Polysaccharide Nanogel-Hybrid Nanofibers by Electrospinning

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ABSTRACT SUMMARY
We proposed hybrid nanofibers consisting of polysaccharide nanogel and gelatin using electrospinning method for novel DDS. The diameters of nanofibers can be controlled from 200 nm to 1 \mu m by changing processing parameters, molecular weight of gelatin and concentration of crosslinker. Fluorescent microscopy showed that a good distribution of the nanogels within the nanofibers. Nanogel/gelatin blend nanofibers have potential applications as novel biomedical materials.

INTRODUCTION
We have developed a new physically cross-linked nanogels produced by self-assembly of hydrophobized polysaccharides. For example, cholesteryl-bearing pullulan (CHP) forms monodisperse and stable nanogels (with diameter 20–30 nm) in dilute aqueous solution. The CHP nanogels complex various hydrophobic drugs and proteins via hydrophobic interactions, and encapsulated substances can be released in its native form. Recently, a bottom-up method for producing such nanogels has been developed using polymerizable nanogels as building blocks. In particular, acryloyl-group-modified CHP nanogels with thiol-group-modified PEG (PEGSH) form nanogel-cross-linked hydrogels. The linkages between the nanogels and PEG are degradable under physiological condition, therefore, the nanogels are gradually released from a cross-linked hydrogel. The hydrogels were found to be useful scaffold for bone regeneration.

Nanofibers have been widely used as functional biomedicines including tissue engineering, sensors, filters, textile, cosmetics, and drug delivery. In particular, electrospinning is a simple method to generate nanofibers using various polymers and composite materials. Advantages of nanofibers including large surface area and high porosity can provide useful scaffolds to mimic the native extracellular matrix (ECM) of tissues. Here we report the preparation of hybrid nanofibers consisting of polysaccharide nanogels and gelatin for novel DDS.

EXPERIMENTAL METHODS
Acryloyl group-bearing CHP (CHPOA) was synthesized as previously reported. Gelatin solutions were prepared in PBS (×10)/ethanol at a ratio of 55/45 with concentrations of 10%. CHPOA/gelatin solutions were prepared by adding CHPOA into gelatin solutions. To obtain water-stable nanofiber, CHPOA/gelatin solutions were prepared in the presence of the chemical cross-linker 1-ethyl-3-(3-dimethyl-aminopropyl)-1-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). The mixtures were stirred for at least 3 h to obtain homogeneous solutions prior to electrospinning.

The resulting solution was loaded into a 1 ml plastic syringe attached to a 23-gauge blunt needle. Electrospinning was done at a voltage of 9-13 kV, a distance from the needle tip to the aluminum cup collector of 10-12 cm, and a solution flow rate of 0.2-1.0 mL/h. The morphologies of the nanofibers were observed by a scanning electron microscope (SEM; S-800, HITACHI Ltd., Chiyoda, Tokyo, Japan) at an accelerating voltage of 10 kV, after sputter coating with Au. In order to test water-stability, CHPOA/gelatin fibers were soaked in water for 24 h. The fiber diameters were determined by using an Image J (National Institute of Health, USA). To

Figure 1. Schematic illustration of polysaccharide nanogel.
confirm the distribution of CHPOA nanogels in the nanofibers, rhodamine modified CHPOA (CHPOA-Rh) nanogels were used to prepare electrospun nanofibers under the same condition. The images of CHPOA-Rh/gelatin nanofibers were obtained from fluorescent microscope (Axiovert 200, Carl Zeiss MicroImaging, Inc., Thornwood, NY).

RESULTS AND DISCUSSION
Electrospun fiber formation is highly dependent on the polymer solution properties including concentration, viscosity, surface tension, and conductivity. Initially, CHPOA nanogel-cross-linked aqueous solution was selected. Under this condition, several droplets were obtained as shown in Figure 2. This results indicate that electrospray occurs due to the low viscosity and high surface tensions of the nanogel solution.

Figure 2. SEM image of nanogel-cross-linked electrospun nanofibers (solvent: mixture of water and DMF at a ratio of 1/1).

Then, we combined with gelatin in order to obtain nanofibers. Gelatin is commonly used biopolymer, since it is a biocompatible and biodegradable in physiological conditions. As CHPOA nanogels and gelatin were mixed, the beads morphology disappeared and obtained smooth nanofibers with the diameter from 200 nm to 1 µm (Figure 3). The fiber diameter could be controlled by changing processing parameters, molecular weight of gelatin and concentration of crosslinker. The fiber morphology was maintained after 1 day soaked in water at room temperature, indicating water-stability of the nanogel/gelatin hybrid nanofibers.

Figure 3. SEM images of nanogel/gelatin fibers; a) before soaked in water, b) after soaked in water at room temperature for 1 day. Scale bar = 10 µm.

Furthermore, nanogels were distributed within the fibers observed by fluorescent microscopy.

Figure 4. Fluorescent microscopy image of nanogel/gelatin nanofibers.

CONCLUSION
Polysaccharide nanogel/gelatin hybrid nanofibers were prepared by electrospinning. Nanogels can be complexed with various proteins, therefore, this system have great potential for biomedical applications. Further studies for tissue culture scaffold are ongoing.

REFERENCES