Inkjet bioprinting as an effective tool for tissue fabrication

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Abstract

Recently, 3D printing and rapid prototyping techniques have been applied in tissue engineering. Many of them are used in fabricating of scaffolds of tissues, which is essential in the conservative scaffold guided tissue engineering. However, this approach using simple scaffold has some limitations to manufacture complex, large-scaled, and multi-type cells composed tissues with good reproducibility.

Then, we have ever explored the application of inkjet technique as the technologies to position and arrange such biological materials as living cells, proteins and growth factors arbitrary onto an intentionally targeted position, in three dimensions. There were several problems to be overcome, when using living cells and biological materials instead of inks. Although we have confirmed that living cells could be safely printed by inkjet with keeping living condition, cells are soon dried and dye, and printed lines are easy to mix together, and 3D structures are never constructed with cells in the liquid medium. Then, we invented inkjet hydrogel forming technique to solve such problems. Applying inkjet hydrogel forming technique, we succeeded to prevent cells from drying, and to avoid from ink mixture, and 3D structures could also be constructed. Inkjet bioprinting has many advantages and much possibility as a tool for 3D tissue engineering.

Introduction

The developments of computer technology and mechanical engineering have brought the innovation in the manufacturing processes in the industrial field. Owing to introduction of computers and manufacturing machines in designing, evaluating, and manufacturing process, the manufacturing of products have been more effectively, more rapidly, and with higher quality and better reproducibility. Then, it is hoped that the high ability of work of computers and machines will lead the innovative progress also in tissue engineering, regenerative medicine and life science field.

Until now, the researches on tissue engineering and regenerative medicine have been developed and successfully achieved to transplant some engineered tissues such as skin, cornea and cartilage. It is hoped that several other tissues and several other organs can be made artificially with tissue engineering and regenerative medicine technologies. However, those successful tissues are only thin and simple tissues and it has not been succeeded yet to produce complicated and thick tissues and organs. Then, many challenging researches have been being made in all over the world.

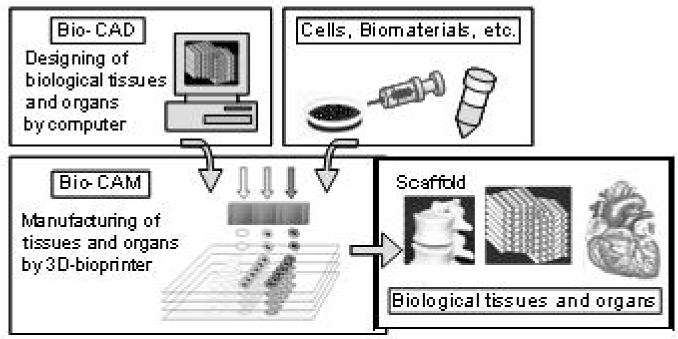

***Figure 1**. Concept of bioprinting*

Bioprinting is one of those challenges, and it is the research aiming to construct biological tissues and finally organs from cells and biomaterials with bottom up procedure using 2D and 3D printing and 3D fabrication technologies. The concept of bioprinting is shown in figure 1. A printer is a machine closely linked to a computer. For this reason, bioprinting also means the concept of the approach that biological tissues are designed in a computer and then constructed by the machines receiving the digital data from the computer. This is just like the approach of computer aided designing (CAD) and computer aided manufacturing (CAM). Therefore, bioprinting means CAD / CAM approach for tissue engineering, too.

Several 2D, 3D printing and rapid prototyping techniques has been applied, such as dip pen, dispenser, inkjet, photo lithography, laser ablation, Laser fabrication, solid free forming, 3D printing, etc. [1-7]. And the researches of bioprinting are aiming to manufacture more sophisticated scaffolds, and biological tissues and organs, and to explore the applications such as for biochips, for bioartificial tissues and organs and for biodevices, too.

Its research field is very wide from basic scientific researches to practical application researches, too. And the related research areas are also wide from basic cell biology, and mechanical and chemical engineering including MEMS and Nanotechnology, to clinical surgery and regenerative medicine.

We have ever investigated and reported the feasibility of inkjet technique for ejecting living cells [8]. It is because inkjet has many advantageous characteristics for an innovative cell seeding or cell positioning method, and because we supposed that living tissues should be construct with living cells directly or simultaneously with construction of scaffold in the case that temporary scaffold is needed. Then, in this study, we challenged 3D construction using inkjet technique and the possibilities of inkjet bioprinting for tissue engineering were discussed.

Materials and Method

Inkjet Gel formation

Through our previous study, we have found that living cells can be safely ejected by inkjet procedure [8]. But some problems were also revealed. We found that inkjet droplets were so small that they dried immediately. For this reason, the cells should be protected from drying during and after printing to lessen the damage. And prevention of ink from blotting and solution against cell migration after printing should also be considered. And we had to construct 3-dimensional structures, too. Then, we tried embedding cells into hydrogel by inkjet. Sodium alginate is well known to react with calcium chloride at once to form alginate hydrogel, which has been used as a biocompatible gel. 0.8% of sodium alginate solution was used as an ink, and ejected into 2.0% calcium chloride solution. We made micro hydrogel beads, micro hydrogel fibers and hydrogel sheet and 3D structures, using an original handmade inkjet bioprinter.

Inkjet Bioprinter

We are developing an original bioprinter. Its photograph is shown in Figure 2. The size and the mechanical system to drive inkjet head was designed and assembled by ourselves. As we have to deal with living cells, bioprinter should be used in a clean environment. Then, we designed the total size on the assumption that it was used in a bio-clean bench. As for the head moving mechanism, inkjet head moves linearly and the paper is sent forward in sequence in usual inkjet printer. But we had to print alginate sodium solution into calcium chloride solution, that is, not onto solid paper but into liquid. For this reason, we designed and made a bioprinter in which printed object was stationary and only inkjet head moved 3 dimensionally. In this study, we used SEAJet inkjet head, which is static electricity actuated inkjet head and was developed by Seiko EPSON [9]. It does not generate any heat, and we confirmed it biocompatible through our previous study using living cells [8].

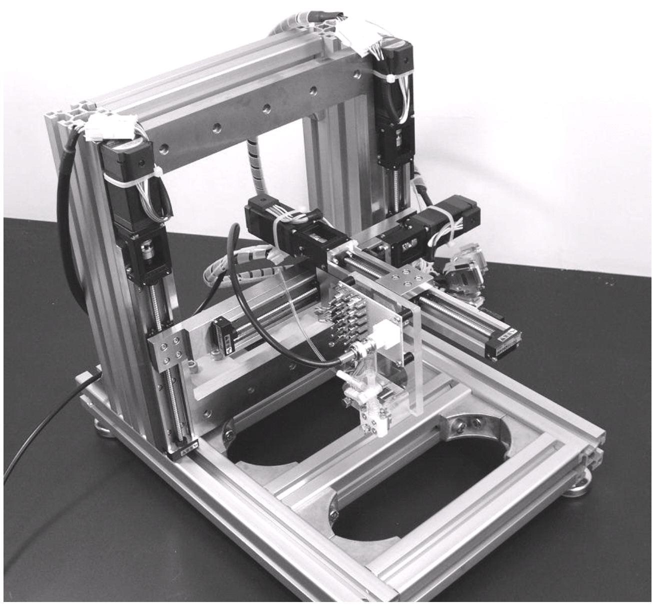

Figure 2. *Experimental bioprinter*

Cell Printing

In the examinations of living cell printing , bovine vascular endothelial cells and HeLa cells were prepared and used. Those cells were suspended in culture medium (MEM) and sodium alginate solution was added and the concentration of sodium alginate was adjusted to 0.8%. Those cell suspended sodium alginate solution was ejected into 2.0% calcium chloride solution.

Results

Micro Gel beads

Alginate sodium solution was ejected into calcium chloride solution and the products were observed with microscope. Particles with almost uniform size could be observed in the calcium chloride solution, that is micro gel beads of alginate hydrogel (Fig. 3, left). Its size were almost uniform and 40μm in diameter. Cell contained micro gel beads could be made, too, when cell suspended alginate sodium solution (Fig. 3, right). Those cells were confirmed alive by time-lapse monitoring.

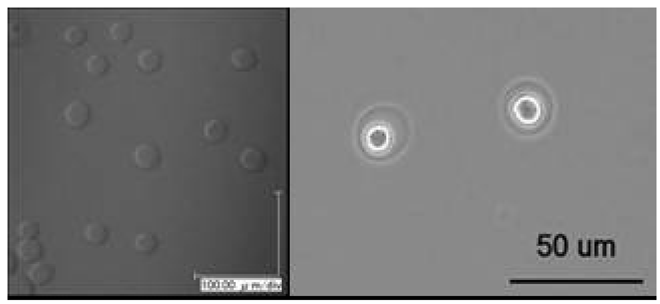

Figure3. *Micro Gel Beads made by inkjet (left), andMicro gel beads containing living HeLa cells(right)*

Micro Gel fiber and Cell Printing

When the lines with alginate sodium solution were printed onto the calcium chloride solution layer on the slide glass, micro gel fibers could be made by optimally controlled ejecting frequency and head moving speed. In the case of using cell suspended alginate solution, cells were embedded in the micro gel

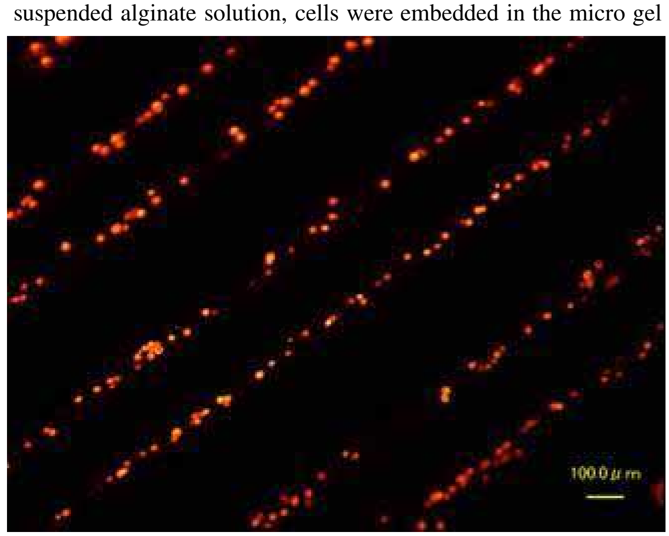

Figure4. *2D Cell printing using micro gel fibers*

fibers (Fig4). The thickness of the micro gel fibers were about 40 to 50μm. Inkjet gel formation was effective in printing cell pattern without blotting even in the calcium chloride solution, or in a wet condition.

2D, and 3D Gel Sheet

Using inkjet bioprinter, 2D gel sheet could be manufactured, too. A square area was painted in a same manner using alginate sodium solution. As a result, 2D gel sheet was produced (Fig. 5). This sheet was composed of densely arranged micro gel fibers made up of micro gel beads. We have also succeeded to layer the gel sheets by printing onto the gel sheet manufactured in advance. It is shown that layer by layer printing was possible by inkjet gel formation.

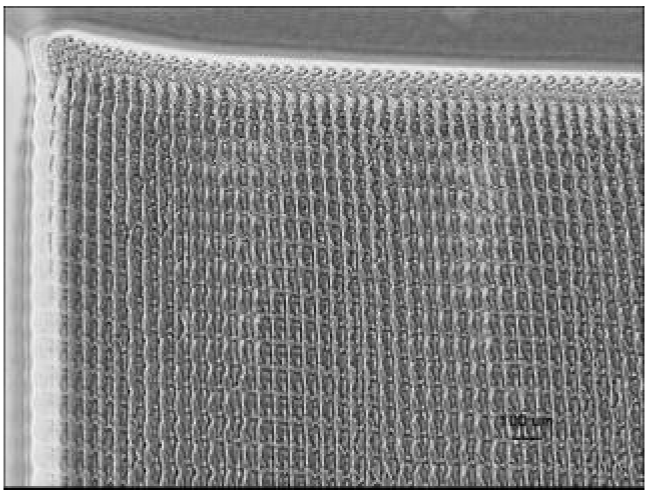

***Figure 5**. 2D Gel Sheet fabricated by inkjet bioprinter*

3D Gel Tube

We challenged to fabricate 3D gel tube, using 3D inkjet bioprinter, too. The fabricated gel tube containing living HeLa cells are shown in Figure 6. The diameter of this tube was 1 mm and the total length was approximately 2 mm. We could fabricate 1mm diameter tube with more than 5mm in length. As HeLa cells were stained with a fluorescent dye, so fluorescent particles represents HeLa cells.

Discussions

Inkjet technique has many advantageous characteristics for tissue engineering, for example, high resolution printing, creating very small droplets with less than a few Pico liters order, color printing, high speed printing, established connection to computers, and printability onto several materials, etc. Exploiting those advantages, several effective procedures for tissue engineering are expected to be innovated, such as arbitrary positioning and arranging living cells, simultaneous building with cells and scaffold, and computer aided 3D fabrication, high throughput printing, etc.[5, 6, 8, 10-12] Inkjet will create many possibilities in tissue engineering.

From our experience of living cell printing, we found that it is not so easy to use inkjet in tissue engineering, although living cells can be safely ejected by inkjet. Then we considered and challenged inkjet gel formation in this study.

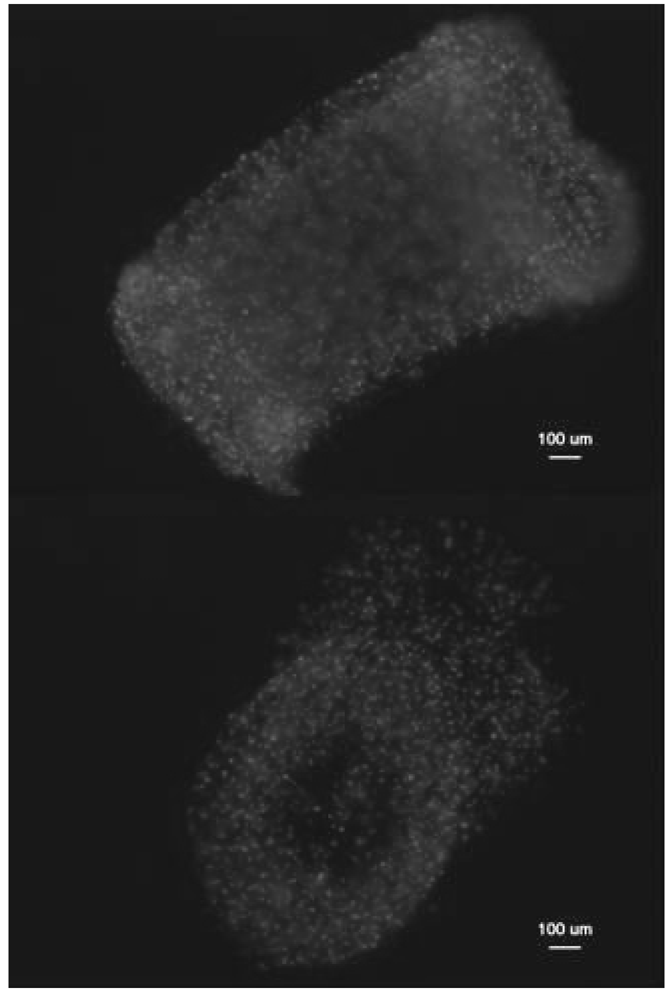

***Figure 6**. 3D Gel tube containing living cells fabricated by inkjet bioprinter*

First we tried alginate gel, because it is most commonly used biocompatible hydrogel in tissue engineering. Alginate gel was found to be useful for inkjet gel forming procedure, because it is immediately formed gel just after mixing with calcium chloride solution. After some preliminary examinations, we decided to use alginate sodium solution at the concentration from 0.8% to 1.0%, considering to the mechanical printing performance of inkjet and the condition of the gel formed by inkjet, as well as biocompatibility for living cells. As alginate sodium solution is somewhat viscous, we experienced the ejecting troubles caused by the obstruction of inkjet nozzles very often when the concentration of alginate sodium solution was more than 1.5%. Such mechanically suitable concentrations were thought to depend on the inkjet system used. If its concentration was lower than 0.5%, the formed gel was too loose to fabricate 3D structures. In addition, we have confirmed cell viability was good when we used alginate sodium solution from 0.8% to 1.0%.

In this study, we found there were several advantages of inkjet gel forming technique. First, we can use biocompatible alginate gel as a gel for inkjet bioprinting. Alginate gel has been used in many medical supplies and apparatuses, and sometimes used as a coating material for cell transplantation and used as one of hemostatic agents. Second, as gel is printed and formed in the calcium chloride solution, the problems of drying and blotting were both completely solved. Therefore, living cell printing was successfully realized. Third, 3D structures can be constructed by gel. As constructed in water, 3D structures can be constructed even with mechanically fragile materials. Forth, as drop on demand 3D fabrication is possible with this method, it will be useful for computer aided digital 3D manufacturing. Fifth, several materials except alginate gel are also expected to be applied, which react to gel by mixture of two solutions. And finally, it is very easy to mix several materials in the gel, such as drugs, growth factors, pigments and dyes, nano-particles including drug delivery systems, etc. by using mixture of them with alginate sodium solution.

Conclusions

We developed our experimental bioprinter according to our experiences of examinations of inkjet gel formation technique. And using this, several constructions, including micro gel beads, micro gel fibers, gel sheet and 3D structures, were fabricated and demonstrated here. Inkjet bioprinting has many advantages and much possibility as a tool for 3D tissue engineering.

Recently, Inkjet printer, 3D printing and several rapid prototyping machines have been applied in tissue engineering. Those machines are indeed established and have high abilities to perform 2D printing and 3D structures. But they are not bioprinters or bio-fabricating machines which are the most suitable machines for dealing with living cells and biomaterials. The true sophisticated bioprinters are needed to be developed for the sake of innovation in tissue engineering and regenerative medicine, as well as in general life science.

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Makoto Nakamura graduated at the School of Medicine, Kobe University (1986). He worked as a clinical pediatrician for 10 years. After received M.D.,Ph.D. (1996), he concentrated his activity on R&D of artificial organs, and worked in National Cardiovascular Center (1996-1999), Tokyo Medical and Dental University as an associate professor (1999-) . Now, his main work has focused on tissue engineering. He started "Bioprinting Project"(2005-) , supported by Kanagawa Academy of Science & Technology .

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