

RAPID CONSTRUCTION OF MULTILAYERED TISSUES ON CURVED SUBSTRATE BY WATER TRANSFER PRINTING

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ABSTRACT

A rapid construction process is necessary for building up numerous cell modules into 3D tissues maintaining the tissue geometries and initial conditions of cells. We proposed new 3D assembly technique to fabricate a hollow tubular tissue structure using by water transfer printing. By utilizing this assembly technique, we discuss the relation between 3D transcriptional body of gel matrix and a developed figure of transfer tissue, and perform fabricating on a hollow tubular tissue. Appropriately simulation of the 3D environment in which tissues normally develop and function is crucial for the engineering in vitro models that can be used for the formation of complex tissues. These artificial hollow tubular tissues would be used for drug efficiency evaluation and operative training as in vitro simulators.

KEYWORDS

Multilayered Tissues, Three-dimensional Assembly, Tissue Printing, Alginate Acid, Fibroblast

INTRODUCTION

Although a recent approach using cell-laden hydrogel matrix does provide ECM [1, 2], a large-scale hollow tubular tissue structure has not yet been achieved because of the module size ($> 500 \mu\text{m}$), uniformity, and throughput limitations. Furthermore, a rapid construction process is necessary for building up numerous cell modules into 3D tissues maintaining the tissue geometries and initial conditions of cells. Recently, we have proposed a multicellular aggregate formation platform [3]. The cellular aggregate formed using this platform has a toroid-like geometry and includes a micro-pore that facilitates the supply of the oxygen and growth factors and expels waste products. By directed assembly of the toroidal multicellular aggregate, this micro-pore can be used to as a communicated channel for vascularization and neurogenesis. However, the assembly technology has a subject and cannot build hollow tubular structure rapidly.

On the other hand, Matsusaki et al, have developed a cell-accumulation technique using highly biocompatible nano-films by layer-by-layer (LbL) assembly for the rapid construction of thick layered tissues with a well-controlled layer number and thickness [4]. Fabricated multilayered tissue has constructed highly developed blood capillary networks (over 1 cm^2 of layered tissues) by sandwiching endothelial cells between the layered tissues. We proposed new 3D assembly technique to fabricate a hollow tubular tissue structure using water transfer printing for the purpose of manipulation of the multilayered tissues. By utilizing this assembly technique, we discuss the relation between 3D transcriptional body of gel matrix and a developed figure of transfer tissue, and perform fabricating on a hollow tubular tissue.

EXPERIMENT

Figure 1 shows the design concept of the rapid construction of multilayered tissues on curved substrate by water transfer printing. Water transfer printing is used on exterior painting from the auto body to the mobile phone. First, a sheet-like multilayered tissue is floated on the water surface and 3D alginate gel matrix is lift up from water to air. A multilayered tissue is transferred on a 3D surface by pulling up alginate gel matrix from the water. The transcribed multilayered tissue on biodegradable material is returned to the culture medium after fixing. After cultured (24 h), a main gel matrix is degraded by using alginate lyase, in order to obtain the multilayered tissue of hollow tubular tissue structure.

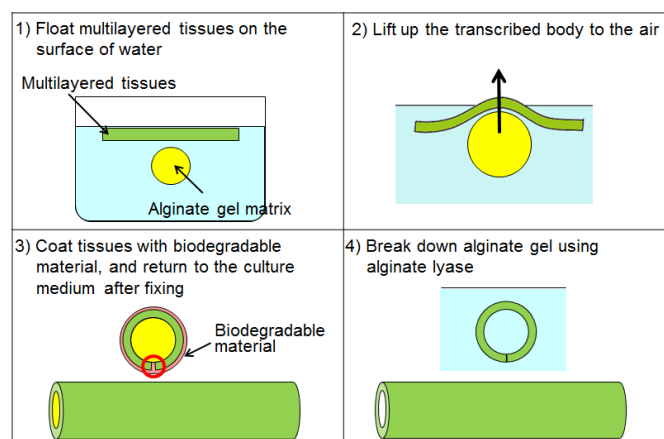


Figure 1: Design concepts of three-dimensional rapid construction of multilayered tissues on curved substrate using water transfer printing.

Multilayered tissues were made by LBL method. LbL method is a thin film fabrication technique. Using this method, we can get uniform thin films on the order of a few nanometers at ordinary temperatures and pressures. The films are formed by depositing alternating layers of oppositely charged materials with wash steps in between. In this paper, we deposited fibronectin-gelatin (FN-G) nanofilms onto single cell surfaces by this method in order to promote cell-cell interactions like natural ECM. Briefly, cells were alternatively immersed with 0.04 mg/ml FN and G in 50 mM Tris-HCl (pH = 7.4). After each procedure, the cells were washed with 50 mM Tris-HCl (pH = 7.4) to remove unadsorbed polymers. Then the coated cells were seeded to culture device at an appropriate density. As a result we can obtain multilayered tissues whose shape corresponding to bottom shape of culture device. The culture device in imitation of commercial culture inserts was made by sandwiching 0.4 μm Nucleporemembrane (GE Healthcare UK Ltd.) between upper and lower parts.

Multilayered tissue is transferred to 1 mm diameter glass capillary tube which dip-coated alginate gel (thickness of 500 μm). So intended multilayered tissue size was determined 10.0-by-6.28 millimeter. Therefore to obtain 50 μm thickness of multilayered tissue, we seeded 1.9×10^6 cells/well of neonatal normal human dermal fibroblasts (NHDF) to the culture device. NHDF cells were maintained in DMEM medium supplemented with 10% FBS at 37 $^\circ\text{C}$ in a humidified 5 % CO_2 atmosphere. The constructed multilayered tissue retrieved from membrane after 4days culture.

RESULTS AND DISCUSSION

To clarify the relationship between 3D transcriptional body of gel matrix and a developed figure of transfer tissue, we evaluated the degree of circularity, surface segmentation, and transcriptional behavior using quasi multilayered tissue (Fig. 2). The developed figure of quasi multilayered tissue was prepared five patterns; Sphere 1 and 2, Ellipsoid, Y-shaped structure 1 and 2. The degree of circularity, following, was made into the parameter for comparing complexity geometry the thought that a round shape is the most stable simple form for a multilayered tissue. The degree of circularity is given by:

$$\text{degree of circularity} = 4\pi S / L^2$$

where S is surface area, L is the nominal perimeter. Table 1 shows the evaluation of developed figures of quasi multilayered tissue. Each degree of circularity of the *Sphere* was 0.2 lower and comparatively complicated geometries. High circularity of transfer tissue demonstrated excellent transcriptional behavior. These results suggested that the geometry of developed figure is closely related to cut line and the direction of pull-up. Furthermore, we showed that it could assemble in the multi-segmentation of part. However, multi-segmentation requires reduction of parts for using a cell laden, resulting in a time-consuming process. These results indicated that the threshold for accurate assembly by water transfer printing depends on both circularity and segmentation.

Figure 3-A) shows microscopic images respectively of the transcribed multilayered tissue retrieved from the water. The size of the multilayered tissue at this time was 9.5 mm \times 6.0 mm. About 35% of contraction was observed from the early stages of cultivation (data not shown). It was shown that multilayered tissues were uniformly transferred on 3D alginate gel matrix. The thickness of the transcribed multilayered tissue was observed as 40 μm from Z-stack confocal images (Fig. 3-B)). Enzymatic degradation by alginate lyase of transcribed hydrogel body in a biodegradable gel fabricated a channel with predetermined 3D hollow tubular structure [5]. The culture device was placed in an incubator (37 $^\circ\text{C}$, 5% CO_2) for 30 min incubation for enzymatic degradation of the hydrogel fiber by alginate lyase (4 mg/mL). The multilayered tissue of hollow tubular structure was capable of connecting both ends with the tube pump and transporting the culture media

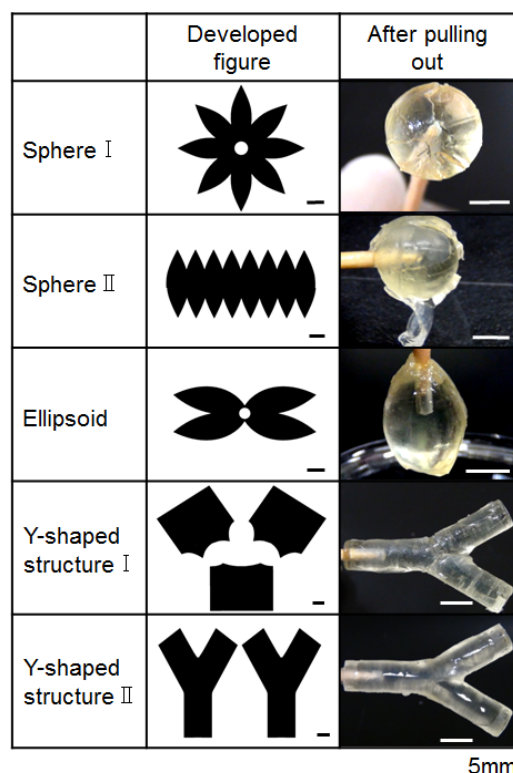


Figure 2: Relationship between 3D transcriptional body of gel matrix and a developed figure of transfer tissue. Evaluation of transcription efficacy by the degree of circularity, surface segmentation, and transcriptional behavior using quasi multilayered tissue.

Table 1: Evaluation of developed figures of quasi multilayered tissue.

	Degree of circularity	Surface segmentation	Transcriptional behavior
Sphere I	0.17	good	good
Sphere II	0.14	good	poor
Ellipsoid	0.25	good	excellent
Y-shaped structure I	0.60	fair (3 parts)	fair (need fixation)
Y-shaped structure II	0.39	fair (2 parts)	fair (need fixation)

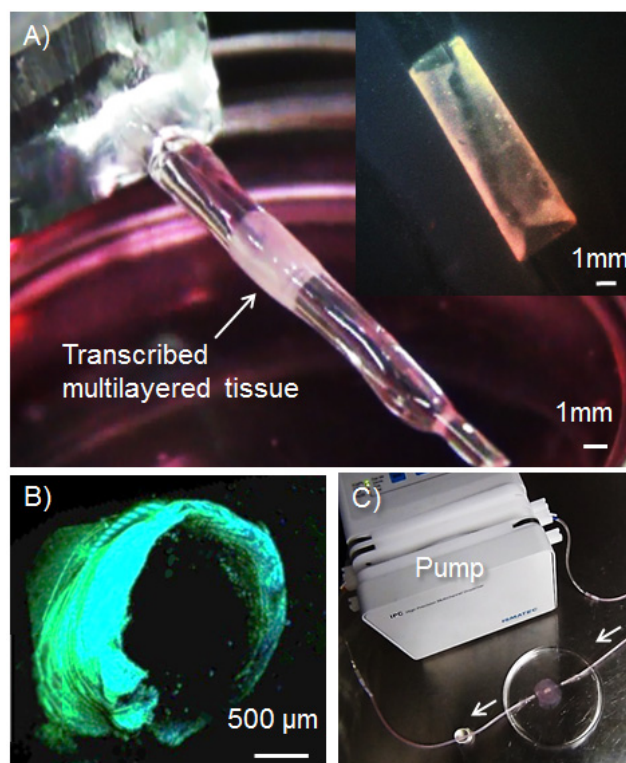


Figure 3: Transcribed multilayered tissue of hollow tubular structure using by water transfer printing. Enzymatic degradation by alginate lyase of transcribed hydrogel body in a biodegradable gel fabricated a channel with predetermined 3D hollow tubular structure.

(Fig. 3-C)). Appropriately simulation of the 3D environment in which tissues normally develop and function is crucial for the engineering in vitro models that can be used for the formation of complex tissues. These artificial hollow tubular tissues would be used for drug efficiency evaluation and operative training as in vitro simulators.

CONCLUSION

Recent trends in tissue engineering have aimed at simulating the physiological environment in vitro. We proposed new 3D assembly techniques to fabricate a hollow tissue construct using by water transfer printing. And it enabled assembling three-dimensional multilayered tissues to curved surface easily. Also we discussed optimum geometry of development figures. Finally, we succeeded in assembling three-dimensional multilayered tissue into tubular construct by this method. The tissue consists of neonatal normal human dermal fibroblasts (NHDF). These artificial hollow tissues would be used for drug efficiency evaluation and operative training as *in vitro* simulators.

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