Selective Fabrication of Hollow and Solid Polysaccharide Composite Fibers Using a Microfluidic Device by Controlling Polyion Complex Formation

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Keywords

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Abstract

Natural polysaccharides are an important class of biomaterials that have attracted significant research interest for biomedical applications because of their high biocompatibility, biodegradability and bioactivity. In this work, we fabricated water-insoluble composite hollow and solid fibers made of polyanion complexes of chondroitin sulfate C (CS) and chitosan (CHI) using a single microfluidic device. A coaxial two-phase microfluidic device was constructed from stainless-steel needles and glass fibers, and CHI solutions and CS solutions were continuously infused into the core and sheath channels, respectively. The obtained fibers were flexible and homogeneous and had diameters of a few hundred micrometers. Hollow fibers were formed using water as the solvent of CS, while core-filled solid fibers were obtained using 20% (v/v) ethanol. The respective mechanisms for the fabrication of the hollow and solid fibers were discussed. An increase in the sheath flow rate or decrease in the core flow rate reduced the diameters of the fibers, while a reduction in the concentration of the CHI solution reduced the thickness of the hollow fibers. Furthermore, bovine serum albumin, used as a model protein, could be incorporated in the hollow and solid fibers by mixing them in the core flow solution. These results suggested the great potential of microfluidic techniques for the preparation of hollow and solid polysaccharide fibers.

Introduction

Fibers, which are two-dimensional bendable materials, have been widely used in the biomedical field. Core-filled solid fibers have been used as not only absorbable surgical sutures but also as component units of cell scaffolds for the construction of more complex objects, such as textile fabrics and three-dimensional structures. For example, three-dimensional scaffolds for cartilage tissue regeneration were fabricated from fibers of bioabsorbable polymers¹, and functional woven tissues were assembled from cell-laden biopolymer fibers.² On the other hand, hollow fibers have been used for regeneration of the peripheral nerve gap,³ construction of artificial blood vessels,⁴ and bioreactors for large-scale cell culture.⁵

Natural polysaccharides are an important class of biomaterials that have attracted significant research interest because of their high biocompatibility, biodegradability and bioactivity.⁶ Fibers have been fabricated from single or multiple polysaccharides. Fibers made of single polysaccharides, such as chitin⁷ and chitosan (CHI)^{8,9}, have been prepared by wet spinning. Fibers and nonwoven fabrics of chitin and CHI have also been prepared by electrospinning.^{10,11} For the fabrication of composite fibers made of multiple polysaccharides, the use of polyion complexes (PICs) of anionic and cationic polysaccharides is a promising approach because it makes water-soluble polysaccharides water-insoluble without the use of cross-linking agents or chemical modification. Composite fibers made of PICs of polysaccharides can be prepared by the interface spinning method¹²⁻¹⁴ and the spray spinning method¹⁵ from anionic polysaccharides, such as gellan gum,¹² gellan sulfate,¹³

chondroitin sulfate C (CS),¹⁴ alginic acid (ALG),¹⁵ and cationic CHI. In the interface spinning method, fibers are spun by withdrawing the films made of their PICs formed at the interfaces between the solutions of anionic and cationic polysaccharides. Although fibers can be obtained without an expensive apparatus, it is difficult to achieve continuous spinning and control the fiber diameter. In addition, hollow fibers cannot be obtained using conventional interfacial spinning methods.

Over the last decade, microfluidic techniques have gained popularity in the biomedical field¹⁶ and have been applied for the continuous fabrication of fibers made of biomacromolecules.^{17–22} In microfluidic spinning, coaxial flows stabilize laminar flows and solidification starts at the interfaces between the core flow and the sheath flow when the respective flows contain molecules and/or ions that react with each other, in some cases with the help of other stimuli, to form insoluble products. Continuous solidification by flowing resulted in the formation of fibers. If solidification occurs only at the very close regions of the interface between the core and sheath flows, hollow fibers are produced. On the other hand, when solidification proceeds into the center of the coaxial flow, core-filled fibers are obtained. Solidification processes have been mainly based on cross-linking, such as enzymatic cross-linking,¹⁸ photopolymerization¹⁹ and the formation of electrolyte complexes, such as ALG and $\text{Ca}^{2+20-22}$ and sodium triphosphate pentabasic and CHI.²³ However, to the best of our knowledge, the fabrication of fibers based on the formation of PICs between anionic and cationic polysaccharides has not been reported. The utilization of PIC formation for continuous fiber fabrication by a microfluidic device is technically challenging because PIC formation must be controlled to prevent clogging.

Here, we first demonstrated that both hollow and solid water-insoluble fibers made of PICs of CS and CHI were successfully prepared using the same microfluidic device. The effects of preparation conditions, such as solvent, concentration and flow rate, on the formability, morphology, and dimensions of the fibers were investigated. Furthermore, the loading of proteins in the fibers was examined using bovine serum albumin (BSA) as a model protein.

Materials and Methods

Reagents

CS (sodium salt, from shark cartilage, molecular weight (MW) *ca.* 20,000), CHI (from crab shell, MW $\geq$ 100,000), fluorescein isothiocyanate (FITC) and other chemicals were purchased from Nacalai Tesque, Inc. BSA, rhodamine B isothiocyanate (RITC) and fluoresceinamine (FA) were obtained from Sigma Co., Ltd. CHI was used after purification (Supplementary Information, method 1), and the other reagents were used without further purification. The distilled water and ultrapure water (18.2 M Ω cm) used in experiments were prepared using Advantec RFD210TA and Advantec RFU414BA, respectively.

Construction of microfluidic device

Set-up, dimensions, and macroscopic and microscopic views of the microfluidic device are shown in Figure 1. A stainless-steel 28G needle (inner diameter: 150 μ m, outer diameter: 350 μ m, length: 28 mm, Kyowa Interface Science, Co. Ltd.) was temporarily fixed inside a capillary tube (inner diameter: 800 μ m, outer diameter: 1300 μ m, length: 55 mm, Sansyo, Co. Ltd.) using a brass tube (inner diameter: 400

μm , outer diameter: $800\ \mu\text{m}$), and the root of the capillary tube was coated with silicon grease to make a cavity to connect with the flow paths. These were set in a mold ($20\ \text{mm} \times 75\ \text{mm} \times 5\ \text{mm}$) and filled with epoxy resin (Nissin resin, Co. Ltd.). After removing the brass tube, flow paths from both sides of the device to the cavity of the root of glass capillary at the angle of 45° were generated using a drill. A stainless-steel 18G needle (Terumo Corp.) was inserted in the path and fixed with epoxy resin.

Preparation of hollow and solid fibers

To prepare the fibers, 2 or 4 wt% CHI solutions in 2 wt% aqueous acetic acid and 4 wt% CS solutions in ultrapure water or 20% (v/v) ethanol were continuously infused into the core and sheath channels, respectively, at the indicated flow rate using syringe pumps (KDS Legato 110, KD Scientific Inc.). The fabricated fibers were injected into a sheath solution in a glass beaker. For fluorescence microscopic observation, fluorescein-labeled CS (FL-CS, Supplementary Information, method 2 and Figure S1(a)) and rhodamine B-labeled CHI (Rho-CHI, Supplementary Information, method 3 and Figure S1(b)) were blended in the sheath and core solutions at 1/100 (w/w) of both polysaccharides, and the fibers were fabricated in the same manner.

Preparation of protein-incorporated hollow and solid fibers

The indicated amount of BSA was dissolved in 2 wt% aqueous acetic acid with CHI. Hollow and solid fibers were then prepared using the same procedure. For fluorescence microscopic observation, FITC-labeled BSA (FITC-BSA,

Supplementary Information, method 4 and Figure S1(c)) was incorporated in BSA at 1/10 (w/w), and the fibers were fabricated in the same manner.

Characterization

The morphologies of the obtained fibers were observed using a fluorescence microscope (ECLIPSE LV100, Nikon Corp.) combined with a precentered fiber illuminator (Intensilight C-HGFI, Nikon Corp.) and NIS-Elements software (Nikon Corp.). For observation of FL-, FITC-, and Rho-labeled molecules, filters (B-3A (excitation filter (EX): 420–490 nm, dichroic mirror (DM): 505 nm, barrier filter (BA): 520 nm) and FITC filter block (EX: 430–480 nm, DM: 505 nm, BA: 535–545 nm) for FL- and FITC-labeled molecules and G-2A (EX: 510–560 nm, DM: 575 nm, BA: 590 nm) for Rho-labeled molecules) were used. The fibers were observed in the sheath solution unless noted otherwise. Plots of the fluorescence profiles were obtained using ImageJ software.²⁴ The diameter and thickness of the fibers were calculated from the cross-sectional images of the fibers obtained using NIS-Elements software. The diameter of the fiber was estimated from the geometric mean of the sides of the bounding box of the fibers, and the fiber thickness was estimated from the arithmetic mean of the thickness of three points on the walls. The diameters and thicknesses of at least three independent fibers were measured, and the results are shown as the mean $\pm$ standard deviation. For scanning electron microscopy (SEM) observations, fibers prepared using ultrapure water as a solvent of CS were rinsed with ultrapure water and freeze-dried after pre-freezing with liquid nitrogen. Fibers prepared using 20% ethanol as a solvent of CS were dehydrated in acetone for 3 h. Cross-sections of the fibers were observed by SEM (JSM-7001F, JEOL Ltd.) with an

acceleration voltage of 20 kV after coating with Pd–Pt using an ion sputter instrument (MC1000, Hitachi Ltd.). Cross-sections that formed during sample handling processes were found in the specimens and observed.

Stability of hollow fibers in solutions

Hollow fibers prepared at a core flow rate of 50 $\mu\text{l min}^{-1}$ and sheath flow rate of 1000 $\mu\text{l min}^{-1}$ were immersed in ultrapure water, phosphate buffer (PB, 10 mM phosphate ion species, pH 5.8, 7.4, 8.0), phosphate buffered saline (PBS, 10 mM phosphate ion species and 137 mM NaCl, pH 7.4), and 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (62.5 mM, pH 6.5) for 24 h and were observed in the solutions under optical microscopy.

Evaluation of release behaviors of FITC-BSA from fibers

FITC-BSA-loaded fibers obtained by spinning for 2 min were immersed in 5 ml of ultrapure water and PBS. The samples were then incubated at 36.5 °C using a dry chamber (DOV-750A, AS ONE). The released amounts of FITC-BSA were evaluated using a UV-vis spectrophotometer (V-660, JASCO Corp.). The absorbance of FITC-BSA at 500 nm was used to calculate the released amounts.

Results

Preparation of CS/CHI composite hollow and solid fibers

A microfluidic device in which the CHI and CS solutions co-axially flow and PICs of CS and CHI can be formed at the interface was designed for the fabrication of hollow and solid polysaccharide composite fibers (Figure 1a). The microfluidic

device with the dimensions shown in Figure 1b was constructed from a stainless-steel needle and a glass capillary (Figures 1c and 1d). First, the conditions under which a stable laminar flow was formed were investigated using 2 wt% CHI solutions in 2 wt% aqueous acetic acid stained with methylene blue (MB) as the core flow and ultrapure water as the sheath flow (Supplementary Information, method 5 and Figure S2). When the ratio of the face velocities of the core and the sheath flows (core/sheath) was large (*ca.* 5 or more), the core flow was unstable. By contrast, when the ratio was small (*ca.* 0.5 or less), the flow was narrowed. Under the conditions in which the ratio was nearly equal to 1, stable laminar flow was observed. Then, 4 wt% CHI solutions in 2 wt% aqueous acetic acid and 4 wt% CS solutions were continuously infused into the core and the sheath channels at 50 $\mu\text{l min}^{-1}$ (face velocity: 2800 mm min^{-1}) and 1000 $\mu\text{l min}^{-1}$ (face velocity: 2500 mm min^{-1}), respectively. Soft fibers with smooth surfaces were obtained by using ultrapure water (Figure 2a) and 20% ethanol (Figure 2b) as the solvent of the sheath solution. To elucidate the distribution of CS and CHI in the fibers, the fibers were prepared by blending FL-CS and Rho-CHI and then observed by fluorescence microscopy. Cross-sectional fluorescence images of the fibers obtained using ultrapure water as a solvent of the sheath solution indicated that the fibers had a hollow structure (Figures 2c and 2e). The fibers were composed of both CS and CHI, and these polysaccharides were distributed homogeneously in the wall of the hollow fiber. The mean diameter of the hollow fibers was $168 \pm 20 \mu\text{m}$ and the thickness of the wall was $15.9 \pm 4.8 \mu\text{m}$. By contrast, the fibers obtained by using 20% ethanol as the solvent of the sheath solution showed a solid structure (Figures 2d and 2f). The fibers were also composed of CS and CHI, and these were distributed homogeneously in the fibers. The diameter

of the solid fiber was $236 \pm 18 \mu\text{m}$. SEM observation of freeze-dried or dehydrated fibers showed hollow and solid structures of the obtained fibers (Figure 3).

The FT-IR spectra of CS/CHI fibers prepared by using ultrapure water (Figure 4c) and 20% ethanol (Figure 4d) showed various peaks originating from CS (Figure 4a) and CHI (Figure 4b), such as the C=O stretching vibration (*ca.* 1650 cm^{-1} , amide I),^{25,26} N-H stretching vibration (*ca.* 1575 cm^{-1} , amide II),^{25,26} C-H symmetric and asymmetric stretching vibrations (*ca.* 2900 cm^{-1})^{25,26} and OH stretching vibration (*ca.* 3400 cm^{-1} , broad)^{25,26} in both CS and CHI, and SO_3^- asymmetric stretching vibration (*ca.* 1220 cm^{-1})²⁶ in CS. In addition to these peaks, a new peak appeared at 1530 cm^{-1} in the both CS/CHI hollow and solid fibers (denoted by an asterisk (*)) in Figures 4c and 4d), which supported ion complex formation between the NH_3^+ and COO^- groups²⁷.

Stability of CS/CHI hollow fibers

Stability of the CS/CHI hollow fibers in various kinds of solvents was investigated. Although morphologies of the fibers did not change after 24-h immersion in PB with pH 5.8 (Figure 5a), 7.4 (Figure 5b) and 8.0 (Figure 5c), PBS (pH 7.4, Figure 5d), and MES buffer (pH 6.5, Figure 5e), the fibers became thicker and partly ruptured after immersion in ultrapure water (Figure 5f).

The effect of the flow rate on morphology, diameter and thickness of CS/CHI hollow fibers

The effects of the flow rates of the core and sheath flows on the morphology and dimensions of hollow fibers were investigated with 20% ethanol used as the sheath

flow solvent. First, the core flow rate was fixed at $50 \mu\text{l min}^{-1}$, while the sheath flow rate was changed. When the sheath flow rate was $500 \mu\text{l min}^{-1}$, the surface of the fibers showed a foam-like structure (Figure 6a). For the flow rates in the $750\text{--}2000 \mu\text{l min}^{-1}$ range, fibers with smooth surfaces were obtained (Figures 6b–d), and their diameters and thicknesses were decreased by increasing the sheath flow rate (Figures 7a and 7c). Then, the sheath flow rate was fixed at $1000 \mu\text{l min}^{-1}$, and the core flow rate was changed. Fibers prepared at a core flow rate of $10 \mu\text{l min}^{-1}$ showed smooth surface, and their diameters and thicknesses were smaller than those obtained at a core flow rate of $50 \mu\text{l min}^{-1}$ (Figure 6e). By contrast, fibers with a hairy surface were obtained at a core flow rate of $100 \mu\text{l min}^{-1}$ (Figure 6f). The fiber diameter and thickness appeared to be constant for the flow rate greater than $50 \mu\text{l min}^{-1}$ (Figures 7b and 7d). Fibers could be fabricated from 2 wt% CHI solutions in 2 wt% acetic acid. Although there was no significant difference in morphology and its diameter, their walls were thinner than those obtained from 4 wt% of CHI (Figure 8).

Incorporation of proteins in the CS/CHI composite fibers

Polysaccharide composite hollow and solid fibers were also formed when BSA was dissolved in 2 wt% aqueous acetic acid at 0.4 wt% with CHI (Figures 9a and 9e). By blending FITC-labeled BSA (FITC-BSA) with BSA, distributions of BSA in the hollow and solid fibers were observed under a fluorescence microscope (Figures 9b and 9f). In the hollow fibers, BSA was loaded in internal aqueous phases of the hollow fibers in addition to their walls (Figures 9b–d and 9d'). In the solid fibers, BSA was distributed homogeneously in the fiber (Figure 9f). The effects of protein loading on the diameter of the fibers were then investigated. Loading of BSA did not

affect the diameter of the hollow fibers (Figure 9g), whereas that of the solid fiber was decreased upon increasing the concentration of BSA (Figure 9h).

Release behaviors of FITC-BSA from composite fibers

Figure 10a shows the release behaviors of FITC-BSA from the FITC-BSA-incorporated hollow fibers (Figure 10b) in ultrapure water and PBS. In ultrapure water, FITC-BSA molecules were released from the fibers within an hour, whereas the release time reached 4 h in PBS. The equilibrium released amounts were almost the same. After 100 h, the fluorescence from FITC-BSA in the fibers was still observed for both cases (Figures 10c and 10d).

Discussion

In the present study, we have continuously fabricated fibers made of PICs of natural polysaccharides, CS and CHI, by utilizing microfluidic techniques. Model experiments using the CHI solution as the core solution and pure water as the sheath solution showed that stable laminar flows were formed when the differences in the face velocity between the core flow and the sheath flow were small (Figure S2). Under optimal flow conditions, it was also confirmed that stable laminar flow was formed at both the point where the two flows merged (Figure S3a) and the terminus of the device (Figure S3b). As expected, hollow fibers were formed by using ultrapure water as a solvent of the sheath solution (Figures 2c and 2e). It was assumed that water-insoluble PICs were rapidly formed at the interface between the core and the sheath flows and blocked further mass transport across the PIC layers, resulting in the formation of hollow fibers (the details will be discussed below). By

contrast, core-filled, solid fibers were formed by using 20% ethanol as the solvent of the sheath solution (Figures 2d and 2f). It has been reported that formation of PIC through electrostatic interaction was weakened in organic solvent such as ethanol.²⁸ Therefore, CS could diffuse into the more inner region of the core flow in the presence of ethanol, resulting in the formation of homogeneous solid fibers. Preparation of hollow fibers using microfluidics sometimes requires the use of a special device such as the device that generates double-coaxial laminar flows.^{2,22} On the other hand, the present results clearly showed that both hollow and solid fibers made of PICs were successfully prepared on demand using the same microfluidic device. SEM images (Figure 3) indicated that both hollow and solid fibers swelled in solutions when prepared and could be handled while maintaining their morphologies in the dry state. FT-IR spectra indicated that both hollow and solid fibers had similar chemical composition and were made of PICs of CS and CHI (Figure 4).

Here, formation of the fibers driven by PIC formation is compared to that driven by electrolyte complex formation of polyelectrolytes and metal ions. In previous reports on ALG- Ca^{2+} fibers, both hollow and solid fibers could be fabricated.²⁰⁻²² Coaxial flow using a core flow of ALG and a sheath flow of Ca^{2+} produced solid fibers²⁰ whereas adverse flow condition (core: Ca^{2+} , sheath: ALG) produced hollow fibers.²¹ The use of three-layered coaxial fluids (central: Ca^{2+} , inner: ALG, outer: Ca^{2+}) also made the fibers hollow.²² This may also be due to high diffusivity of Ca^{2+} . Ca^{2+} can diffuse fast and ALG are solidified, and therefore the obtained fiber reflected the shape of ALG flow. For fiber formation by PICs, once PIC walls were formed at the interface, polysaccharides with high MW could not diffuse across the PIC walls, resulting in formation of hollow fibers.

The obtained CS/CHI composite hollow fibers showed high stability in a variety of biological solutions except ultrapure water (Figure 5). This is important for the use of these fibers as biomaterials. Instability of the fibers in ultrapure water may be attributed to rupture after the uptake of abundant water.

An increase of the flow rate of the sheath CS solution or a decrease of the flow rate of the core CHI solution reduced the diameters and thicknesses of hollow fibers (Figures 6 and 7). The decrease in the diameter with increasing sheath flow rate and decreasing core flow rate have been reported for other fibers prepared with microfluidics,^{18,21,29,30} and it can be explained that the increasing difference in the face velocities of the core and the sheath flows induced a larger drag force and stretched the core solution. If the difference in the face velocities was too large, the laminar flow was destabilized and transformed to turbulent flow (Figure S2). Instability of the flow resulted in the formation of fibers with foam-like or hairy surfaces (Figures 6a and 6f). Furthermore, the thickness of the hollow fibers depended on the concentration of the CHI solutions (Figure 8b). These results suggested that the diameter and thickness of hollow fibers can be controlled by changing the flow conditions within the limits of a certain range.

Polysaccharide composite hollow and solid fibers containing proteins were also prepared by adding the proteins into the core flow of the CHI solutions. The proteins were loaded in PICs by electrostatic interaction and hydrogen bonding, and the system was widely used for protein delivery and their controlled release.³¹ We have demonstrated that BSA were loaded in PIC complex gels of CS and CHI by mixing BSA in CHI solutions (unpublished data). Since BSA with isoelectric point of 4.7³² were positively charged in 2 wt% acetic acid solutions, complexes of BSA and CHI

were not formed in the CHI solutions. During fiber formation, BSA interacted with CS or became negatively charged in the neutral solution and interacted with CHI. Alternatively, BSA was simply trapped in the PIC networks prior to diffusing out of the networks because of their relatively rapid formation. Morphological observations showed that incorporation of BSA into the fibers did not change their surface. In the hollow fibers, FITC-BSA was incorporated not only in the walls but also in the internal aqueous phases (Figures 9b–d). This may be because unloaded BSA molecules were trapped in the internal aqueous phases of the hollow fibers. These results also supported the fast formation of PICs at the interface between the core and sheath flows. By contrast, FITC-BSA molecules were distributed homogeneously in the solid fibers (Figure 9f). The mean diameter of the solid fibers was decreased by increasing the loaded concentration of BSA (Figure 9h), whereas that of the hollow fibers was not changed (Figure 9g). Incorporation of BSA can affect the electrostatic interaction between CS and CHI, which may induce a decrease in fiber diameter.

Regarding the release behaviors of FITC-BSA loaded in hollow fibers, release in ultrapure water was faster than that in PBS whereas their equilibrium release amounts were almost the same. Fast release in ultrapure water appeared to be due to a burst release of FITC-BSA from the internal aqueous **phase** of the hollow fibers and correlated with the difference in the stability of the fibers in ultrapure water and PBS. The fact that FITC-BSA remained in the fibers after 100 h immersion in PBS and ultrapure water (Figures 10c and 10d) indicated that the FITC-BSA molecules located inside the wall of fibers may strongly interact with the PICs. Continuous, long-time release of proteins may be achieved if we can gradually weaken the interactions.

Although compounds with low MW, such as MB, could also be loaded in the

CS/CHI hollow fibers, these were released to the coagulation solution immediately after spinning (Supplementary Information, method 6 and Figure S4). These results suggested that release from the fibers were correlated with the interaction between the model drugs and polysaccharides and the diffusivity of model drugs in the polysaccharides PICs. In fact, we previously demonstrated that MB loaded in CS/CHI composite films prepared by hot-pressing PIC gels were readily released from the films within one hour,³³ in contrast to FITC-BSA released continuously for up to 2 weeks. One possible solution for the loading of small MW compounds in the present fibers is the use of nanosized reservoirs. For example, coumarin 6 was successfully incorporated in the hollow fibers by loading coumarin 6 in polylactic acid nanoparticles that added in the core flows (Figure S5). Since polylactic acid is biodegradable, such a system can be expected to realize a sustained release of small MW drugs. The ability of CS/CHI fibers to stably hold proteins and nanomaterials allows the functionalization of the fibers with bioactive molecules such as growth factors, cell adhesion proteins and drugs. These molecules should be released according to their desired profiles based on the knowledge of the design and fabrication of various drug delivery systems.

Conclusion

Although polysaccharides and their PICs are promising materials for biomedical applications, their fabrication processes are limited. In this study, we demonstrated that distinct hollow and solid fibers made of PICs of CS and CHI could be fabricated using the same microfluidic device by merely changing the sheath flow solvent. The diameters of the fibers were also controllable depending on the flow rate.

Furthermore, proteins could be incorporated in the hollow and solid fibers by mixing them in the core flow solutions. CS-based materials have been used for regeneration of wide range of tissues, cartilage,³⁴ bone,³⁵ skin,³⁶ and nerve;³⁷ therefore, CS/CHI composite fibers are potent materials for use in tissue engineering. We are currently conducting cell culture experiments using the present fibers. PICs of polysaccharides have been prepared from not only CS but also other glycosaminoglycans (GAGs) such as hyaluronic acid and heparin with CHI.³⁸ This means that hollow and solid fibers with various biological functions could also be fabricated from other GAGs using microfluidic techniques.

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Supplementary information is available at Polymer Journal's website

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Figures

Figure 1.

Figure 2.

Figure 3.

Figure 4.

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Figure 8.

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Figure 10.

Titles and Legends to Figure

Figure 1. The microfluidic device used in this study. (a) Schematic illustration of the microfluidic device and the preparation of fibers, (b) sectioned illustration of the flow exit, and (c, d) macroscopic (c) and magnified (d) views of the microfluidic device.

Figure 2. Microscopic images of CS/CHI composite fibers. Optical images (a, b) and fluorescent images ((c, d): FL-CS, (e, f): Rho-CHI) of CS/CHI fibers prepared using water (a, c, e) and 20% (v/v) ethanol (b, d, f) as a solvent of sheath solution. Flow conditions are as follows. Core: $50 \mu\text{l min}^{-1}$, sheath: $1000 \mu\text{l min}^{-1}$. Scale bars: $500 \mu\text{m}$ (a, b) and $200 \mu\text{m}$ (c–f).

Figure 3. SEM images of the cross-section of CS/CHI fibers prepared using water (a, b) and 20% (v/v) ethanol (c, d) as a solvent of the sheath solution. (b) and (d) are magnified images of (a) and (c), respectively. Scale bars: $50 \mu\text{m}$ (a, c) and $10 \mu\text{m}$ (b, d).

Figure 4. FT-IR spectra (left) with zoom in the region of $1800\text{--}400 \text{ cm}^{-1}$ (right) of CS (a), CHI (b), and CS/CHI fibers prepared using water (c) and 20% (v/v) ethanol (d) as a solvent of the sheath solution. Dashed lines show that the position of the peak originated from NH_3^+ , which is also denoted by an asterisk (*) in (c) and (d).

Figure 5. Stability of CS/CHI hollow fibers in various kinds of solvents. Microscopic

images of CS/CHI composite fibers after 24-h immersion in phosphate buffer of pH 5.8 (a), 7.4 (b) and 8.0 (c), PBS (pH 7.4, d), MES buffer (pH 6.5, e) and ultrapure water (f). Scale bars: 500 μm .

Figure 6. Microscopic images of CS/CHI composite hollow fibers prepared under various flow conditions. (a) core: 50 $\mu\text{l min}^{-1}$ (face velocity: 2800 mm min^{-1}), sheath: 500 $\mu\text{l min}^{-1}$ (face velocity: 1200 mm min^{-1}), (b) core: 50 $\mu\text{l min}^{-1}$, sheath: 750 $\mu\text{l min}^{-1}$ (face velocity: 1800 mm min^{-1}), (c) core: 50 $\mu\text{l min}^{-1}$, sheath: 1000 $\mu\text{l min}^{-1}$ (face velocity: 2500 mm min^{-1}), (d) core: 50 $\mu\text{l min}^{-1}$, sheath: 2000 $\mu\text{l min}^{-1}$ (face velocity: 4900 mm min^{-1}), (e) core: 10 $\mu\text{l min}^{-1}$ (face velocity: 570 mm min^{-1}), sheath: 1000 $\mu\text{l min}^{-1}$, (f) core: 100 $\mu\text{l min}^{-1}$ (face velocity: 5700 mm min^{-1}), sheath: 1000 $\mu\text{l min}^{-1}$. Scale bars: 500 μm .

Figure 7. Effects of the sheath flow rate (a, c) and core flow rate (b, d) on the diameter (a, b) and thickness (c, d) of the composite hollow fibers. The core flow rate in (a) and (c) was 50 $\mu\text{l min}^{-1}$, and the sheath flow rate in (b) and (d) was 1000 $\mu\text{l min}^{-1}$, respectively. Error bars indicate the standard deviation (S.D.) ($n>3$). *significant ($p < 0.05$, Student's t-test).

Figure 8. Effects of concentration of CHI used as the core flow on the diameter (a) and thickness (b) of the composite hollow fibers. The core flow rate was fixed at 50 $\mu\text{l min}^{-1}$, and the sheath flow rates were 1000 and 2000 $\mu\text{l min}^{-1}$. Error bars indicate the standard deviation (S.D.) ($n>3$). N.S.: not significant using the Student's t-test.

Figure 9. FITC-BSA-loaded CS/CHI composite fibers. (a–f) Microscopic images of CS/CHI composite fibers prepared using water (a–d) and 20% (v/v) ethanol (e, f) as a solvent of the sheath solution. (a–d) Optical images (a), and fluorescent images of FITC-BSA (b), Rho-CHI (c), and the overlay image (d). Plots of fluorescence profiles in (d) are shown in (d'). (e, f) Optical images (e), and fluorescent images of FITC-BSA (f). (g, h) Effect of BSA concentration on diameter of the composite fibers prepared using water (g) and 20% (v/v) (h) as the solvent of the sheath solution. Scale bars: 200 μm . Error bars indicate the standard deviation (S.D.) ($n>3$). *significant ($p < 0.05$, Student's t-test). N.S.: not significant using the Student's t-test.

Figure 10. Release behaviors of FITC-BSA from the FITC-BSA-incorporated hollow fibers in PBS and ultrapure water. (a) Released amount of FITC-BSA from hollow fibers, (b–d) fluorescence microscopy images of FITC-BSA-incorporated hollow fibers before (b) and after immersion in PBS (c) and water (d) for 100 h. Scale bars: 500 μm .