* directions; 7. mechanical z-stage; 8. light).

inkjet phenomena took place. After the demonstration, we printed structures that contained cells utilizing the electrostatic inkjet.

2. Electrostatic inkjet phenomena

2.1. Experimental set-up

An experimental set-up illustrated in Fig. 1 was constructed to investigate the characteristics of the formation of droplets in the electric field. A tube filled with conductive pigment ink was mounted perpendicular to a plate electrode made of stainless steel. Voltage was applied between the tube and the plate electrode by a function generator (Iwatsu, Tokyo, SG-4105) and a high voltage amplifier (Matsusada Precision Inc., HEOP-10B2). The plate electrode was moved in *x* and *y* directions by using linear stages and a PC. The formation of the droplet at the tip of the tube was observed with a high-speed microscope camera (Photron Inc., Japan, FASTCAM-MAX 120K Model 1) and a light (Sanei Electric Inc., Japan, XEF-501S).

2.2. Mode of drop formation and print demonstration

Fig. 2 shows the current–voltage characteristics of the water pin electrode [8]. This figure indicated that the formation of the droplet was classified into the following three modes corresponding to the discharge modes. In MODE 1, the diameter of the drop was several times larger than that of the tube diameter and the drop period was long, more than a second. In MODE 2, a Taylor cone [9] was formed at the end of the tube and the tip of the cone periodically separated to form a very small droplet. The diameter of the droplet was in the order of several tens of microns. This MODE 2 is suitable for precise inkjet printing. The frequency of droplet ejection was from several hundred Hz to several kHz. In MODE 3, the Taylor cone changed to hemispherical and the droplet became relatively large,

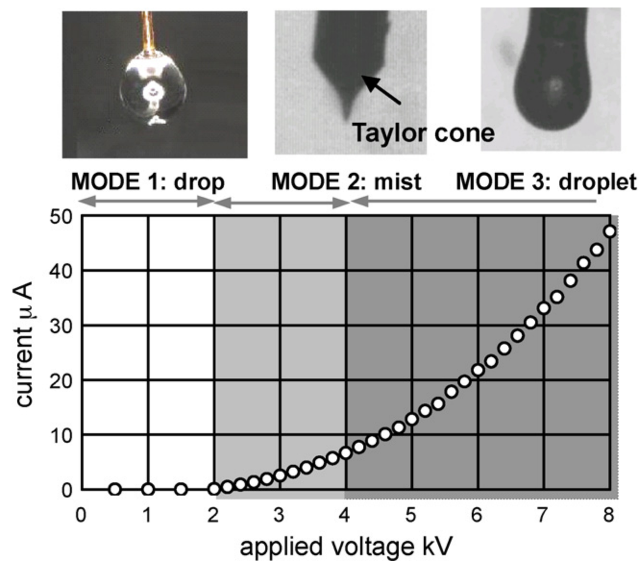

Fig. 2. *V*–*I* curve in pin-to-plate electrode system and modes of drop formation (3 mm air gap).

Table 1
Specifications list of inkjet technology.

	To eject living cell	High resolution	High viscosity
Piezo	○	○	○
Thermal	○	○	○
Electrostatic	○	◎	◎

nearly the same as the tube diameter. The frequency of droplet ejection was less than several hundred Hz. This MODE 3 has possibility of inkjet printing, however it is difficult to print high resolution image.

Fig. 3 shows the print sample utilizing MODE 2 region. Maximum resolution of these samples was approximately 2700 dpi. Resolution of print samples utilizing the electrostatic inkjet was higher than that of print samples utilizing commercial printer. The electrostatic inkjet have potentials to realize 3D printing with emulsified liquid that consisted largely of nano-particles, because highly viscous liquid, more than 30,000 mPa s, were ejected by the electrostatic inkjet. Compositions of the liquid we have synthesizes for the 3D printing were 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°. Fig. 4 shows the demonstrated 3D lines in case that the time to print is changed. The height of the 3D line increased when the time to print was increased.

Table 1 is a specifications list of the inkjet methods. Every inkjet method is able to apply for ejecting cells without killing. As previously mentioned, the electrostatic inkjet has two merits those are high resolution to print and ability to print high

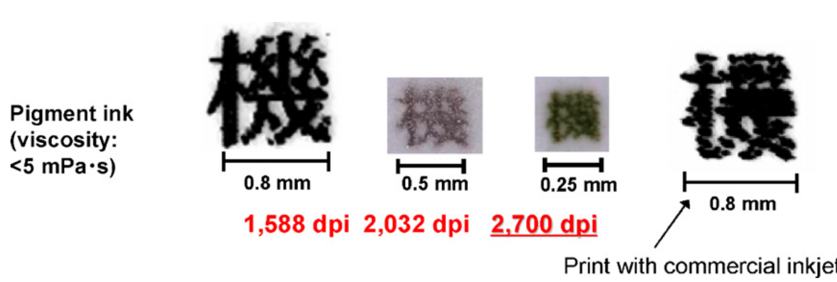

Fig. 3. Print samples of Chinese character "mecha" by using the electrostatic inkjet and a commercial inkjet (material: pigment ink).