CELL FIBERS:
CONSTRUCTION OF CENTIMETER-SCALE 3D TISSUES BY WEAVING
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ABSTRACT
This paper describes “cell fibers” for engineering centimeter-scale 3D heterogeneous artificial tissues. Cells (both cell lines and primary cells) proliferate in our collagen/alginate core-shell hydrogel fibers, and completely fill the core space to form cell fibers. We found that the cell fibers keep their fibrous shapes even after removing the shell alginate hydrogel, and that they still maintain their bioactivities. As a demonstration, we constructed (i) tissue fabric sheets by weaving the cell fibers and (ii) 3D heterogeneous coil-like tissue tubes by reeling up two different cell fibers. These cell fibers would be an extremely effective tool as building elements for constructing large-scale functional tissues.

KEYWORDS: Hydrogel fiber, Cell encapsulation, Woven structure, 3D structure, Tissue Engineering

INTRODUCTION
Building up a 3D tissue from elements (cell-encapsulating hydrogel beads [1], spheroids [2], cell sheet [3]) have been extensively attempted to construct functional tissues such as livers, pancreas and hearts. As one of the building elements, we have been focused on cell-encapsulating hydrogel fibers [4], since many tissues in our human body are fibrous shapes (blood vessels, muscles, spinal cords). Several cell-seeded hydrogel fibers have been reported [5,6], however, handling of the cell-seeded fibers haven’t been achieved because of its fragility.

Here we propose a collagen/alginate core-shell hydrogel microfiber, providing both sufficient mechanical strength for handling and appropriate environments for cell growth (Figure 1). Encapsulated cells in the core collagen can proliferate so that the core space is filled with cells to form “cell fiber”. This cell fiber keeps their fibrous shapes even after we remove the shell alginate hydrogel, since the cells in the fiber are tightly connected to each other. Using these cell fibers, we demonstrate to construct large area 3D tissues by weaving or reeling up the cell fibers.

CELL-ENCAPSULATING COLLAGEN/ALGINATE CORE-SHELL HYDROGEL FIBERS
Cell-encapsulating collagen/alginate core-shell hydrogel fibers were continuously formed in a double coaxial laminar flow microfluidic device that we previously reported [4] (Figure 2(A)). Briefly, the device was composed of pulled glass capillary tubes. We used 0.2% collagen solution with suspended cells (~10^8 cells/ml), 1.5 wt% sodium alginate solution and 100 mM calcium chloride with 3 wt% sucrose solution as core, shell and sheath solution, respectively. Flow rates are 75 µl/min (core), 75 µl/min (shell) and 3.6 ml/min (sheath). At this condition, cells were successfully encapsulated in the collagen core (diameter of the core: ~80 µm), and the alginate shell (diameter of the shell: ~150 µm) protected the collagen core (Figure 2(B) and (C)).

The fabricated core-shell fibers were moved to Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution, and then cultured at 37 °C, water-satulated 5% CO2 environment as well as normal tissue culture. Encapsulated cells (for example, HepG2 cells) proliferated...
and filled the space of the collagen core to form “cell fiber” at 11 days of culture (Figure 3(A-C)). Interestingly, the cell fiber kept their fibrous shape even after removing the alginate shell with enzymatic digestion (Figure 3(D)), indicating that the cells form strong cell-to-cell connections to each other.

We found that various types of cells are applicable to our core-shell hydrogel fibers. As well as HepG2 cells (Figure 4(A)), HeLa (Figure 4(B)) and Min6 (Figure 4(C)) cells formed cell fibers. In addition, we also succeeded forming cortical cell fiber by using primary cells dissected from rat brain (Figure 4(D)). We think that this applicability of primary cells to our fiber is particularly important for practical applications such as transplantation.

**BIOACTIVITIES OF THE CELL FIBERS**

We next confirmed the bioactivities of the grown cell fibers. In the cortical cell fiber, we investigated Ca\(^{2+}\) oscillation of the cortical cells with Fluo4-AM staining (Figure 5(A) and (B)). Many cortical neurons exhibited spontaneous synchronized Ca\(^{2+}\) oscillations over the cortical cell fiber. This result proves that neuronal activities were kept in the cortex cell fibers. We also evaluated lactic acid secretion for HepG2 cell fibers. As shown in Figure 5(C), the HepG2 cell fiber secreted lactic acid as it grows, meaning that the fiber keeps their metabolic activity.

**3D TISSUES BY WEAVINB OR REELONG UP THE FIBERS**

Finally we demonstrated a weaving and reeling the cell fibers to build centimeter-scale patterned 3D tissues. As we reported previously [4], cell fibers can be handled in an aqueous solution by fluid flows generated with narrow silicon tubes (typical inner diameter: 0.5-1 mm). Using this handling technique, HeLa cell fibers were woven in culture medium in the size of ~1 cm × ~2 cm (Figure 6(A) and (B)) and 6-by-5 fabric-like cell structure (Figure 6(C) and (D)). Heterogeneous 3D coil-like tissue structure was constructed by reeling HepG2 and Min6 cell fibers (Figure 6(E)). This coil tissue can grow after removing the alginate shell from the cell fibers: We embedded the coil tissue structure in collagen hydrogel, and then removed the alginate shell with alginate lyase. As shown in Figure 6(F), the coil-like tissue grew and merged the border of two different cell fibers after 14 days of culture. This result indicates that constructed tissues made

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**Figure 3.** (A-C) Growth of the encapsulated HepG2 cells in the core-shell fiber. At 11 days of culture, the core was completely filled with the cells and formed a “cell fiber.” (D) This cell fiber can keep its shape after removing the alginate shell with alginate lyase.

**Figure 4.** Various types of cells proliferated and formed cell fibers. (A-C) Cell fibers of HepG2, Min6 and HeLa cells. All of them are common cell lines. (D) Rat primary cortical cells also grew and formed cell fibers.

**Figure 5.** Bioactivities of the cell fibers. (A)(B) Phase and fluorescent image of the cortical fiber at day 14. Cortical cells were dye with calcium indicator, Fluo4-AM. Synchronized calcium oscillation was successfully observed over the cortical cell fiber. (C) Lactic acid secretion from HepG2 fibers at day 1, 7 and 9. As the HepG2 cells grow, the secreted lactic acid increases.
of our cell fibers maintained their bioactivity.

CONCLUSION

We proposed a cell-encapsulating collagen/alginate hydrogel fibers for constructing 3D patterned centimeter-scale tissues. This proposed hydrogel fibers provide both sufficient mechanical strength by alginate shell and preferable cell growth condition by collagen core. Using this cell fibers, we demonstrated woven centimeter-scale tissue structure and heterogeneous coil-like tissue structure, and proved that the constructed tissues keep their bioactivities. We believe that our cell fiber approach would pave the way for building 3D-patterned functional artificial tissues.

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REFERENCES


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