ABSTRACT

We proposed a 3D assembly technique and a perfusion cultivation system. The technique uses water transfer printing to fabricate a multilayer structured tubular tissue. By the perfusion cultivation system, this study was aimed to determine whether the tissue-engineered structure maintains the normally mechanical property as the development and function. In this work, we demonstrated that fabricated tissues could rapidly assemble into aligned tubular tissue in the appropriate conditions and circularly cultured the multilayer structured tubular tissue.

KEYWORDS: Multilayer Structured Tubular Tissues, Three-dimensional Assembly, Tissue Printing, Fibroblast

INTRODUCTION

There are many studies about engineered tissue structures to study and mimic the biological and mechanical function of many native tissues in vivo. Our proposing technique differs from previous study [1], and does not require a solid biodegradable scaffold [2, 3]. Therefore, this approach can simply assemble fabricated tissues into a functional 3D structure and circularly culture the tubular tissue in a suitable tubular tissue environment.

THEORY

Figure 1 shows the design concepts of three-dimensional rapidly tubular tissues on a curved substrate using water transfer printing. Firstly, tissues are made by Layer-by-Layer (LbL) technique [4, 5]. Using this technique, we deposited fibronectin-gelatin (F-G) nano films onto single cell surfaces in order to promote cell-cell interactions like natural ECM. We seeded $1.0 \times 10^5$ cells/well of neonatal normal human dermal fibroblasts (HDFs). After 3 days cultured, a sheet-like tissue is floated on the water surface. The tissue is transferred on a 3D surface by lifting up a supporting body from water into the air. After transfer and construct the tubular tissue, by the assistive jigs, the configuration is maintained and the supporting body is removed from the tubular tissue. To evaluate this tubular tissue with culturing, we propose the custom-designed perfusion cultivation system with various stimuli and monitoring.

![Figure 1: Design concepts of three-dimensional rapidly constructed tubular tissues and schematics simulated the perfusion cultivation system. A fabricated tissue is transferred onto a 3D surface using water transfer printing. This system was composed of a centrifugal pump, a tube-installed chamber and a reservoir, which were connected to each other with silicon tubes to form a closed circuit.](image)

EXPERIMENTAL

We fabricated sheet-like tissues and constructed tubular tissues, approximately 10 mm in length. Double glass capillaries were used as supporting bodies. By the assistive jigs, the inner glass capillaries were removed from the tubular tissue (Fig. 2). And the tubular tissues were capable of connecting both ends of the outer glass capillaries to the silicon tubes easily. After that, the constructed tubular tissues were installed with the assistive jigs in the chamber. And a closed circuit was assembled, such a perfusion cultivation system was filled with medium.

Figure 3 shows a tube-installed chamber with an inserted tubular tissue. This system was composed of a centrifugal pump, a tube-installed chamber and a reservoir, which were connected to each other with silicon tubes to form a closed circuit. In this chamber, we did perfusion cultivation the tubular tissue.
RESULTS AND DISCUSSION

Figure 4 shows fluorescent Z-stack confocal images of transferred tubular tissue. The tubular tissues was uniformly transferred to the 3D supporting body. It consisted of 2 layers and the thickness of the transferred tubular tissue was observed to be 50 μm. Moreover, by using water transfer printing, we were able to get multilayer structured tubular tissue rapidly. Blood vessels have multilayer structures, so we succeeded in biomimetic vessels to fabricate multilayer structure. Although contraction of approximately 35% was observed from the early stages of cultivation, fabricated tissues were wide enough to allow formation of the tubular tissue structure. However, multi-segmentation requires the reduction of parts for using a cell laden construct which is a time-consuming process. These results indicated that the threshold for accurate assembly by water transfer printing depends on both circularity and segmentation. Figure 5 shows a perfusion experiment with fluorescent beads. Blue color is the tubular tissue, and green color is fluorescent polystyrene beads. In the tubular tissue, medium was transported using the flow by the centrifugal pump (Flow rate; 20 μL/min). Furthermore, we measured the mechanical property of tubular tissue by the artificial circulatory system (Fig. 6).

Figure 4: Fluorescent images of transcribed tubular tissue, which has a hollow 2 layers structure using by water transfer printing. Scale bar is 100 μm.

Figure 5: Images of a perfusion experiment of the tubular tissue (blue) with fluorescent beads (green). The tubular tissue was transporting medium by the centrifugal pump (Flow rate; 20 μL/min).
Figure 6: Images of tubular tissue walls stretch by perfusion and impressed pressure graphs during perfusion. The graph shows max impressed pressure is about 1,000 Pa by pressure sensor and the stretch of tubular tissue walls is about 0.4 μm. Scale bar is 1 mm.

Simulation of the 3D environment in which tissues normally develop and function is crucial for the engineering of in vitro models that can be used for the formation of complex tissues [6, 7]. Developing 3D cell-laden constructs will be essential for creating tissue-engineered organs in vitro with cell volumes similar to those of organs. Arteries have thick walls made up of connective tissue on the outside, smooth muscle in the middle and endothelium on the inside. These structures are there in order to withstand the pressure created by the heart, known as blood pressure. Capillaries are the smallest blood vessels in the body, so that blood can sometimes only move through them in a single file. Capillaries have very thin walls and are purely made up of endothelium, allowing oxygen, nutrients, to diffuse in and out of them. Layer-by-layer technique can readily create normalized tissue structure such as blood vessels [5].

CONCLUSION
Recent trends in tissue engineering have aimed at bionic simulating the physiological environment in vitro. We proposed a new 3D assembly technique to fabricate a hollow tissue construct using water transfer printing. This proposed technique can be used to create customized, macro-scale, 3D tubular tissue and also is applicable to many different cell types similar to structure of blood vessels. This technique would be useful for creating in vitro simulators for drug efficiency evaluation and operative training. More in-depth investigation using this perfusion cultivation system potentially increase our knowledge about complex cell-cell interaction and organization within 3D cell populations in well-defined microarchitectures.

ACKNOWLEDGEMENTS
This research is supported by a Grant-in-Aid from The Ministry of Education, Culture, Sports, Science and Technology for Scientific Research (23106002 and 24106506) and the Nagoya University Global COE program for Education and Research of Micro- and Mechatronics.

REFERENCES

CONTACT
*Y. Yamagishi, tel: +81-52-789-5220; yamagishi.yuka@biorobotics.mech.nagoya-u.ac.jp

2027