Microfluidic Control for Biological Cell Orientation

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Abstract: There is a great demand to manipulate biological cell autonomously since biologist should spend much time to obtain skillful manipulation techniques. For this purpose, we propose a cell chip to control, carry, fix and locate the cell. In this paper, we focus on the cell rotator to rotate individual biological cell based on a micro fluidics technology. The cell rotator consists of injection hole and rotation well to rotate a biological cell properly. Under the variation of flow rate in injection hole, the angular velocity of a biological cell is evaluated to find the feasibility of the proposed rotation method. As a practical experiment, Zebrafish egg is employed. Based on this research, we find the possibility of non-contact rotation way that can highly reduce the damage of the biological cell during manipulation. To realize an autonomous biological cell manipulation, a cell chip with manipulation well and micro channel in this research will be utilized effectively in near future.

Keywords: Biological cell, Cell manipulation, Cell rotator, Microfluidic, Orientation, Zebrafish egg.

1. INTRODUCTION

Recently, the characterization and micromanipulation of individual embryo cells become a challenging issue in biomedical applications such as cloning, gene expression analysis, and cell replacement therapy (CRT). Especially, cell manipulation has become a significant work in the agricultural industry and allows approaches based on diagnosis or pharmaceutical test [1]. Nevertheless, most bio manipulation tasks such as gene injection, in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) are usually carried out by the experienced operators relying on the visual information from the microscope. In case of manipulation for the biological cell, one of biomedical objects, many operators should spend over a year to perform reliable cell handling. In addition, the size of biological cell is not same and deformable easily. Moreover, they can be seriously damaged during the manipulation and treatment [4]. Also, the liquid flow due to the micropipette motion in petri dish acts as a disturbance to the cell behavior. Therefore, the manual handling of cells is not reliable even when the manipulation is carried out by skillful operators [5, 6].

Previous efforts for realizing an autonomous cell manipulation system are as follows. Tan and Ng [1] tried a computer-controlled cell injection using a piezoelectric actuator. Yanagida *et al.* [2] applied the piezo-driven micromanipulator to ICSI system for the manipulation of human ovum. Nakayama *et al.* [3] also used the piezo-micromanipulator to improve the IVF performance. Sun and Nelson [5] suggested the visual-served cell manipulation system that the position of a generative cell and pipette end-point are identified using the visual information from the optical microscope. Some of the above researches are based only on the piezo-electric manipulator in order to reduce the impact of cell during the injection. However, the orientation and position control of generative cells has been overlooked by many researchers [7].

Figure 1 shows the overall biomanipulation system we have developed, where the microfluidic cell rotator to be discussed in this paper has been integrated in the cell chip. In Fig. 1, cells are monitored using the microscope and the operator can sense the cell penetration force when the micropipette injects the cell through the haptic interface [8,9].

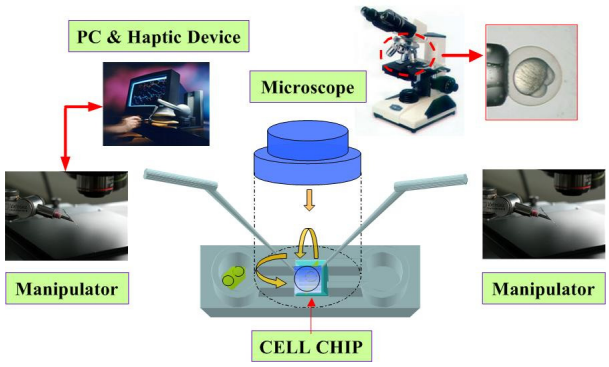

Fig.1.Biomanipulation system.

In this paper, we propose a microfluidic system to control the orientation of cells properly. Our system consists of four parts such as rotator well, microchannels, injection hole, and holding hole. Also, we used the syringe pump to control the flow rate. This paper is organized as follows. In Section 2, we propose a microfluidic cell rotator which actually has the function of cell orientation control in the cell chip and the fabrication process is discussed in Section 3. Section 4 explains the experimental results and Section 5 concludes this paper.

2. CELL ROTATOR

Generally, most bio cell manipulation tasks are carried out by experienced operators relying on visual information from the microscope only. But, there are many problems. For example, if the cell escapes in the field of view of microscope, the operator has to find the cell by manual operation of the moving stage.

On the other hand, previous cell manipulation methods are classified into two groups. The first method is a direct contact method and the second one is a non-contact method. The cell manipulation using the glass pipette is a representative contact

type cell manipulation method. This method has the merit of handling cells easily, however, may give rise to cell damages. Cell trapping or cell particle aggregation using laser tweezer and ultrasound belongs to the non-contact type cell manipulation method, which cause less damages to the cell than contact methods. But the non-contact method requires much manipulating skills.

In order to increase the success rate of development in cell injections, it is very important to inject the cell with good orientations. Taking the example of Zebrafish egg, if we inject gene into the yolk directly as shown in Figure 2, we can expect much higher success rate of development.

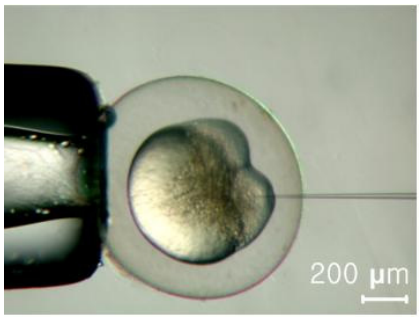

Fig. 2 Best orientation for higher success rate

Figure 3 show the cell chip we are developing to control, isolate, carry, fix, and locate the cell. The cell chip is comprised of microactuated PolyPyrrole (Ppy) valve, microchannels, and a cell rotator using microfliudics. The cell population is inserted into the inlet and the cells are transported into the cell rotator through the microchannel and the Ppy valve controls the individual cell sequentially. Then, a single cell is held at a suction hole in the cell rotator using the microfluidic suction flows.

The cell rotator denoted in Figure 4 is an apparatus for injecting specific parts of the cell after holding it. In this paper, we focus a cell rotator using microfluidics, which can control the cell orientation. It is composed of a well, a holding hole, an injection hole, and microchannels. The speed of fluid in the cell rotator should be slow not to give a cell damages. We fabricated the device in Figure 4 in the next section by micro machining process. By controlling the speed of injection fluid of micro syringe, we can rotate individual cell.

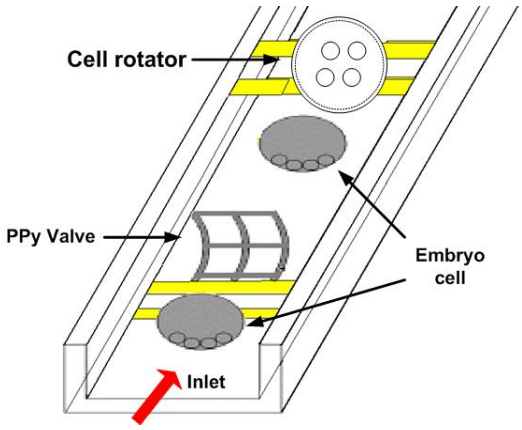

Fig. 3 Conceptual diagram of the cell chip

3. FABICATION PROCESS

As a target cell for microfluidic cell rotate on, we selected the Zebrafish egg cell. The diameter of the Zebrafish egg is about 800~1200 μm depending on the embryological stage. As an average value, we assume that the diameter is 1000 μm and the mass is 1.286 mg.

Considering the physical data of the Zebrafish egg, we designed and fabricated five cell rotators with different well sizes between 1200 and 2000 μm. We precisely fabricated two holes for cell holding and flow injection respectively in the lower part of the wells.

While, the injection hole is off-centered to create a rotation flow over the cell and the two holes are connected with the microchannels perpendicularly. Also, the microchannels are connected with micro syringe pump.

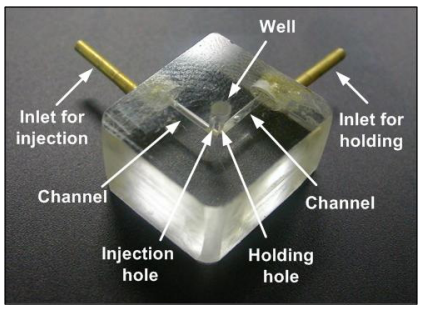

Fig. 4 Photos of the fabricated cell rotator

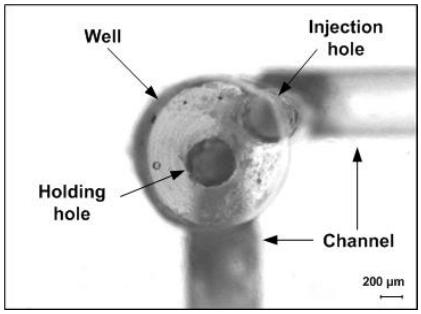

Fig. 5 Top view of the fabricated cell rotator

Figures 4 shows the fabricated cell rotator whose material is Acryl. As shown in the top view of the cell rotator of Figure 5, the two holes in the device are connected with the micro channels.

4. EXPERIMENTS

Figure 6 shows the experimental setup of the system to manipulate the Zebrafish egg where the device is located on the microscope table actuated by the precision linear stages. The orientation of the Zebrafish egg was controlled by changing the flow rate in the micro channel using the micro syringe pump and a cell tram oil device. The micro syringe pump (VIT-FIT model of LAMBDA Inc.) has the function of infusion and withdrawal. Flow rate can be controlled from 0.1 to 100 mm/min. The cell tram oil device (eppendorf AG) is manually operated with infusion and withdrawal function. The visual information on the Zebrafish egg's orientation was obtained from the microscope vision system.

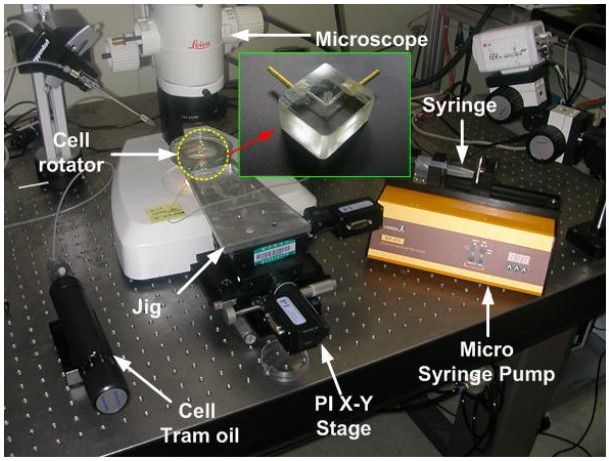

Fig. 6 Experiment system setup

The procedure of cell rotation experiment is given as follows.

First, suck the Zebrafish egg through the holding hole in the center of the well by using the cell tram oil device.

Second, provide microfluidic flow to the off-axed injection hole located at the lower part of the well

Third, by controlling the micro syringe pump, change the flow rate from 1 to 30 mm^3 /s, observe the orientation change of the cell through the vision system.

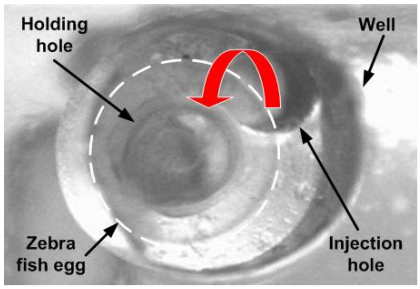

Fig. 7 Zebrafish egg in the cell rotator

Figure 7 shows that the Zebrafish egg is rotating in the cell rotator and Figure 8 corresponds to the one cycle of rotation. Relying on this figure, we could compute the RPM of cell rotation.

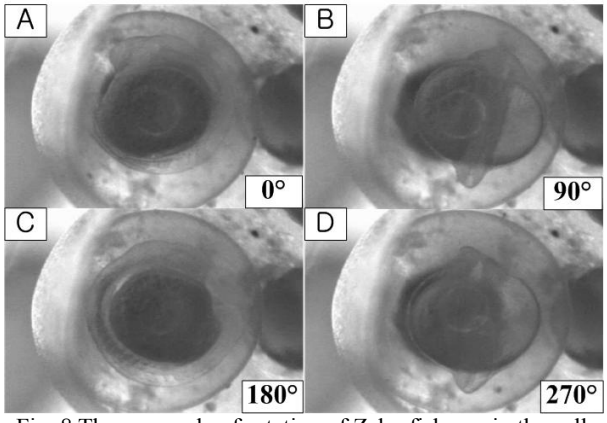

Fig. 8 The one cycle of rotation of Zebrafish egg in the cell rotator frame by frame

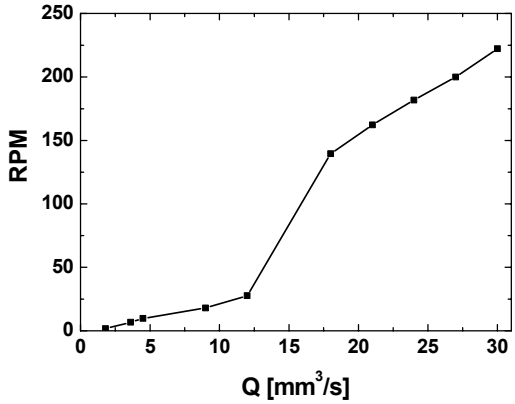

Fig. 9 The relationship between flow and RPM

After many trials and data conversion, we finally obtained the experimental results in Figs. 9 and 10. Figure 9 shows the relationship between flow rate and RPM of cell rotation, where the RPM data was determined by searching the image files saved during cell rotations. In the cell rotation experiment, we changed the flow speed by micro syringe pump from 6 to 100 mm/min which is correspondent to from 1.74 to 28.97 mm^3 /s in Fig. 9.

In the low speed zone in Fig 9 where the flow rate is from 1.74 to 4.35 mm^3 /s, the speed of the orientation change was linearly increased as the flow rate increases and we could easily control the position and orientation of the cell. However, in the high speed zone where the flow rate is more than 8.7 mm^3 /s, it was very hard to control the cell orientation. In fact, the most effective range of flow rate for the cell orientation was from 1.74 to 3 mm^3 /s.

Conclusively, we could observe that the flow rate of 1~3 mm^3 /s is feasible range for controlling the orientation of Zebrafish egg where the cell rotation speed was proportionally increased with respect to the flow rate (Q).

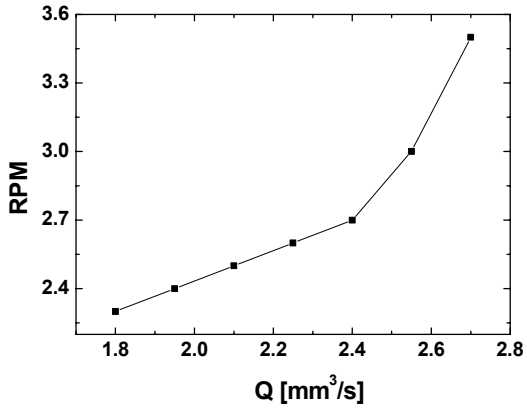

Fig. 10 The relationship between flow and RPM

5. CONCLUSIONS

A microfluidic cell rotator with cell holding and rotating capabilities was fabricated and experimentally tested. As a case study of cell rotation, we manipulated Zebrafish eggs. In consideration of the Zebrafish egg size, the diameter of the injection hole and that of the rotation well were determined as 400 μm and 1800 μm, respectively. We obtained the experimental result that the injection flow rate of 1~3 mm^3 /s is a feasible range for controlling the orientation of Zebrafish egg. Currently, we are developing a cell rotation device for mouse embryo cells with about 70 um diameters based on the laser machining technology. Our final goal is to construct an autonomous biomanipulation factory with the cell rotation device, which can manipulate bio cells, inject biomaterials such as DNA, protein and etc., and moreover characterize the cell properties for cell therapy.

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