2014 IEEE International Conference on Robotics & Automation (ICRA)
Hong Kong Convention and Exhibition Center
May 31 - June 7, 2014. Hong Kong, China

Fluidic Self-assembly of Multilayered Tubular Microstructures by Axis Translation inside Two-layered Microfluidic Devices

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***Abstract*—Microfluidic devices provide efficient approaches for building cellular tubular structures for *in vitro* tissue models in tissue engineering. In this paper, we report a novel method of constructing three-dimensional (3D) multilayered tubular structures based on axis translation of two-dimensionally (2D) microstructures inside microfluidic devices. The on-chip fabrication of movable 2D microstructures embedding fibroblasts (NIH/3T3) based on Poly (ethylene glycol) Diacrylate (PEGDA) was reported. Novel two-layered microfluidic devices were fabricated by Polydimethylsiloxane (PDMS), for conducting the fluidic self-assembly of the 2D microstructures. The self-assembly process was experimentally demonstrated. For improving the assembly results, a funneled structure and 3 micro grooves were added inside the microfluidic channel. Improved self-assembly result of constructing a multilayered tubular microstructure with higher efficiency was demonstrated.**

I. Introduction

Recently the construction of three-dimensional (3D) cell structures is one of the major challenges in tissue engineering, since it can provide effective approaches of constructing implantable *in vitro* artificial tissues [1, 2]. The cells inside real tissues and organs are arranged according to certain shapes, such as neural cells with line shape, skin with reticular shape and blood vessel with tube shape [3]. For building artificial tissues, important issues are how to immobilize cells inside certain structures for encapsulation and how to assemble these structures to form different shapes as cell scaffolds [4, 5]. Moreover, long-term co-culture of these immobilized and assembled cells is the key way to construct functional artificial tissues [6].

For cell immobilization, approaches such as aspiration, pressure of solution and fluidic structure are used [7]. The aspiration and pressure have the advantages of the large fixing force, while the disadvantage is cell damaging [8]. Cells are immobilized inside special microfluidic device. The immobilized cells are difficult to be further analysis [9]. On-chip fabrication based on photo-crosslinkable resin via UV illumination is a creative way for immobilizing cells [10].

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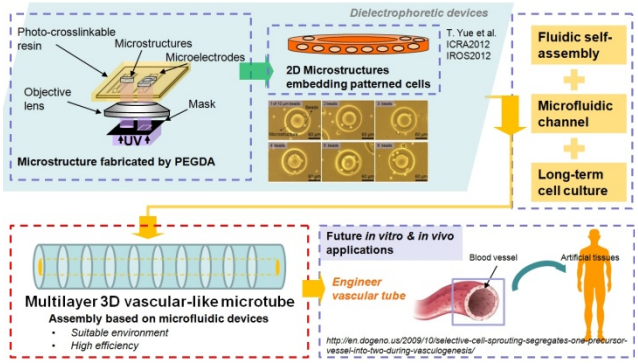

Fig.1 A schematic drawing of the fabrication and assembly method

In our previous works, cells which were patterned inside the microfluidic channel were directly immobilized, maintaining their patterns. Several advantages such as high-speed, low-cost and arbitrary shaped are with the on-chip fabrication method [11].

There are several methods for assembling 3D cell structures. Scaffolds are used for supporting cells to generate complex and arbitrary 3D structures [12]. However, the small vascular-like networks are difficult to be generated. Constructing 3D structures after cell encapsulation is a good way for building 3D cell structures. Hydrogel fiber embedding several kinds of cells and co-culture of them is demonstrated [13]. Shape of these hydrogels is difficult to control. Cell embedded microstructures are used as components for constructing larger 3D cell structures as a bottom-up approach [14]. Approaches of assembling these cell embedded microstructures include direct manipulation and fluidic assembly [15].

In our previous work, a dielectrophoretic microfluidic device was developed and two-dimensional (2D) microstructures embedding controllable particles were fabricated on-chip [16]. Based on these 2D microstructures, some preliminary experiments of constructing 3D multilayered structures were conducted inside microfluidic channels. However, there were many limitations in our previous methods [17].

Self-assembly is a suitable approach for high-throughput assembly of these microstructures, because of its automatic process and short operating time [18]. Self-assembly method is applied in many researches. 3D structures can be assembled by air [19]. Furthermore, inside microfluidic device, self-assembly based on fluidic dynamics provides good methods for constructing 3D structures [20]. Different 3D

microstructures are self-assembled based on functional channel structures, which shows great potential to be used in large amount cell assembly [21].

In this paper, we developed a novel method of constructing 3D multilayered tubular structures based on 2D microstructures inside two-layered microfluidic devices, aiming for applying in building engineered vascular-like tubular structures in the future. The whole research procedure is shown in Fig. 1. Compare with the previous assembly method [17], the axis translation of 2D microstructures was achieved, and the assembly with higher efficiency and longer tubular structures were experimentally demonstrated.

The on-chip fabrication of movable 2D microstructures embedding fibroblasts (NIH/3T3) based on Poly (ethylene glycol) Diacrylate (PEGDA) was reported. PEGDA has low toxicity. Cell viability is confirmed inside several kinds of PEGDAs such as molecular weight with 700 and 3400 [22]. Novel two-layered microfluidic channels with a micro well and micro grooves were presented for conducting fluidic self-assembly. The two-layered microfluidic devices were fabricated by Polydimethylsiloxane (PDMS). The axis translation of 2D microstructures inside the micro well and the fluidic self-assembly process were demonstrated. For improving the assembly results, the funneled structure and 3 micro grooves were added in the microfluidic channels. Improved self-assembly result of constructing multilayered tubular microstructures with higher efficiency was demonstrated.

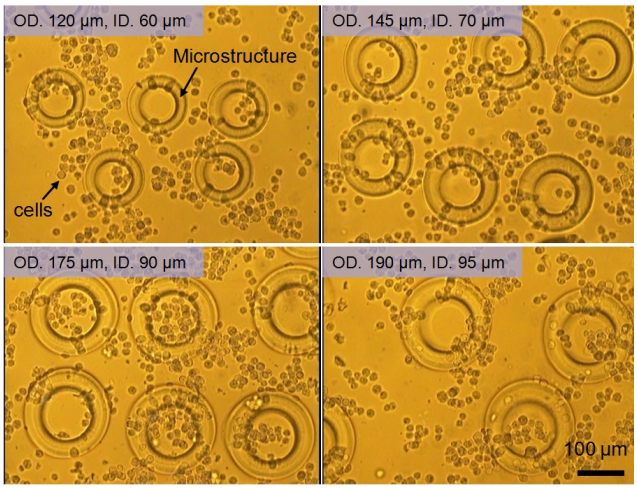

Fig. 2 Fabricated movable microstructures embedding fibroblasts (NIH/3T3), with different outer diameter (OD.) and inner diameter (ID.)

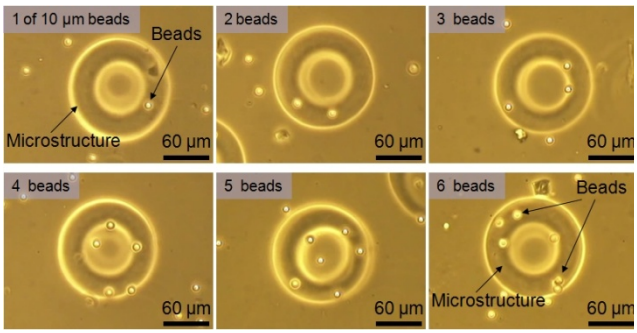

Fig. 3 Movable microstructures embedding microbeads of which the concentration (number) is controllable

II. On-chip Fabrication of Movable Microstructures Embedding Cells

A. On-chip fabrication method

The on-chip fabrication method for arbitrary-shaped microstructures is shown in upper left part of Fig. 1. Optical microscope (IX-71, Olympus) was used for observing samples and exposing UV on samples. X-Y stage and height of the objective lens (Z-axis) were controlled for adjusting fabrication positions. PET (polyethylene terephthalate) masks were set between UV lamp and objective lens for patterning UV light. Several objective lens were used, including oil immersion objective UPLFLN 100XOI2, 60X and 40X (Olympus). UV was illuminated by the mercury lamp (USH-103tems) and controlled by the shutter (BSH-RIX, Sigmakoki). The microfluidic device was set on the X-Y stage.

The patterns on the PET mask are able to be arbitrary-shaped, depending on the designs. UV was illuminated through the mask from bottom of the objective lens into the PEGDA (molecular weight 200, 700, Sigma Aldrich). UV was patterned by the masks. Then the PEGDA was polymerized and the arbitrary shapes of microstructures were fabricated inside the solution [11]. The position of the microstructure is confirmed by the stage. Microstructures were fabricated by illuminating the UV-ray for 0.2 second via the objective lens of 100X and 40X. By different masks, we could easily fabricate different microstructures with large amount in short time. Furthermore, by programming control software for UV lump shutter and stage of microscope, it is possible to conduct the automatic on-chip fabrication.

During the fabrication, when the surface of substrate is glass, the fabricated microstructure adheres on the glass surface. When the surface of substrate is PDMS, the fabricated microstructure is able to move in the non-polymerized resin freely [10]. Therefore, a PDMS (SILPOT 184 W/C, Dow Corning Toray) surrounded microfluidic channel was prepared for fabricating movable microstructures.

B. Movable 2D microstructures embedding controllable cells

After fibroblasts (NIH/3T3) were cultured inside the incubator, collected cells were mixed with PEGDA solution (including 20% PEGDAs (molecular weight 700, Sigma Aldrich), 0.5% photoinitiator (Irgacure 2959, Ciba), and PBS (Phosphate Buffered Saline, Wako)). Solution was injected inside the PDMS coated channel, and then movable 2D microstructures embedding cells (NIH/3T3) were fabricated directly inside microfluidic devices as shown in Fig. 2. As the donuts shaped mask was used, the donut-shaped

microstructure was fabricated. Donut-shaped microstructure could be used as the building layers of the tubular structures as mentioned in Fig.1.

The diameters of the cell embedded microstructures were controlled by the masks (able to be form 100 μm to several hundred microns. The cells inside the microstructures are randomly distributed and uncontrollable. However, in natural way, cells inside body are not randomly distributed but with different distribution in different locations. Therefore it is important to control the cell concentration inside certain areas for *in vitro* culture. In our previous work, a microfluidic device integrated with dielectrophoresis was developed and microstructures embedding controllable numbers of particles were fabricated on-chip [16]. Fig. 3 shows the fabricated microstructures with different numbers of microbeads. This method is able to be used for fabricating the desired 2D microstructures as the building blocks for 3D structures.

III. Fluidic Self-assembly For Multilayered Microstructures

A. Design and fabrication of the 2-layer PDMS microfluidic device

In view of constructing 3D tubular structures via on-chip fabricated 2D movable microstructures, the fluidic self-assembly based on axis translation was involved. A microfluidic device with 2 PDMS layer (upper part and bottom part) was presented for constructing 3D structures, including a micro well for axis translation and a micro groove for releasing the solution, as shown in Fig. 4. This device is for conducting both on-chip fabrication and self-assembly of microstructures inside one microfluidic channel, which provides an efficient way of constructing tubular structures as a factory assembly line.

The upper part is commonly designed with a 500 μm width channel for providing the space of microstructure on-chip fabrication. There is a micro well inside the bottom part for assembling the movable microstructures by axis translation. The micro groove is for releasing the solution and stopping the assembled microstructures. The thickness of the fabricated microstructures is controlled by the depth of the channel in the upper part, which is about 35 μm, and then the thickness of fabricated microstructures will be about 30 μm [16]. The depth of the well is 100 μm and the length is 500μm. The width of the groove is 35 μm. The concept of the axis-translation self-assembly is described as follow. The fabricated microstructures are flowed horizontally with the solution, into the micro well. Following the flow direction, the microstructures are rotated 90 degrees (axis translation from the original position to the assembly position) to be vertical inside the micro well and stopped at the end of the micro well, because they are not able to go into the micro groove. The stopped microstructures will be assembled one by one. Finally, a tubular 3D structure embedding large amount of cells is formed inside the micro well.

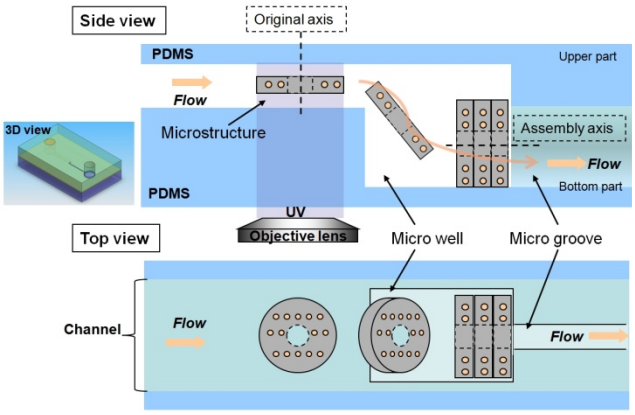

Fig.4 A schematic drawing of the two-layer microfluidic device for self-assembly of movable microstructures

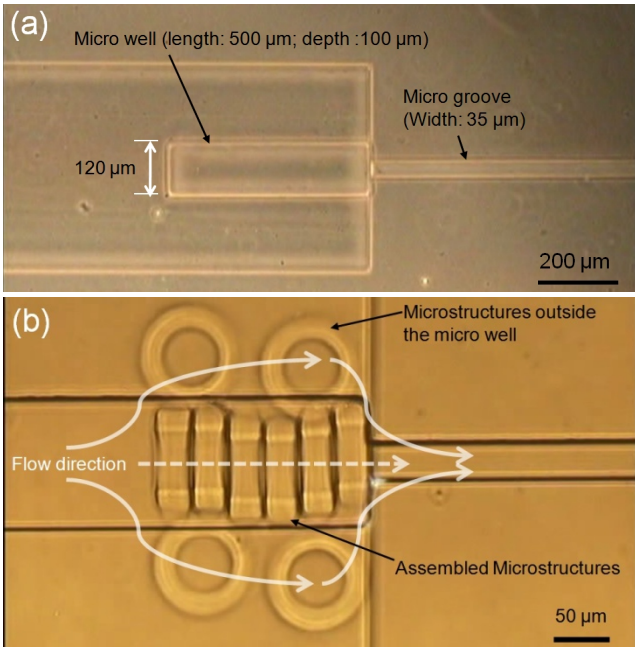

Fig. 5 Fabricated microfluidic channel and the preliminary assembly result

The fabrication method of this 2-layer microfluidic device is based on the conventional photolithography method. Here the two PDMS parts were bonded together and the channels were aligned under microscope manipulation stage. The fabricated upper and bottom PDMS parts are bonded together to form the micro channel. The top views of the microfluidic channels are shown in Fig. 5(a). It is for testing the self-assembly as preliminary experiments. The width of the micro well and groove is about 120 μm and 35 μm respectively. And the depth of the upper channel and micro well is 35 μm and 100 μm respectively. All the parameters are acceptable according to the design.

B. Experimental results of self-assembly

For the assembly tests, the microstructures without cells or beads were used. Sample solution was injected to the inlet of the channel. Negative pressure was given from the outlet by a syringe pump. The on-chip fabrication and fluidic

self-assembly were conducted inside the device. The experimental results of self-assembly are shown in Fig. 5(b). Movable 2D microstructures were continually fabricated inside upper part channel and then moved with solution flow into the micro well of bottom part. The microstructures rotated 90 degrees and became vertical, assembled at the entrance of the micro groove. The axis translation was succeeded. 6 layers were assembled to form a tubular structure. The great advantage of this method is that the whole assembly process is performed without any outside manipulation. However, this is the preliminary results with some problems. As shown in Fig. 5(b), there are some microstructures outside the micro well and failed to be assembled. Improvements are needed.

IV. Improvements of the Microfluidic Channel for Self-Assembly

A. Improved fabrication channel with a funneled structure

In the previous channel, the width of the fabrication channel is larger than the micro well so that some donuts are failed to be assembled. We added a funneled structure at the end of the fabrication channel to make sure that the fabricated movable microstructures go into the micro well smoothly. As shown in Fig. 6(a), all the 2D microstructures are flowed into the micro well and the axis was translated. Fig. 6(b) shows the fabricated microfluidic channel with a funneled structure. The width of the end of the fabrication channel is the same as the micro well. Microstructures are able to flow into the micro well smoothly via this funneled structure, for avoiding the previous problem in Fig. 5(b).

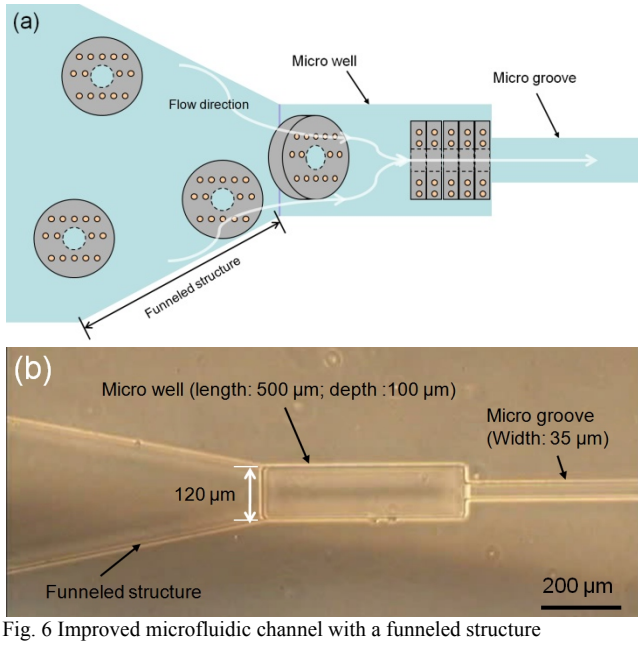

Fig. 6 Improved microfluidic channel with a funneled structure

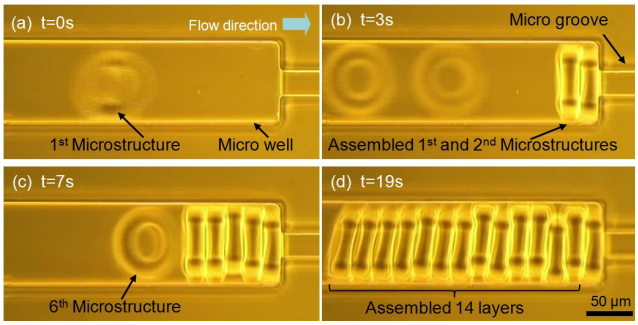

Fig. 7 Improved self-assembly result of constructing multilayered tubular microstructures

B. Experimental results

The improved self-assembly was conducted by donut-shaped microstructures inside this improved channel, as shown in Fig. 7. All the microstructures were successfully flowed into the micro well and assembled. The axis translation of each 2D layer was succeeded. A multilayered tubular microstructure with 14 layers was assembled in 19 seconds (about 0.7 layers per second, averagely). The fabrication time was not considered here and just the assembly time was counted, for evaluating the assembly efficiency. The inner hollow of this tubular structure is shown clearly in the micrograph. The result demonstrated the function of the funneled structure for the assembly in the channel.

C. Improved channel with 3 micro grooves

Based on the experimental results, we think the assembly efficiency may be influenced by the flow velocity, which means that if the flow velocity is higher the assembly time can be shorter. The inner hollow of this tube-shaped structure and the section size of the micro groove influence the flow velocity. However, there is only one micro groove in the previous microfluidic channel. The channel was improved by adding 2 more grooves inside, as shown in Fig. 8(a). The area for releasing the solution was increased.

Fig. 8(b) shows the calculation results of the section size of the micro grooves in both cases (the previous case with 1 groove and the improved case with 3 grooves). The section size of the improved channel (5000 μm^2) was about 5 times larger than the previous one (800 μm^2). It is expected that the flow velocity will be increased in the improved channel. Fig. 9(a) shows the fabricated channel with 3 micro grooves.

D. Experimental results

Fig. 9(b) shows the comparison results between the previous and the improved channels. During about 3 seconds of assembly process, 4 layers were assembled in the improved channel, while just 3 layers were assembled in the previous channel. Besides, the alignment of the assembled structures in the previous channel was not as good as the ones in the improved channel.

In the improved channel, it was possible to assemble more than 1 layer within 1 second (about 1.3 layers per second, averagely). It was faster than the previous result (0.7 layers per second). Fig. 9(c) shows a 3D tubular structure with 16

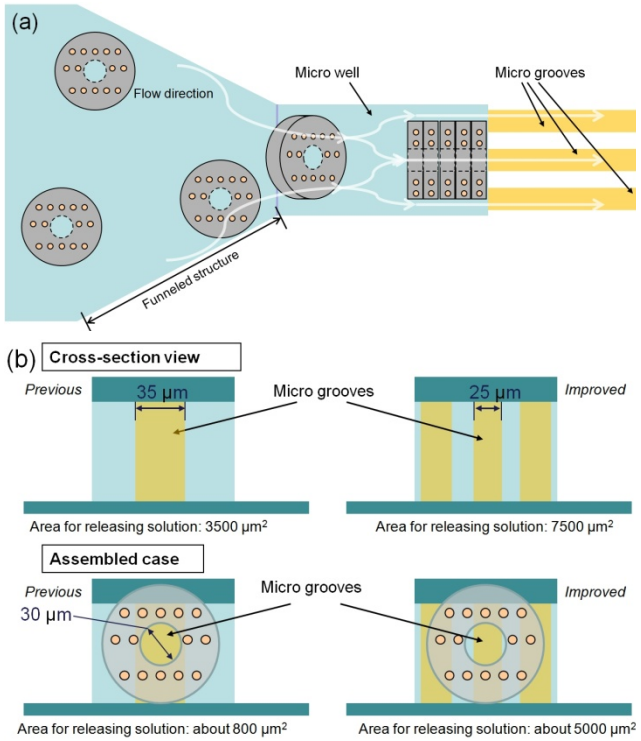

Fig. 8 Improved microfluidic channel with 3 micro grooves and the caculation of the the section size for releasing the solution

layers, constructed in the channel. Both the higher efficiency and the closed environment are able to be obtained in the presented device. Based on this improved microfluidic channel, the assembly of cell embedded microstructures was conducted. Fig. 10 shows the assembly result. Cells were encapsulated inside the donuts and assembled as a tubular structure based on the axis translation method. The result shows great potential to be used in high-throughput construction of 3D tubular structures for building cellular structures or *in vitro* tissue models.

E. Discussion

Self-assembly of the microstructures was demonstrated. However, the experiment result is still preliminarily, because of several unsolved problems. One is how to connect the assembled layers after assembly. The other one is how to remove the assembly structures from the microfluidic channel to the outside.

For solving the first problem, a secondary UV exposure is a good choice. After assembly, the solution in the channel can be replaced to water, which is not photo-crosslinkable. Then secondary UV exposure will be given to the whole assemble structure. Then the layers will be firmly connected as one tube, which has been demonstrated in [15]. One thing should be noted is that the UV is not good for cells. The cell viability should be evaluated after secondary UV exposure. For solving the second problem, we think we can directly separate the 2 layers to open the channel and collect the assembled tubes. However, the opened devices cannot be used again. Other methods for collecting the assembled tubes need be developed.

Fig. 9 Experimental results of self-assembly via the improved microfluidic channel with higher efficiency.

The alignment of assembled layers was not good enough. There is displacement between some layers. The reason is that the diameter of microstructures is smaller than the width of micro well. It may be solved by modifying the size of the channel. Besides, there were no cells inside the used microstructures in assembly experiments. For the next step, assembly of cell embedded structures should be conducted.

V. Conclusion

In this paper, we reported a novel method of constructing 3D multilayered tubular structures based on axis translation of 2D microstructures inside microfluidic devices. The

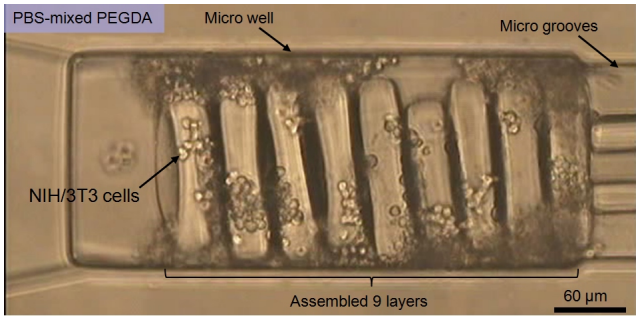

Fig. 10 The assembly result of cell embedded tubular structures based on the axis translation self-assembly method.

on-chip fabrication of movable 2D cell embedded microstructures based on PEGDA was reported. Novel two-layered microfluidic devices with a micro well and micro groove were fabricated. The self-assembly and the axis translation of the 2D microstructures were experimentally demonstrated. For improving the assembly results, the funneled structure and 3 micro grooves were added in the channel. The improved self-assembly of constructing 3D multilayered tubular microstructures with higher efficiency was demonstrated. The tubular microstructures were able to be used as engineered vascular-like tubes for *in vitro* applications. The result shows great potential to be used in high-throughput construction of 3D tubular structures for building *in vitro* tissue models.

For the future works, the cell viability will be confirmed after assembly. Microfluidic devices for self-assembly should be improved and the on-chip extraction function should be added.

ACKNOWLEDGMENT

This work was partially supported by Japan MEXT KAKENHI (Hyper Bio Assembler for 3D Cellular Systems (BioAssembler)) Grant Number 23106006, and Nagoya University Global COE Program (COE for Education and Research of Micro-Nano Mechatronics).

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