MICROBIALY-FABRICATED CELLULOSE MICROSTRANDS IN THE CORE OF HYDROGEL FIBERS

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ABSTRACT
This paper describes rapid fabrication of bacterial cellulose (BC) microfibers using cellulose producing bacteria, Acetobacter xylinum. We successfully fabricated BC microfibers (100-200 μm in diameter) consisting of cellulose nanofibrils by sealing and culturing A. xylinum within the core of the hydrogel fiber. The BC microfibers were formed within 24 - 48 hours after the fabrication. This process is remarkably fast considering the conventional method takes 3-7 days to obtain BC scaffolds. The cellulosic biofilm retain its structure even after removal of the shell. We found that the our microfibers have high potential as micro-scaffolds for making “cell tube”.

KEYWORDS: Hydrogel fiber, Bacteria, Bacterial cellulose, Scaffold

INTRODUCTION
Bacterial cellulose is a biodegradable natural polymer of glucose secreted from the cell wall of A. xylinum. The bacteria synthesize cellulose nanofibrils and form complicated cellulose networks as they proliferate. This BC is known to have unique properties such as high biocompatibility, high water retaining capacity, high crystallinity and high mechanic strength [1,2]. By virtue of these properties, BC has already been widely used in foods, audio speakers, for wound-dressings, and for manufacturing high strength papers. Moreover, several researches have been attempting to use BC as a scaffold for constructing 3D cell structures such as blood vessels. However, to the best of our knowledge, the obtained structure is far from tube-like morphology. It is possibly due to their relatively large diameter (0.5-10 mm), surface tension, and surface structure of the BC scaffolds. Here we present a novel method to fabricate BC microstrands which have high potential as micro-scaffold for tissue engineering.

FABRICATION OF BACTERIAL CORE-SHELL FIBERS
The bacteria encapsulating hydrogel fibers were continuously extruded from the outlet of a double coaxial laminar flow device we previously reported [3] (Figure 2A). Using this device, bacterial core-shell microfibers with desired diameters were easily fabricated.

Figure 1: Conceptual illustration of this study. (A) Illustration of a bacterium used in this study. The bacteria Acetobacter xylinum(A. xylinum) secrete cellulose nanofibrils (bacterial cellulose; BC) from the cell wall and eventually they form hydrogels composed of cellulose network. (B) Illustration of a A. xylinum/alginate gel core-shell fiber and a process to construct “cell tube”.

Figure 2: Fabrication of bacterial core-shell fibers. (A) Illustration of the microfluidic device to fabricate hydrogel core-shell fibers. Bacterial core-shell fibers are rapidly fabricated through double coaxial microfluidic device. (B) A phase contrast image of the fabricated fibers. Bacteria are observed as white dots within the core. Scale bar: 200μm. (C) An actual photo of fabricated bacterial core-shell fibers placed in a dish. Scale bar: 3.5 mm.
fabricated at meter-scale length. The device was constituted from three glass tubes which forming core flow (bacterial suspension), shell flow (1.5wt% sodium alginate), and sheath flow (100 mM calcium chloride).

The core of the hydrogel fiber kept its fluidic nature which enables the bacteria to move freely forming a fine cellulose network within it. The bacteria within the core of the hydrogel fibers were cultured simply by putting the microfiber into the culture medium (Figure 3A). The culture medium was suggested to diffuse into the core of the fibers because the proliferation of the bacteria was clearly observed 24 hours after the fabrication (Fig. 3B-E).

**BACTERIAL CELLULOSE MICROSTRANDS**

Then we immersed the cultured fiber into citrate solution to remove the alginate gel. The core structure retained its shape after the removal of the shell (Figure 4 A, B). After the removal of the shell, the fiber was boiled with 1 M NaOH to remove proteins and bacteria from BC. This is the prevailed method to purify BC from the biofilm of *A. xylinum* [4]. The mesh-like structures of the core were observed by microscopy after the treatment (Figure 4 C, D). The obtained microfibers were confirmed as BC by X-ray diffraction patterns (Figure 5) which shows three characteristic peaks for cellulose of BC ($2\theta = 14.5, 16.6,$ and 22.5).

To see the surface morphology of the purified BC microfiber and bulk sample, we observed them using scanning electron microscopy (SEM) (Figure 6 A-D). The bulk sample was made by a common method to
fabricate BC hydrogel described elsewhere [5]. Briefly, a single colony of \textit{A. xylinum} was inoculated into 10 mL of culture medium and cultivated until the BC hydrogel became visible (for 5 days). The SEM images indicated the BC microstrands had high porosity compared to the bulk sample. This micro-porosity rose possibility that this BC microstrands could be a good material for mammalian cell attachment [5].

**CELL SEEDING EXPERIMENT**

Finally, to test if mammalian cells actually adhere to the BC microfiber, 3T3 cells were seeded on the BC microstrands. As a result, cells attach well to the BC fiber 24 hours after the seeding (Figure 7A). To cover BC microstrands with cell, 3T3 cells were seeded for the second time after 24 hours incubation (Figures 7B). 24 hours after the second cell seeding, tube-like structures of cells were partially formed around the BC microstrands (Fig. 7C, D).

**CONCLUSION**

We proposed an efficient method to fabricate BC microstrands. The obtained BC microstrands possessed high porosity and demonstrated to have high affinity with mammalian cells such as 3T3. This BC microstrands could work as a scaffold for constructing blood vessels. We believe this novel BC microfibers has high potential as a scaffold for tissue engineering.

**REFERENCES**


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