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Characteristics on micro-biofabrication by patterning with electrostatically injected droplet

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ABSTRACT

Micro biofabrication technologies have been developing aiming to fabricate 3D artificial organs, 3D scaffolds, and complex tissue structures. We are now developing a new inkjet bio-printing method via electrostatic phenomenon. The merits of the new method are of high resolution, and of ability to eject highly viscous liquid and media. In this paper, we attempted to apply the proposed method for precision printing cells and biomaterials. Living cells and scaffolds have successfully been printed and the biochemical characteristics have been investigated. A 3D cell structure which had a cavity to create blood vessels has also successfully fabricated by this method.

1. Introduction

Biomanufacturing technologies are emergently pursued to improve QOL (Quality of Life) by replacing original parts in human body with artificial ones fabricated by means of engineering methodologies. There are several examples developing artificial joints and implants using bio-compatible materials with specific surface processing methods [1–4]. On the other hand, in order to fabricate three-dimensional (3D) artificial organs containing live cells with fine internal structures, new micro biofabrication methods must be developed. MEMS technology [5], in general, is suitable to fabricate complex 3D micro/nano structures. This technology needs large scale fabrication facilities and also photo masks to generate micro/nano scaffold structures by lithography. Moreover, a vacuum condition is not preferable for keeping cells alive during putting them into the micro scaffold structures. Among another technology, 3D Collagen Sponge Method [6] is suggested to fabricate 3D cell structures. When a 3D collagen sponge is put into a medium with cells, cells are attached to the surface of the sponge. This technology is applied for investigation of growth of neuron cells. However, it is difficult to control the amount in attachment and position of the cells. Cell Sheet Technology [7] is now being applied to fabricate 3D cell structures instead. When several kinds of cell sheets are laminated, a simple 3D cell structure is fabricated. Blood vessels play an important role in our body because nutrition is transported through blood vessels. In case that cells are 200 μm far from blood vessels, they will die because of nutrition shortage. Thus, the position of cells must be controlled precisely to fabricate complex 3D cell structures.

Under those backgrounds, printing technology is recently highly focused to solve those mentioned subjects. Direct Printing Method [8] is suitable to print cells and biomaterials (scaffolds) in terms of resolution and positioning precision, even though viscosity of biomaterials is relatively high. Furthermore Drop-On-Demand (DOD) printing [9,10] is possible to fabricate precision bio structures. Thus, an inkjet printing technology can be used for 3D fabrication to pile 2D printed layers. Skull and born have successfully been fabricated by using inkjet technology [11]. Piezo and thermal printing types belong to DOD printing. It has been found that ejected cells had been alive in spite of high pressure or heat during printing. Commercial piezo inkjet printing technology was tried to be applied for 3D positioning of calcium alginate which contained living cells [12], though, it could eject only scaffolds with low viscosity [13].

Precision artificial organs can be fabricated when high resolution to print in control of cell position and ability to eject highly viscous liquid can be achieved due to high viscosity of most biomaterials to be used. We are now developing an innovative inkjet bio-printing method via electrostatic phenomenon to solve the problem. Patterning with electrostatically injected droplet (PELID) method as a new bio printing has been proposed to give merits on 3D bio fabrication; those are of high resolution in printing cells and biomaterials and of ability to eject highly viscous liquid [14,15].

In this study, we investigated the characteristics in droplet formation mode of this new printing method. Applying this method, 3D cell structures which consist of living cells and scaffolds have successfully been fabricated. It has also been confirmed that the cells could be alive and even grow after the fabrication of the 3D cell structures by this method.

2. Methodology and experimental procedure

2.1. Fundamental characteristics

An experimental set-up illustrated in Fig. 1 was constructed to investigate drop ejection modes in patterning phenomena with electrostatically injected droplet. The capillary tube made of silica

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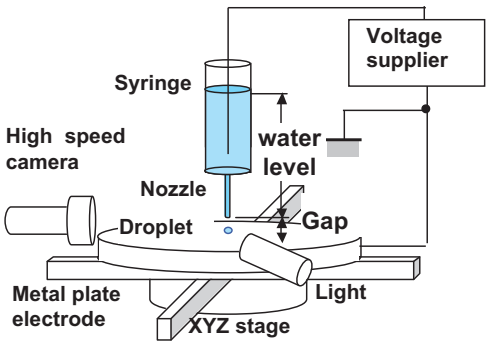


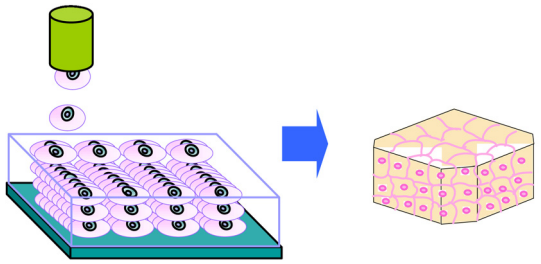
Fig. 1. Experimental set-up.

coated by polyimide was equipped with a bottom of a syringe. Ion-conductive liquid is poured into the syringe. This tube with the liquid is hanged down perpendicular to a plate electrode made of stainless steel. Voltage is applied by a function generator and a high voltage amplifier; the capillary tube was charged positively, the plate electrode was charged negatively. The formation of droplet was observed by a high speed camera with lighting to observe transient formation and ejection of droplets. The gap between the capillary tube and the plate electrode was adjusted by a Z-stage and the plate electrode was moved in *X* and *Y* directions with two linear motors.

2.2. Application for biofabrication

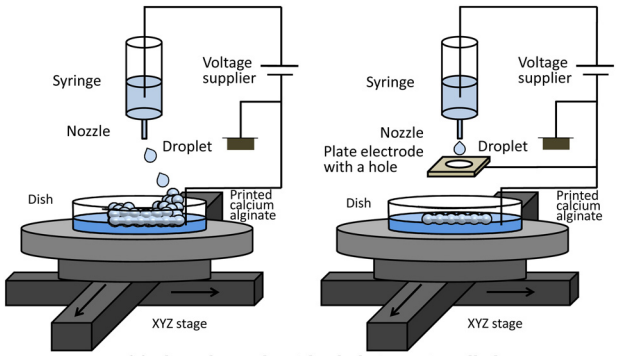
The experiment was conducted to observe the suitability of the new method in printing cells precisely and ejecting highly viscous scaffolds. Fabrication steps are shown in Fig. 2. A 3D structure can be fabricated by the patterning cells and scaffolds together with a cultivation process.

The experimental set-up to pattern 3D cell structure is modified from Fig. 1. As shown in Figs. 3 and 4, dish which is filled with medium is set on the plate electrode. When syringe is filled with cells and/or collagen, 2D printing is carried out. In case that syringe



(a) Pattern cells and scaffolds (b) Cultivate the patterned structure

Fig. 2. Fabrication process of 3D cell structure. (a) Pattern cells and scaffolds. (b) Cultivate the patterned structure.



(a) plate electrode with a hole is not installed
(b) plate electrode with a hole is installed

Fig. 3. Experimental set-up to pattern 3D cell structure and printed structures. (a) Plate electrode with a hole is not installed. (b) Plate electrode with a hole is installed

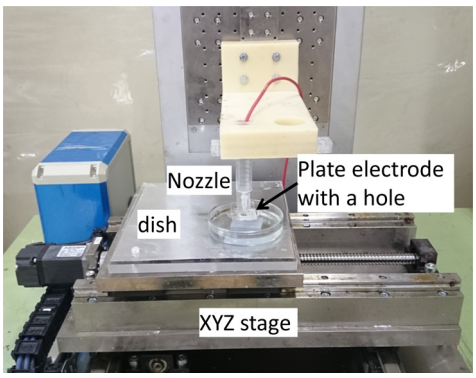


Fig. 4. Photograph of the experimental set-up in case that the plate electrode with a hole is installed.

is filled with aqueous solution of sodium alginate and dish is slightly filled with aqueous solution of calcium chloride, calcium alginate gel is generated in dish when the droplets of aqueous solution of sodium from the syringe are ejected to the dish filled with the calcium chloride.

When Ca ion was put into the aqueous solution of sodium alginate, gelification was took place because of cross-linking. The viscosity of the aqueous solution of sodium alginate (10 wt%) was 30 mPa s at 20 °C.

Even when the printed 3D cell structure becomes thick, the air gap is controlled to be constant by Z-stage. When the air gap between nozzle and the printed structure is short, the Taylor cone is inclined to the edge due to the electric concentration. From this reason, it is difficult to fabricate complex 3D structure precisely in a normal way. As to solve this problem, a plate electrode with a hole is devised between the nozzle and the dish in order to control the droplet direction more precisely. Precision print has successfully achieved though the air gap is short because the electric field around the tip of nozzle is controlled to be constant with the hole of the plate.

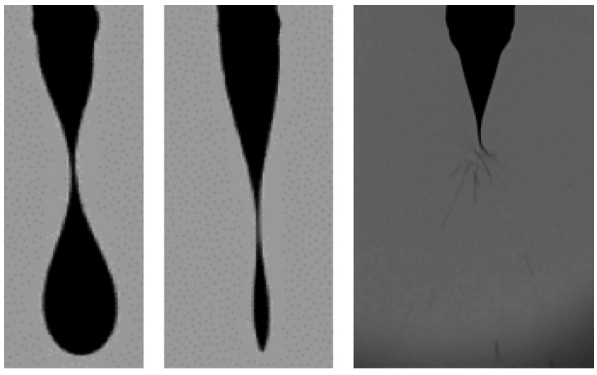
3. Experimental results and discussions

3.1. Fundamental characteristics

Firstly we classify the droplet formation into following three modes shown in Fig. 5. These three modes are determined by the applied voltage and the air gap.

Drop mode: At the dark discharge region, 3–4 kV, a drop is formed at the tip of the tube. This becomes large gradually and drops finally. The diameter of the drop is several times larger than that of the tube diameter.

Droplet mode: At the beginning of the corona discharge, 4–4.5 kV, a Taylor cone [16] is formed at the end of the tube and the tip of the cone periodically separates from the cone to form a very



(a) Drop mode (b) Droplet mode (c) Spray mode

Fig. 5. Mode of droplet formation (nozzle size is 200 μm). (a) Drop mode. (b) Droplet mode. (c) Spray mode