Alginate Gel Honeycomb Structures Fabricated with the Bio-printer

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Abstract

We are developing the bio-printer, which can fabricate three-dimensional (3-D) structures containing living cells. The cultured cells with sodium alginate solution are ejected through the inkjet printer nozzle into the mixture of calcium chloride solution and polyvinyl alcohol. The ejected droplets could gel into a rigid, 3-D structure with living cells. The position of the inkjet nozzle can be controlled three-dimensionally with linear stages and stepping motors, and the resolution of the positioning is 2 um in all 3 dimensions. In this study we have tried to fabricate the honeycomb structure using our developing bio-printer, considering constructing hepatic lobules to regenerate the tissue of a liver. As preliminary specimens, the sodium alginate solution without living cells was ejected into the mixture of calcium chloride solution and polyvinyl alcohol, and the small honeycomb structures of 3, 6, 9 and 12 hexagonal compartments were attempted. The length of a side of the hexagon in the honeycomb was set to 1 mm on the control system of the bio-printer. As a result, 5—10 mm high honeycomb structures of 3, 6 and 9 hexagonal compartments were successfully fabricated with the bio-printer. We are, at present, trying to utilize these honeycomb structures as a bioreactor containing several different kinds of cells, and it would lead to the regeneration of hepatic lobules.

Introduction

An organ transplant or an artificial organ is a possible treatment option to replace or repair the recipient's damaged organ. The organ transplant is always facing the donor shortage problem, and it also comes with the side effect of infection due to immune system suppressor drugs. As for the artificial organs, some artificial replacements have been developed for kidney, bone, heart, and so forth, but they still require the improvement of their endurance and reliability. While the problem of endurance and reliability is resolved in the future, artificial organs cannot grow with the patient's age. Regenerative medicine/tissue engineering is rapidly developing involving multidisciplinary fields of medicine, biology and engineering to replace the lost organ functions [1]. So far, regenerative medicine has achieved some practical results for thin or simple organs with less blood perfusion, such as skin and cartilage. At present, researchers are trying to regenerate the critical organs. This kind of critical organ has its 3-dimensional (3D) microstructure, is composed of various different types of cells, and has a rich capillary network to secure blood perfusion for its function.

In order to regenerate the critical organs, we have to develop the following technologies:

* 3D precise disposition of cells in the order of several micrometers,
* disposition of several kinds of cells, and
* composing vessel and capillary structures in the 3D regenerated tissues.

Our group is trying to apply the inkjet technology into regenerative medicine to realize these 3 key technologies. Commercial inkjet printers have nozzles driven by the piezoelectric material, and the printhead can eject the small droplet down to 1 pL. Color printhead could achieve the ejection of several kinds of cells. Piling thin 3D living layers would realize the arbitrary 3D tissues containing the vessel-like structures by analogy with the rapid prototyping techniques.

Figure 1 shows the bio-printer we are developing. The printhead has the piezoelectric inkjet chip. This inkjet chip has 4 nozzles, so it can eject a total of 4 kinds of cells or materials. The position of the printhead can be adjusted three-dimensionally with linear stages and stepping motors, and the resolution of the positioning is 2 um.

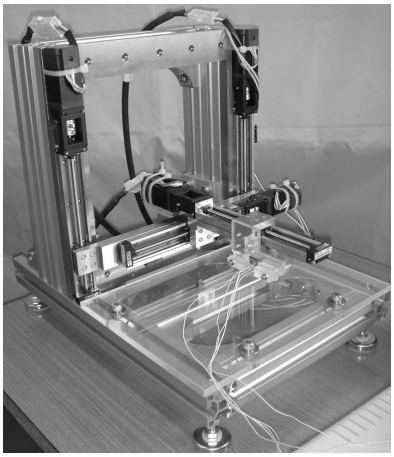

***Figure 1**. Bio-printer*

The droplet including sodium alginate solution and living cells are ejected from the inkjet nozzle into the pool of polyvinyl alcohol (PVA) solution with calcium ions. The alginate gel beads comprise the 3D structures with the help of viscosity of PVA solution, and the living cells are fixed in each gel bead of the 3D structure [2]. Using this bio-printer, we have already succeeded in forming the simple 3D structures, such as a thin sheet, stacked sheets and multilayer tube, with alginate gels containing living cells [2,3].

In this study, the construction of some complicated 3D structures was attempted with the bio-printer. The sodium alginate solution without living cells was ejected into the mixture of calcium chloride solution and PVA from the inkjet printhead, and the small honeycomb structures of 3, 6, 9 and 12 hexagonal compartments were tried to demonstrate the potential of our bio-printer.

Materials and Methods

Small droplets were ejected through the inkjet printhead supplied by Fuji Electric System Co., Ltd. Japan. The droplets usually contain both living cells and sodium alginate solution in our system, but in this study the sodium alginate solution without living cells was ejected to confirm whether the bio-printer could build up the honeycomb structure. The droplets of sodium alginate solution were ejected into calcium chloride solution. The droplets gelated to beads, adhered to each other, and drew 2D (or monolayer) structure in calcium chloride solution by changing the position of the printhead appropriately. By repeating the construction of 2D structures, the layers also adhered to each other to make up a 3D structure. To pile up this monolayer, the 2D structure must slowly go down in calcium chloride solution, and PVA was mixed in calcium chloride solution to adjust the viscosity of the solution and the resultant sink speed of the structure made of alginate gel beads. The details are described in Reference [2].

In this study the small honeycomb structures of 3, 6, 9 and 12 hexagonal compartments were attempted. In order to build up the honeycomb structure of 3 hexagonal compartments, the motion of the printhead was controlled to sketch the drawing shown in Figure 2. The printhead started to move at "Start/End" point according to the direction shown by the arrows and their numbers,

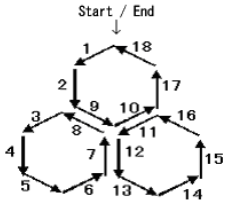

Figure 2. *Motion of the printhead to construct honeycomb with 3 compartments*

and returned to the start point after the 18th movement. The length of a side of the hexagon in the honeycomb was set to 1 mm on the control system of the bio-printer. At the doubled path, such as #8 and #9, the sweep speed of the printhead was also doubled to obtain the same density of the ejected droplets along a side of the hexagonal compartment. Repeating the printhead movement shown in Figure 2 would lead to the 3D honeycomb structures of 3 hexagonal compartments.

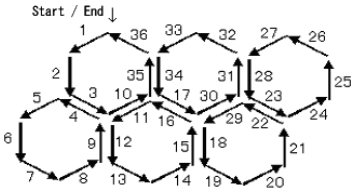

Figure 3. *Motion of the printhead to construct honeycomb with 6 compartments*

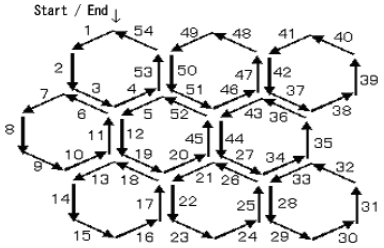

Figure 4. *Motion of the printhead to construct honeycomb with 9 compartments*

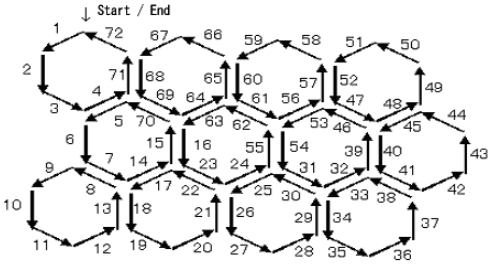

Figure 5. *Motion of the printhead to construct honeycomb with 12 compartments*

Fabricating the honeycomb structures of 6, 9 and 12 hexagonal compartments was also attempted, and the movements of the printhead were shown in Figures 3—5. One layer of the honeycomb structures of 6, 9 and 12 compartments was drawn with 36, 54 and 72 steps, respectively. Then, this monolayer was stacked with the help of viscosity due to PVA in calcium chloride solution, and the layers comprised the resultant 5—10 mm high honeycomb structures.

Results

Figure 6 shows the 3D honeycomb structure with 3 hexagonal compartments made of alginate gel beads. The honeycomb structure with 3 compartments was successfully fabricated, and it wound and taperd a little bit. From the longitudinal picture, the adhesion between layers was loose. As compared to the simple

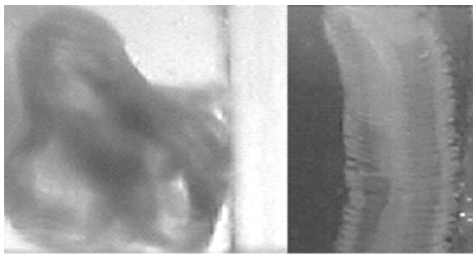

Figure 6. *3D honeycomb structure with 3 hexagonal compartments (Left: cross section showing 3 hexagons, Right: longitudinal picture)*

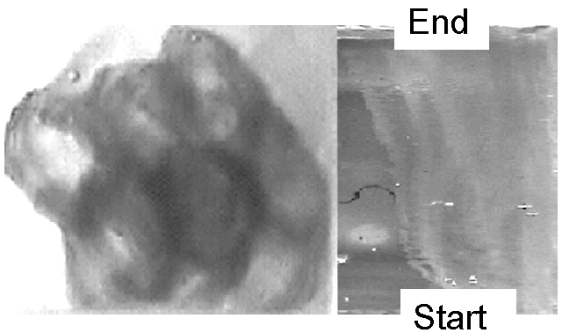

Figure 7. *3D honeycomb structure with 6 hexagonal compartments (Left: cross section showing 6 hexagons, Right: longitudinal picture)*

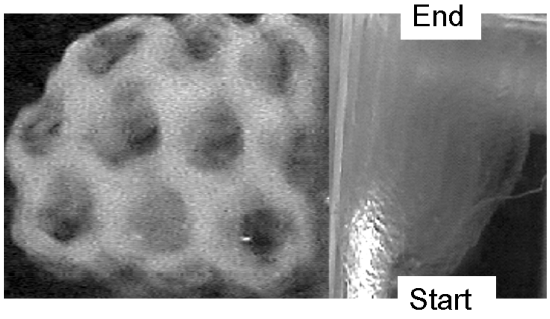

Figure 8. *3D honeycomb structure with 9 hexagonal compartments (Left: cross section showing 9 hexagons, Right: longitudinal picture)*

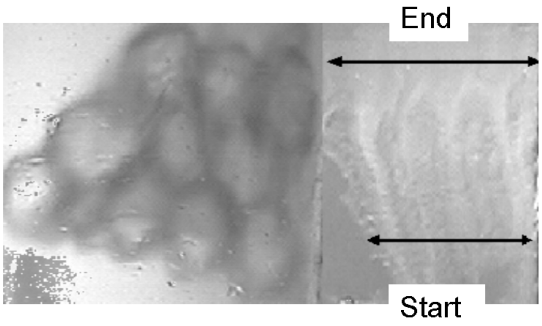

Figure 9. *3D honeycomb structure with 12 hexagonal compartments (Left: cross section showing 12 hexagons, Right: longitudinal picture)*

tube fabricated with the bio-printer which is described in Reference [2], it took longer time to draw a single layer of the honeycomb structure. Before the droplet of sodium alginate solution was ejected to draw the upper layer, the alginage gel beads on the under layer completely gelated and then the adhesion between the under and the upper layers was so loose. For the firm adhesion between layers, the speed of gelation must be controlled by adjusting the concentration of calcium chloride solution.

The honeycomb structures with 6, 9 and 12 hexagonal are shown in Figures 7, 8 and 9, respectively. The more compartments the honeycomb structure has, the more severe taper it has. In Figure 9, only 11 compartments could be established. The honeycomb structure with 12 compartments were tried several times, but all 3D structures were missing one or two compartments. This was probably due to the longer time to complete one layer. In addition to controlling the speed of gelation by adjusting the concentration of calcium chloride solution, the higher scanning speed of the printhead would be also effective.

Conclusion

Using our developing bio-printer, the sodium alginate solution without living cells was ejected into the mixture of calcium chloride solution and PVA, and the small honeycomb structures of 3, 6, 9 and 12 hexagonal compartments were attempted. The length of a side of the hexagon in the honeycomb was set to 1 mm on the control system of the bio-printer. As a result, 5—10 mm high honeycomb structures of 3, 6 and 9 hexagonal compartments were successfully fabricated with the bio-printer. We are, at present, trying to utilize these honeycomb structures as a bioreactor containing several different kinds of cells.

Acknowledgement

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References

[1] Regenerative medicine NIH definition, http://ptei.org/index.php

[2] Yuichi Nishiyama, Makoto Nakamura, Chizuka Henmi, Kumiko Yamaguchi, Shuichi Mochizuki, Hidemoto Nakagawa, Koki Takiura, "Development of a three-dimensional bio-printer: Construction of cell support structures using hydrogel and state-of-the-art inkjet technology, " ASME Journal of Biomechanical Engineering, *in press*.

[3] Paul Calvent, "Printing Cells," Science, 318, pp.208-209. (2007)

Author Biography

Koki Takiura received B.S. and M.S. degrees in Aeronautics and Astronautics from the University of Tokyo, Japan, in 1991 and 1993, respectively. In 1996 and 1997, he was a research fellow at National Cardiovascular Center Research Institute, Japan. In 2003, he received Ph.D. from the University of Tokyo, Japan. From 2004 to 2006 he was an associate professor in Tohoku University Biomedical Engineering Research Organization, Japan, and since 2006 he has been an associate professor in Yamagata University, Japan. His research interests include artificial hearts, medical device development and biomedical measurements by optical observation.