MICROFLUIDIC SYNTHESIS OF COMPLEX ALGINATE FIBERS FOR THE DIRECTION CONTROL OF CELL GROWTH

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ABSTRACT

We report herein a simple system for continuously synthesizing complex Ca-alginate fibers using a uniform-depth, co-flowing microchannel. The continuous flow of sodium alginate (NaA) solution is gradually gelled in the microchannel by the diffusion of Ca^{2+} ions from the outer gelation solutions. Introduction of thin buffer layers between the NaA and the gelation solutions could regulate the gelation speed of alginate and prevent the microchannel clogging, and the highly viscous multiphase flows were stabilized with a help of a thickener. We applied the presented system for fabricating complex alginate fibers with a cross section composed of multiple regions, including two or three-component fibers, spiral fibers composed of solid and soft regions, and fibers incorporating oil droplets. In addition, the microscopic solid-soft anisotropy of the hydrogel fibers made from NaA and propyleneglycol alginate (PGA, a non-gelling derivative of alginate) was utilized to control the direction of cell growth, demonstrating a unique application for the formation of linear tissues or cell networks.

KEYWORDS: Alginate Hydrogel, Fiber, Multiphase Flow, Tissue Engineering

INTRODUCTION

Hydrogels are versatile materials for biomedical applications, including scaffolds for tissue engineering, matrix for cells immobilization, and vehicles for controlled delivery and release of drugs. Among various shapes of hydrogel materials, fibers are highly useful because of the easiness in operation/immobilization, and the efficient supply of oxygen and nutrients compared to the bulk-scale hydrogels. Researchers have reported on the synthesis of alginate[1,2] or chitosan[3] hydrogel fibers using either a micro-nozzle or a double capillary. Although these studies demonstrated the synthesis of isotropic fibers with typical diameters of 10~1000 μm, it was impossible to obtain complex hydrogel fibers composed of different components in the cross section. Here we propose a simple microfluidic system for fabricating complex Ca-alginate fibers, which would be useful as a new type of highly-functional hydrogel materials. In this study, we demonstrated the fabrication of complex fibers composed of 2 or 3 regions, and examined the factors affecting the fiber diameter and its uniformity. Also, to demonstrate the applicability of the presented system as unique a material for tissue engineering, we fabricated solid-soft-solid fibers and controlled the direction of cell growth inside them to form linear colonies.

EXPERIMENTAL

The microfluidic system for synthesizing alginate fibers is shown in Fig. 1. Microchannels having 5~7 inlets are used. $CaCl_2$ solutions, buffer solutions, and sodium alginate (NaA) solutions are respectively introduced from the outer, middle, and inner inlet channels. By introducing NaA solutions with different compositions, complex fibers are obtained. Also, introduction of oil phase between the NaA solutions, we could prepare droplet-incorporating fibers. Two critical points exist to stably obtain Ca-alginate fibers; (1) the introduction of thin buffer layers between NaA and $CaCl_2$ solutions, which regulates the rapid gelation speed of Ca-alginate hydrogel, and prevents the hydrogel formation and channel clogging at the confluence, and (2) the use of a thickener (dextran, average Mw of 500,000) to balance the viscous NaA solution and the buffer/gelation solutions.

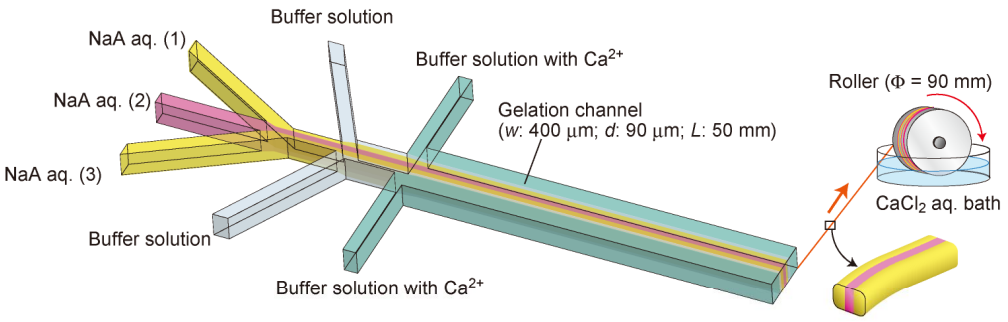

Figure 1: Schematic diagram showing the microfluidic system for synthesizing Ca-alginate hydrogel fibers. In the gelation channel, Ca^{2+} ions diffuse into the central NaA phase, continuously forming the Ca-alginate fibers.

PDMS microdevices were fabricated by using standard soft lithography and replica molding techniques. 10% dextran solutions with and without containing 0.1 M $CaCl_2$ were respectively used as the gelation and buffer solutions. The solutions were continuously introduced into the microchannel by using syringe pumps. The obtained fibers were recovered by using a roller partially dipped in a gelation solution. For cell cultivation, mouse fibroblast (NIH-3T3) cells or human cancer (HeLa) cells were used. These cells were maintained in DMEM supplemented with 10% FBS, and confluent cells were harvested from 100-mm dishes and used for the experiment.

RESULTS AND DISCUSSION

We initially examined if the alginate fibers are actually synthesized, by using 1.8% NaA solutions with different-color particles and a microchannel having 6 inlets. Fig. 2 (a) shows the micrographs of the multiphase flow in the microchannel. The central NaA solution was transformed into gel at a point ~25 mm from the confluence. The obtained fibers were so long, keeping the anisotropy in the cross section (Fig. 2 (b)). Also, the diameter of the obtained fibers could be accurately controlled by changing the flow rate of the NaA solutions; the horizontal width of the fiber was slightly larger than the vertical width, as shown in Fig. 2 (c). When much broader microchannels (both the width and depth of 800 μm), we obtained broader fibers with diameters up to 200 μm. Also, we found that the lower flow rate of the buffer solutions, Q_2, resulted in the higher uniformity of the width of the obtained fibers.

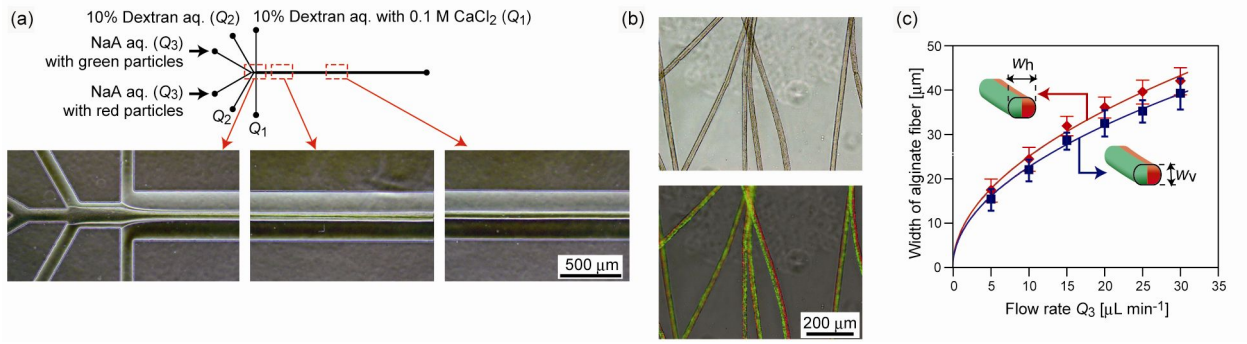

Figure 2: Synthesis of anisotropic Ca-alginate fibers. Q_1 and Q_2 were 100 and 10 μL/min, respectively. (a) Parallel flows (and a gelling fiber) formed in the microchannel at the confluence, and 5 and 25 mm from the confluence, and (b) optical and fluorescence micrographs of the fibers. In (a) and (b), Q_3 was 15 μL/min. (c) Relation between the alginate flow rate Q_3 and the horizontal (w_h) and vertical widths (w_v) of the obtained fibers.

As applications for the highly functional fibrous materials, we fabricated variety of fibers as shown in Figure 3. Fibers composed of three regions (with different color particles) were synthesized using 7-inlet microchannels, with the widths of the each region accurately controlled by changing the inlet flow rates of the NaA solutions (Fig. 3 (a, b)). Then, by utilizing propyleneglycol alginate (PGA; a derivative of alginate that does not form hydrogel), spiral fibers were obtained composed of solid (2% NaA) and soft (1.5% PGA + 0.5% NaA) regions, as shown in Fig. 3 (c). Both the width of the fiber and the radius of the curvature could be controlled by changing the flow-rate ratio of the NaA solutions. In addition, droplet-incorporating fibers were obtained by using 7-inlet microchannels and by introducing an oil phase (olive oil containing 1% lecithin from soy bean) between the NaA phases (with green particles). As shown in Fig. 3 (d), oil-incorporating fibers were successfully obtained. Even when the relative flow rate of the NaA solution was much lower than the flow rate of oil phase, the fiber did not release the trapped droplets, and were stable at least for several month when stored in 0.1 M $CaCl_2$ aq. This unique fibrous material would be useful as storing devices of droplets for the controlled drug elution.

The solid-soft anisotropy of the complex fiber was further utilized as a cell-cultivation platform in a heterogeneous microenvironment. We fabricated solid-soft-solid complex fibers incorporating cells in the soft core, and observed the direction of cell growth. The soft core was made from 0.9% PGA and 0.3% NaA, while the solid shell from 2% NaA (Fig. 4 (a)), and the obtained fibers were coated with poly-L-lysine. In the anisotropic fibers, mouse fibroblast (NIH-3T3) cells, initially located in the core (Fig. 4 (b)), grew along the soft region and formed linear colonies after 6 days of cultivation (Fig. 4 (c)). While in the control (isotropic fibers totally made from 2% NaA), the colony shape was spherical (Fig. 4 (d)). Similar results were obtained when HeLa cells were cultivated (Fig. 4 (e)). The microscopic anisotropy of the hydrogel fibers would therefore be useful as a unique cell cultivation platform, and could be applied for constructing linear tissue models including neural networks or tubular blood vessels.

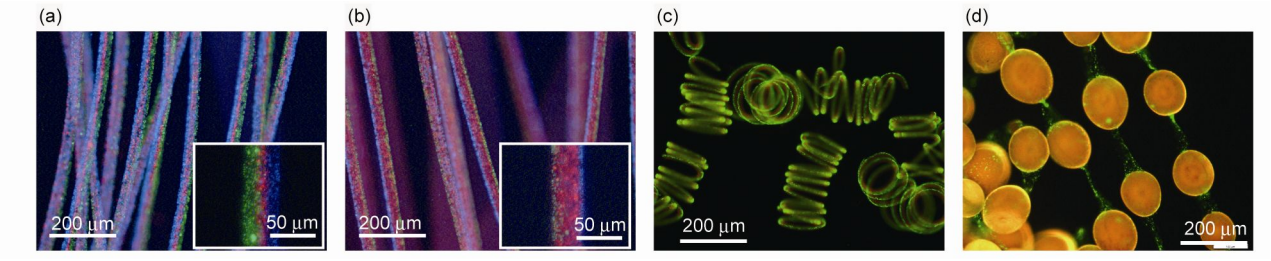

Figure 3: Complex Ca-alginate fibers fabricated by using the presented system. (a, b) Fibers composed of three regions with different-color microparticles, obtained by using a 7-inlet microdevice. The flow rates of the NaA solutions were 20, 10, and 20 μL/min for (a) and 10, 30, and 10 μL/min for (b). (c) Spiral-shape fibers composed of solid (2% NaA; w/ green particles) and soft (0.5% PGA + 1.5% NaA; w/ red particles) regions, obtained by using a 6-inlet microdevice and cut into 1 cm-long fragments. Q_1, Q_2, Q_3, and Q_4 were 100, 10, 10, and 25 μL/min, respectively. (c) Fibers incorporating oil droplets, made from 2% NaA aq. (with green particles) and olive oil containing 1% lecithin and 0.01% nile red, obtained by using a 7-inlet microdevice. Q_1, Q_2, Q_3 (NaA, ×2), and Q_4 (oil, introduced from the center inlet) were 100, 10, 5, and 20 μL/min, respectively.

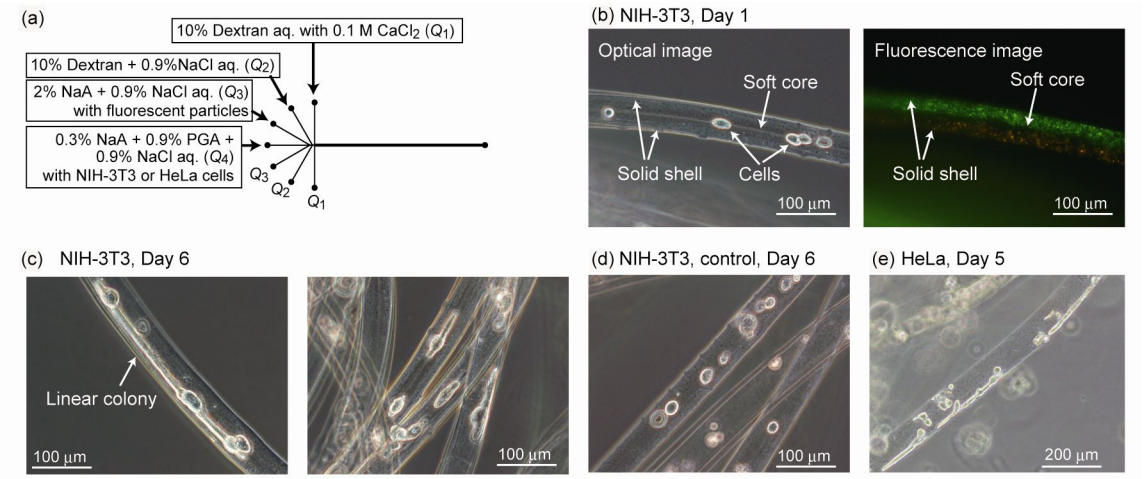

Figure 4: Direction control of cell growth in the complex fiber, in which the soft region (core) is sandwiched by solid regions (shell). (a) Schematic diagram showing the flow rate conditions. Green/red fluorescent particles were suspended in 2% NaA solutions, to observe the positions of the shells and core. (b, c) Optical and fluorescence images of the anisotropic fibers incorporating NIH-3T3 cells, on (b) day 1 and (c) day 6, respectively. (d) NIH-3T3 cells grown in homogeneous fibers on day 6 as control. (e) HeLa cells cultured in complex fibers for 5 days. Q_1, Q_2, Q_3, and Q_4 were 100, 5, 10 (or 20 for (e)), and 10 μL/min, respectively.

CONCLUSIONS

A simple but highly versatile microfluidic system was presented for continuously preparing complex Ca-alginate hydrogel fibers. These functional fibers would be available as new materials for constructing tissue-engineering scaffolds, functional microenvironments for the guided cell growth, and biocompatible vehicles for storing and gradually eluting bioactive compounds.

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