FABRICATION AND SELF-ASSEMBLY OF MOVABLE MICROSTRUCTURES EMBEDDING CELLS INSIDE MICROFLUIDIC DEVICES

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ABSTRACT

Currently the research about large quantities cells assembly is seriously regarded, since it can provide high efficiency methods for artificial tissue engineering. In this paper, we report a cell assembly method based on cell immobilization by photo-crosslinkable resin and microfluidic self-assembly inside microfluidic devices. The on-chip fabrication of movable microstructures embedding yeast cells (*W303*) based on Poly (ethylene glycol) Diacrylate (PEGDA) was reported. A 2-layer microfluidic device was fabricated by Polydimethylsiloxane (PDMS) and self-assembly method of the fabricated movable microstructures via this device was preliminarily demonstrated.

KEYWORDS

On-chip fabrication, Microfluidic device, Self-assembly, Tissue engineering.

INTRODUCTION

The cells inside real tissues and organs are usually arranged with large amount according to certain patterns and shapes, such as neural cells, skin and blood vessel. For artificial tissue engineering, an important issue is high-throughput cell assembly. [1] The convenient methods for cell assembly are based on such as optical tweezes, surface adhesion and fluidic channel structures. Optical tweezers is low-damage and easy to be controlled. Automatic cell manipulation system is constructed. But the manipulation force is weak and efficiency is low. [2] By using surface adhesion and fluidic channel structures, it is able to assembly cells with high efficiency. While the position of cells is not flexible since surface properties and channel structures are not easy to change. [3][4]

On-chip fabrication based on photo-crosslinkable resin via UV illumination is a creative way for immobilizing cells. Microstructures are directly fabricated inside microfluidic channel within short time. [5] If the cells are mixed inside the photo-crosslinkable resin in advance and then injected inside microfluidic channel, the cells are able to be directly immobilized inside the microstructures. These microstructures could be arbitrary shaped and they are the components for assembling 3D cell structures. There are several advantages of the on-chip fabrication method such as high-speed, low-cost and arbitrary shaped. [6]

Self-assembly is a suitable approach for high-throughput cell assembly because of its low time consuming. [7] Self-assembly method is applied in many researches. 3D structures can be assembled by air. [8] Furthermore, inside microfluidic devices, different 3D microstructures are self-assembled based on special channel which shows great potential to be used in large amount cell assembly. [9-11]

In this paper, we report a cell assembly method based on cell immobilization by on-chip fabrication and microfluidic self-assembly inside microfluidic devices. The whole research procedure is shown in Figure 1. There are 3 advantages in this research. First, the arbitrary shaped microstructures are able to be efficiently on-chip fabricated inside microfluidic chips. Second, the large amounts of cells are able to be immobilized inside microstructures. Third, the assembly of microstructures embedding cells is with high efficiency because of the microfluidic self-assembly.

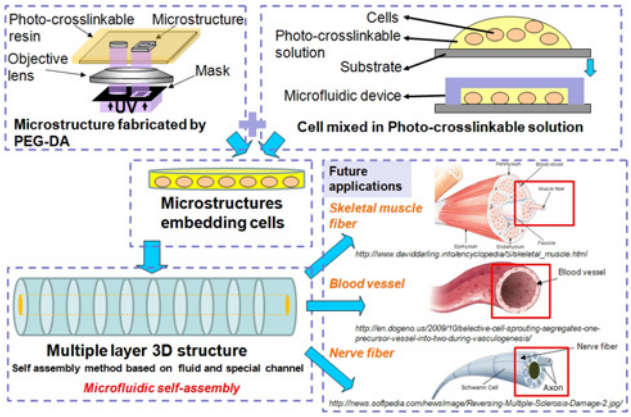

Figure 1. A schematic drawing of the cell assembly method

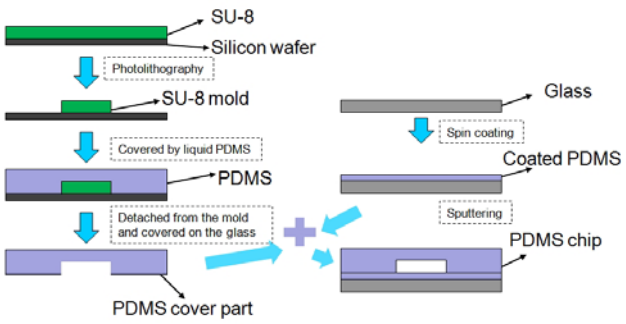

Figure 2. Fabrication procedure of PDMS microfluidic chip with PDMS coated bottom surface

Movable microstructures embedding yeast cells (*W303*) based on Poly (ethylene glycol) Diacrylate (PEGDA) was reported. A 2-layer microfluidic device was fabricated and self-assembly method of the fabricated movable microstructures via this device was preliminarily demonstrated.

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EXPERIMENT AND RESULTS

Microfluidic chip made of PDMS is a key component for many microfluidic applications as well as the used on-chip fabrication method [12]. The fabrication procedure for PDMS (SILPOT 184 W/C, Dow Corning Toray) microfluidic chip with PDMS coated bottom surface is shown in Figure 2. Fabrication method is also based on photolithography. The mold of the microfluidic channel was made by photoresist SU-8. The silicone tube was put in the hole and the solution was injected to the channel by the negative pressure.

On-chip fabrication was conducted inside this chip. As shown in Figure 1, via the microscope, the patterned UV-ray was illuminated through the mask to the photo-crosslinkable resin which was injected into the microfluidic device made of Polydimethylsiloxane (PDMS). The photo-crosslinkable resin, which is Poly (ethylene glycol) Diacrylate (PEGDA), has low toxicity. Cell viability is positively confirmed inside several kinds of PEG-DA such as molecular weight 700 and 3400. [13] This resin was polymerized and microstructures with arbitrary shape were directly fabricated at desired place inside the channel of microfluidic device. The fabricated microstructures on the PDMS surface can move in the non-polymerized resin freely. [14]

By mixing yeast cells (*W303*) inside PEG-DA solution and PDMS coating layer on the channel bottom, movable microstructures embedding cells were fabricated directly inside microfluidic chips as shown in Figure 3. The fabrication time is 0.2 second. Because of the used donuts shaped mask, the donuts shaped microstructure was fabricated. The inner and outer diameter of the microstructure was about 40 μm and 100 μm.

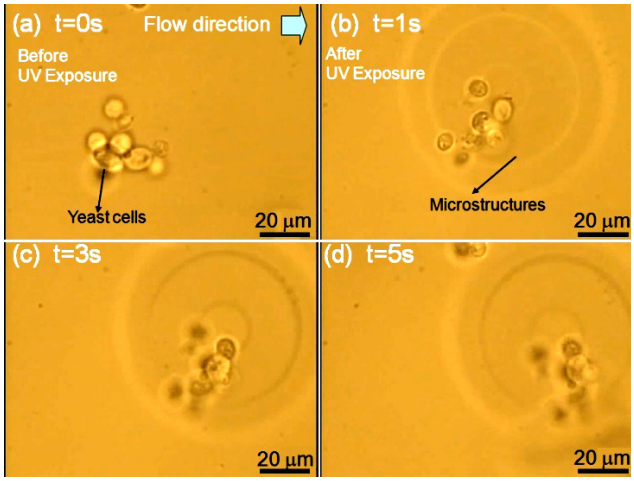

Figure 3. Fabricated movable microstructure embedding yeast cells

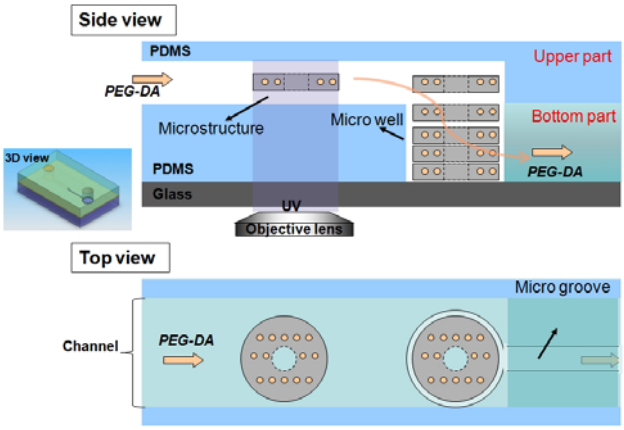

Figure 4. Schematic of a microfluidic device for self-assembly of movable microstructures

For constructing cell 3D structures based on the fabricated movable microstructures, a microfluidic device with 2 PDMS layer (upper part and bottom part) was presented as shown in Figure 4. The upper part is commonly designed with a 500μm width channel for providing the space of microstructure fabrication. There is a micro well inside bottom part for assembling the fabricated movable microstructures and a micro groove for releasing solution but microstructures. The diameter of the well is 130 μm and the width of the groove is 40μm. The depth of the well is 50μm and the gap between upper and bottom parts is 10μm. As shown in Figure 4, the fabricated movable microstructures will flow with the solution and go into the micro well, then stop at the top of the micro groove. Therefore, the stopped microstructures will be assembled from bottom to the top one by one. Finally, it will form a tube shaped 3D structure with large amount of cells.

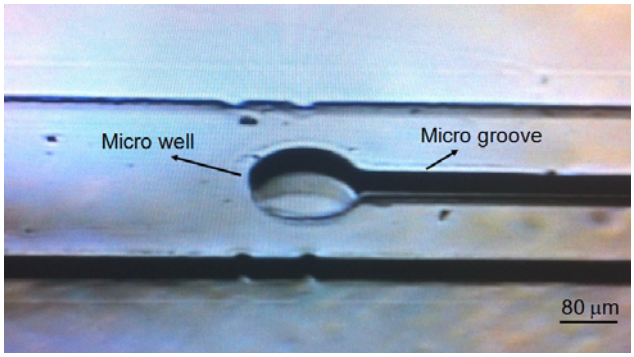

Figure 5. Fabricated bottom part of the PDMS microfluidic device

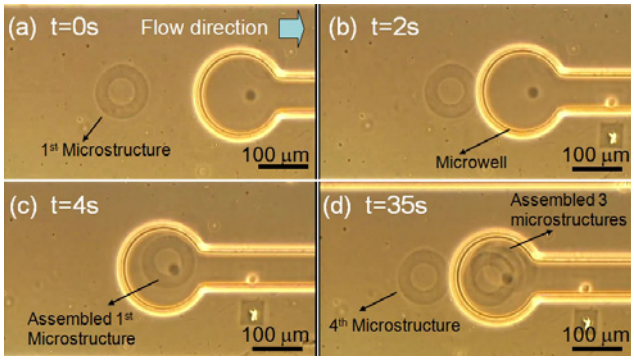

Figure 6. Self-assembly result of microstructures inside microfluidic channel

The fabrication method is the same as shown in Figure 2 and the fabricated bottom part is shown in Figure 5. The size of the micro well and groove is 135μm and 43μm. Depth of micro well is 53μm. The experimental result is shown in Figure 6. The fabricated first microstructure successfully went into the micro well as shown in Figure 6(c). Then the second and third one also went into and assembled as shown in Figure 6(d).

DISCUSSION

The cells inside the fabricated movable microstructures are randomly distributed without control. It is very important to control the cell concentration inside certain area for in vitro culture. Therefore, the approach for controlling cell concentration or pattern inside on-chip fabricated movable microstructures will be a suitable research topic for the next step.

Self-assembly of donuts shaped microstructures is demonstrated. The assembled microstructures contain 3 layers was succeed. However, the experiment result is not good because of the difference of the size between the fabricated microstructures and the micro well. As shown in Figure 6(d), the assembled 3 layers are not in the same axis. Next time the micro well should be fabricated close to the size of the microstructures and make sure the assembled ones are able to be in the same axis.

These movable microstructures are able to be further assembled to be large 3D structures. By continually fabrication of movable microstructure, a tube shaped structure is able to be constructed. By culturing cells, it could become functional component of artificial tissue. Also, microstructures with different shapes should be fabricated to form different functional cell 3D structures.

CONCLUSION

In this paper, we reported a cell assembly method based on cell immobilization by photo-crosslinkable resin and microfluidic self-assembly inside microfluidic devices. The on-chip fabrication of movable microstructures embedding yeast cells (*W303*) based on PEGDA was presented. A 2-layer microfluidic device was fabricated by PDMS and self-assembly method of the fabricated movable microstructures via this device was demonstrated.

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