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Concurrent connection of embryonic chick heart using a microfluidic device for Organ-Explant-Chip

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Abstract

We propose a concurrent microvascular connection method called suction-induced vascular fixation (SVF) method for the achievement of Organ-Explant-Chip which is a biologically-designed simulator having biological materials such as cells, tissues, and organs. The advantages of proposed method with using a microfluidic device are as follows: (1) operation of flexible objects (blood vessels), (2) alignment the blood vessels concurrently, and (3) reduction of the DOFs of the blood vessels. From the experimental results, we confirmed that four cardiovascular of the explanted embryonic chick heart can be induced into the fabricated microfluidic device concurrently. We have also succeeded in construction of hybrid circulatory system between artifacts and embryonic chick heart, and monitoring the response of the heart of chick embryo by supplying the culture medium.

Keywords: Bionic Simulator, Microvascular Connection, Microfluidic Device, Chick Embryo

1. Introduction

In regenerative medicine and drug discovery research, in vitro biological models (bionic simulator) are wildly desired for understanding the biological function as well as getting more efficient research [1]. Bionic simulator means a biologically-designed simulator which has biomaterials such as cells, tissues, and organs. Until now, biological analysis of living cells or tissues in the context of whole living organs remains dependent on animal studies, which have time-consuming and ethical problems. Therefore, new experimental techniques without using conventional large animals have been required [2].

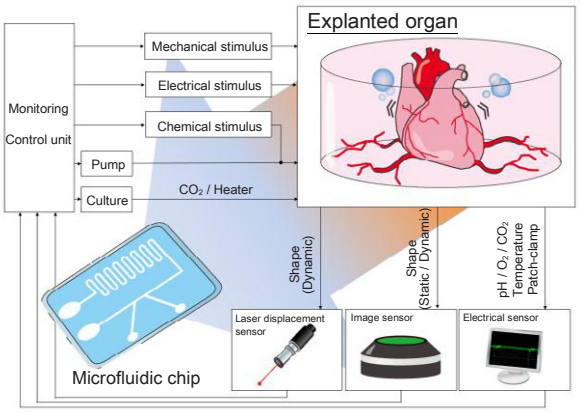

Fig. 1 The concept of Organ-Explant-Chip

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The main alternative approaches for studying the bionic simulator are called “Organ-on-a-chip [3-6].” This approach reconstitutes cells as the structural tissue arrangements and functional complexity of living organs that are cultured in a microfluidic chip [3]. D. Huh et al. creates co-culture system of epithelial cells and endothelial cells by using a microfluidic chip which has stretch microchannels in order to simulate the human lung function [4]. By contrast, langendorff perfusion system has been used for a long time as a simulator with using the organs extracted from the organisms [7-9]. In this method, an extracted heart from a rat or a rabbit is used as a model for a diverse range of studies of the heart, including the evaluation of the therapeutic interventions, clinical hypertension, and cardiac physiology. These two approaches are quite notable research, however, it is difficult to construct the bionic simulator that can observe and evaluate the function of the three-dimensional biological organs in vitro environment for a long time.

Based on the background, we newly propose the bionic simulator system called “Organ-Explant-Chip” using isolated organ tissue from the ethically-acceptable small organism. An explanted organ is maintained by perfusion culturing in a microfluidic chip, while monitoring and evaluating the function of explanted organ in real-time. The advantages of proposed approach are that we can use living 3D organs for bionic simulator and that we can observe the function of organs in vitro. We believe Organ-Explant-Chip will be a new simulator system that contributes to on-chip drug simulator, in vitro disease model, and evaluation of the interaction between the other organs.

To achieve the Organ-Explant-Chip, we use a chick embryo as a simulator model [2], and the following three functions are mainly needed for construction of the Organ-Explant-Chip;

1) Connection of the organ-artifact and/or organ-organ unit for incorporating into a simulator system as well as supplying the nutrition, oxygen, and drugs.
2) Control of the state of organ and environment for the long-term culturing and creating the arbitrary environment.
3) Evaluation the response of the explanted organ for understanding the biological function.

First of all, we are focusing on the connection technique because we need to reconstruct the extracted tissues and organs as parts of the bionic simulator by connecting with the external environment, other tissues, and organs. However, because of the size of the organ tissue extracted from the developing chick embryo, it is difficult to perform the vascular connection. In general, microvascular anastomosis (< 1 mm in diameter) with using a suture is difficult, and there are the time-consuming and the skill-dependent problems in the surgery region [10-13]. Therefore, it is necessary to simplify the task of vascular connection technique.

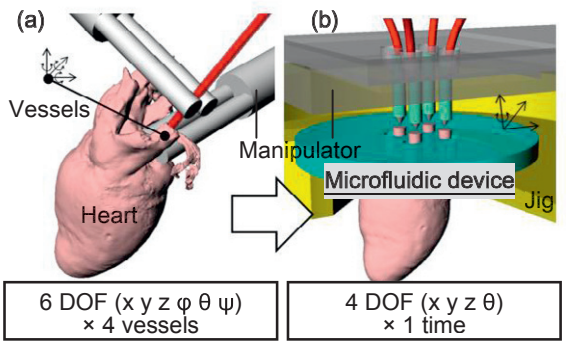

Fig. 2 The concept of the microvascular connection of the extracted organs with using a microfluidic device

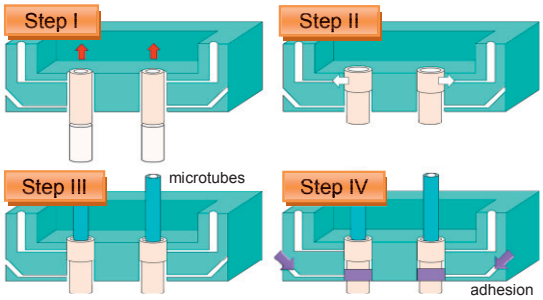

Fig. 3 Procedure of the suction-induced vascular fixation (SVF) method with using a microfluidic device

In this study, we propose the simplified method for vascular connection by fixing and positioning the flexible vessels with using a microfluidic device, and discuss how to connect to the artifact and the microvascular led to a living organ.

2. Concurrent connection of embryonic chick heart using a microfluidic device

We have proposed suction-induced vascular fixation (SVF) method [14] for concurrent microvascular connection of the chicken embryonic heart. The concept of the SVF method is shown in Fig. 2. Blood vessels of the chicken embryonic heart are small in diameter, flexible with multi-DOFs, and concentrated in one place, which make it difficult to connect the blood vessels. In SVF method, we use a microfluidic device with suction mechanism for fixing the position and orientation of the vessels. By sucking blood vessels through the device, it is possible to perform positioning and fixing the vessels easily and concurrently for the vascular connecting operation. We can also decrease the DOFs of the vessels compared to those without fixation (24 → 4 DOF). By using this method, we can shorten the working hours of vascular connecting procedure as well as ease the operation.

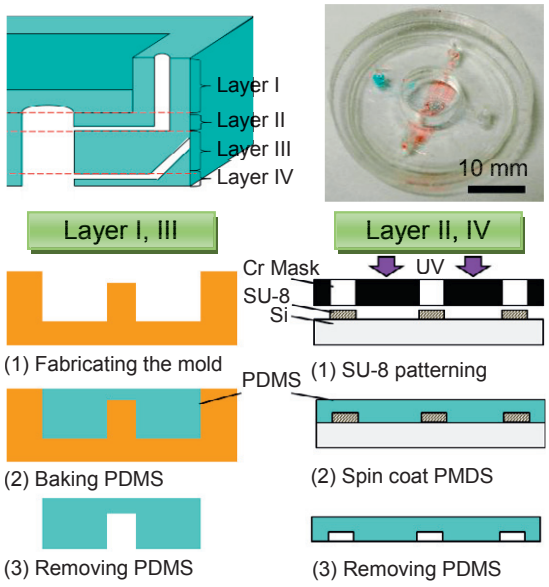

Fig. 4 Process flow of the microfluidic device for SVF method

The procedure of connecting blood vessels with SVF method is summarized as follows, as shown in Fig. 3;

Step I: sucking from the vertical direction for positioning the blood vessels,

Step II: sucking from the horizontal direction for fixing and expanding in diameter of the blood vessels,

Step III: inserting the artificial tubes into the blood vessels with using micromanipulators,

Step IV: pouring the adhesive for securing the connection.

Figure 4 shows the fabrication process of the microfluidic device with suction mechanism. This device is fabricated by stacking PDMS (Polydimethylsiloxane) layers which are made by photolithography (μm order in 2D resolution) and molding (mm order in thickness) according to the required processing accuracy. This device has two types of sucking mechanism for induction and fixation of the vessels.

To operate the blood vessels efficiently by suction, it is important where to place the holes patterned on the microfluidic device. In this study, we set the simple geometric models of the chicken embryonic heart with four blood vessels and the microfluidic device, as shown in Fig. 6(a). The parameter of the microfluidic device is determined by the basic experiment written in the following chapter. D_1, d_1 is the diameter of the blood vessels led to the heart of chick embryo and the holes patterned on the microfluidic device, D_2, d_2 is the distance of between the vessels and between the holes, and t is the thickness of the microfluidic device.

Figure 5 shows the experimental set up. To support the vascular connection, we have developed the master-slave micromanipulator. Falcon 3D haptic interface (Novint) is used for the master, and automatic xyz stages are used for the slave of the micromanipulator.

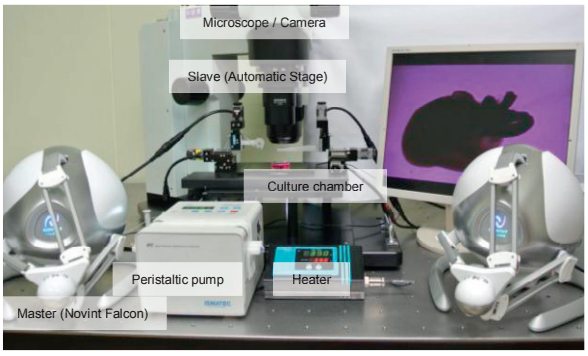

Fig. 5 Experimental set up for vascular connection

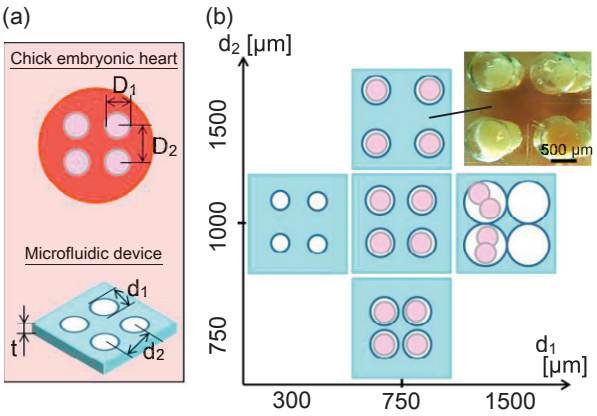

Fig. 6 Evaluation of the induction efficiency of the blood vessels by using SVF method

3. Experimental results

In order to determine the parameter of the microfluidic device, we performed the suction of the blood vessels through the microfluidic device with variety of shapes in Step I. In this experiment, we incubated fertilized chicken eggs for 11 days (Hamburger and Hamilton stage [HH] 37) [15], and then isolated the heart from developing chick embryo. There being individual variability of the chick embryo, D_1, D_2 is about 750 μm, 1000 μm, respectively.

Figure 6(b) shows the relationship between the induction efficiency of blood vessels and the parameter of the microfluidic device. Considering the length of the blood vessels extracted from the developing chick embryo, we set the thickness of the microfluidic device (t) is 1.5 mm constantly. If the size of the holes (d_1) are large, several blood vessels are induced into the same hole, and blood vessels are not induced into a hole if small. In addition, the distance between the holes (d_2) does not significantly affect the induction efficiency of the blood vessels. From the experimental result, we determine the parameter of

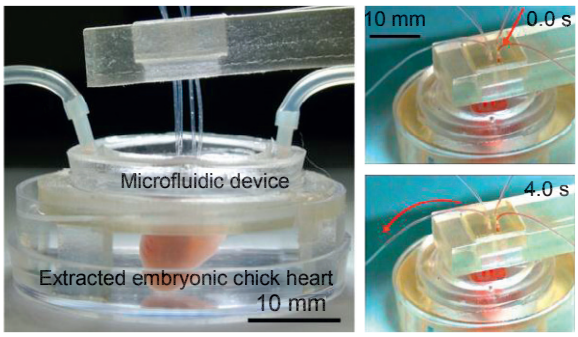

Fig. 7 Vascular connection experiment between blood vessels and artificial tubes

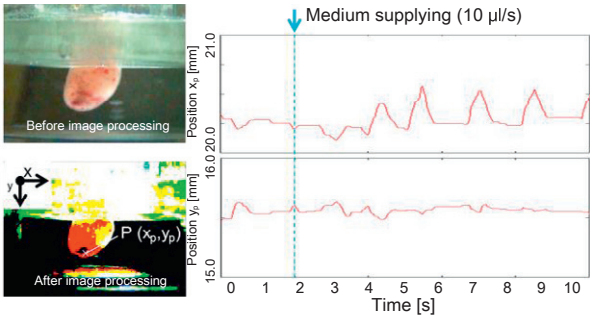

Fig. 8 Time lapse analysis of the heart beating by image processing

the microfluidic device (d_1=750 μm, d_2=1500 μm). We confirmed that four blood vessels can be induced into the each hole patterned on the microfluidic device. Moreover, by using the microfluidic device, the problem of the interference between the blood vessels can be solved, which assists the simple task for the connection of the multiple vessels.

Next, by sucking from the six channels arranged in the circumferential direction of the vessels during the Step II, we confirmed that vessels can be secured to the sidewall of the microfluidic device while expanding the diameter of the vessels about 10 %. This result suggests that we can insert the artificial tubes to the vessels easily in the following steps.

Figure 7 shows the result of connecting artificial tubes and the vessels connected to a heart of the chick embryo. We have succeeded in construction of hybrid circulatory system between artificial tubes and the living heart.

The advantage of the connection with organ and external system is that we can supply the nutrition or administer the drugs into the organ through the blood vessel. Figure 8 shows the result of time lapse analysis of the heart beating by image processing. We succeeded in observing the heart beat continuously while supplying the culture medium. From this result, Organ-Explant-Chip can control the environment and monitor the reaction of the extracted organ, which makes it useful in bionic simulator.

4. Discussions

To realize the Organ-Explant-Chip using an isolated organ, vascular connection is important for supplying nutrition, oxygen, and drugs as well as incorporating into a simulator system. However, because of small size of the tissue especially extracted from an ethically-acceptable small animal like a developing chick embryo, it is difficult to handle the tissue. In this study, we propose the concurrent microvascular connection technique without using a conventional suture method. With using a microfluidic device, we can operate the blood vessels concurrently by negative pressure.

We believe that this connection technique can also be applied not only to organ-artifact but also to organ-organ connection. Furthermore, combining the 3D cell culture such as cellular built up approaches [16], it is possible to contribute significantly to the improvement of the flexibility of the Organ-Explant-Chip for regenerative medicine and drug discovery.

5. Conclusions

In this study, in order to achieve Organ-Explant-Chip using an explanted biological organ, we newly propose the concurrent connection of embryonic chick heart using a microfluidic device. From the experimental result, we confirmed that the vessels of the chicken embryonic heart can be concurrently connected to the artificial tubes and the solution was circulated through the heart from the artificial tube connected to the vessel while monitoring its macroscopic behavior.

In the future, mechanical properties of the connection portion, instantaneous response, and long-term maintenance of the explanted organs will be evaluated. Organ-Explant-Chip will support development biology and drug screening in various stimulated environments to investigate organ tissues.

Acknowledgements

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