Biomacromolecules **2006,** *7,* 3291−3294

Notes

Chitosan Nanofiber

Kousaku Ohkawa,* Ken-Ichi Minato, Go Kumagai, Shinya Hayashi, and Hiroyuki Yamamoto

Institute of High Polymer Research, Faculty of Textile Science and Technology, Shinshu University, Tokida 3-15-1, Ueda 386-8567, Japan

Received May 6, 2006
Revised Manuscript Received September 1, 2006

Introduction

Nanofiber technology forms an important object for recent material research studies. An elegant method for nanofiber production is now well-known as “electrospinning”,[1,2] which allows fabricating a fine and dense meshwork of given polymer fibers directly from its solution in the presence of an electric field.[1] The history of electrospinning, its rediscovery and popularization, and theoretical aspects associated with the electrospinning phenomenon have been described, especially by a pioneering research group,[3−6] and several review articles are available for current trends in nanofiber technologies.[1,7,8]

Among the possible industrial expectations of the electrospun nonwoven fabrics, here, two of the particular applications of nanofibers are considered highly potent for natural polysaccharides; one is to use the nanofiber network space as a novel separation material,[9] and the other is as biomedical scaffolding materials for tissue cultures.[7] The biomedical approaches have been described for the numerous combinations of the nanofiber materials and the culturing cells. The relationships between the nanofiber morphologies and the cell culturing properties have not yet been systematically clarified. Only the successful aspects of the cell growth properties are focused, along with the nanofabric preparation of a specified biocompatible polymer. As for the separation technology, the diameters and the mechanical strength of the electrospun nonwoven fabrics are critical factors for their material properties. Recently, a cellulose nanofiber membrane for affinity separation of the biological molecules has been reported.[10] Natural polysaccharides were of the promising classes of polymeric materials for both the biomedical and separation technologies. The stable production of nanofibrous materials from natural polysaccharides can be recognized as a breakthrough technique for inspiring a new frontier based on the present nanofiber material research.

Chitosan, a natural cationic polysaccharide, is still an interesting polymer based on its physicochemical properties, including its solid-state structure and the chain conformations in the dissolved state.[11] In the molecular structure of chitosan, the hydroxyl and amino groups were regularly arranged at the equatorial positions in the β(1,4)-linked D-glucosamine repeating units.[12] The hydroxyl groups contribute by forming relatively rigid crystallites (forms I and II) in the solid state.[13] In the solution state, hydrogen bonding between chitosan molecules drives the formation of microfibrils, depending on the concentrations.[14] These characteristics of chitosan will inspire the methodology for successful electrospinning of this material and will further form an interplay between basic polymer chemistry and advanced nanomaterial science.

Recently, interpretations of the epectrospinning phenomena have been more advanced from the rheological aspects, and the chain configurations of given specific polymer molecules in the prespun solutions are being related to the postspun fiber morphology. Long et al. have developed an empirical equation for poly(ethylene terephthalate-*co*-ethylene isophthatate), fiber diameter D (μm) $= 0.18(C/C_e)^{2.6}$, allowing a prediction of the electrospun fiber diameters, based on the normalized concentration, C/C_e, where C_e is the entanglement concentration.[15] Later, they found that hydrogen bonding between polymer side chains in a specific solution state rendered the fibers thicker than those predicted from the equation.[16] They also reported the effect of the intermolecular association state of a polyelectrolyte leads to production of a thicker electrospun fiber, and the addition of neutral salt reduced the electrostatic interaction between the polyelectrolyte molecules, resulting in the decreased critical concentration for the electrospinning procedure.[17]

Wnek et al. demonstrated that the entanglement and the weight-average molecular weights determine the average fiber diameter.[18] These reports clearly indicated that the intermolecular interaction in the prespun solution is one of the crucial factors for the successful production of the homogeneous fiber network via electrospinning. On the other hand, Rutledge et al. experimentally found that solution elasticity, especially the Deborah number, unequivocally determines the morphology of the electrospun fibers of poly(ethylene oxide)/poly(ethylene glycol), i.e., the “beads-on-fiber” or the homogeneous fiber.[19]

On the basis of the solution properties of chitosan, we have already succeeded in preparing nonwoven fabrics of chitosan.[20] In our previous study, a commercial chitosan sample, chitosan10 (Wako Pure Chemical Ind., Osaka, Japan), was employed. The viscosity average molecular weight of the chitosan10 was determined as 21×10^4, and the degree of acetylation was 0.77.[19] For the electrospinning of the chitosan10, we examined the destruction of the rigid crystalline structure that allows the chitosan molecules to be electrospun more easily. The tested solvents were the diluted hydrochloric acid, acetic acid, neat formic acid, and trifluoroacetic acid. Among the tested solvents, only trifluoroacetic acid (TFA) produced a fibrous material on the collector. The addition of dichloromethane to the chitosan/TFA solution improved the homogenity of the electrospun chitosan fibers. The electrospun chitosan fibers having the mean diameter of 330 nm were successfully prepared at 8 wt % in TFA:dichloromethane = 70:30.[20]

The average diameter of the electrospun chitosan10 fiber in our previous study was, however, in sub-micrometer size rather than being a nanofiber, which is frequently defined as having a diameter equal to or less than 100 nm.[8] Here, the final goal of the present study is to prepare ultrathin fibers, especially in the nanoscale range, from a natural polysaccharide, chitosan. To achieve this, we focused on the solution viscosity as a critical parameter for the electrospinning nanofiber production.[21,22]

---

* To whom correspondence should be addressed. Telephone: +81-268-215573. Fax, +81-268-215571. E-mail: kohkawa@giptc.shinshu-u.ac.jp.

10.1021/bm0604395 CCC: $33.50

Experimental Section

Electrospinning. Commercial chitosan samples having different average molecular weights, chitosan10, chitosan100, chitosan500, and chitosan1000, were purchased from Wako Pure Chemical Ind. (Osaka, Japan) and used for the experiments as received. The chitosan samples were dissolved in TFA at room temperature and then subjected to electrospinning experiments under the apparatus conditions, in which the tip-to-collector distance was 150 mm and the applied voltage was 15 kV. A copper plate wrapped with aluminum foil was used as the collector, which was connected to the ground. No pumping pressure was applied to the chitosan solution placed in the syringe tip.

Viscosity Measurements. A Ubbelohde viscometer was employed for the determinations of viscosity average molecular weights[23] of the chitosan samples. The viscosity measurements of the prespun chitosan solutions were performed using a rheometer (DV III, Brookfield Co., USA) equipped with a spindle no. 14 at 300 rpm at 25 °C.

Characterization of Nanofibers. The morphologies of the electrospun deposition were observed using a scanning electron microscope (SEM; Hitachi SN-2380N, Japan). The average fiber diameters and their distributions were measured with the SEM photographs. For detailed observations of the electrospun nanofiber surfaces, the field-emission-SEM images were taken using a Hitaichi S5000 (Japan) apparatus. The sample surfaces were coated with platinum−palladium (thickness, approximately 3.0 nm)

Results and Discussion

First, the effects of the solution viscosity on the electrospinnability were examined, and the resulting morphologies of the electrospun deposition were observed to distinguish the spinnabilities of the following three classes: (i) coexistence of beads and fibers, (ii) homogeneous fiber (very few or no beads), and (iii) failure of the spinning jet. TFA was employed as the spinning solvent throughout the present experiments. Four kinds of commercial chitosan samples were tested, and they were chitosan10 (viscosity average molecular weight, $M_v = 21 \times 10^4$), chitosan100 ($M_v = 131 \times 10^4$), chitosan500 ($M_v = 158 \times 10^4$), and chitosan1000 ($M_v = 180 \times 10^4$). The chitosan samples were dissolved at room temperature and then subjected to electrospinning experiments.

Figure 1 represents the relationship between the chitosan concentration in weight percent (wt %) and the solution viscosity in centipoise. The viscosities of all four chitosan samples exponentially increased with the concentration. In Figure 1, three kinds of areas were indicated, according to the resulting morphologies of the electrospun depositions: area A (approximately 200−800 cP), fibers and coexisting beads; area B (approximately 800−1000 cP), deposition of homogeneous fiber network; area C (>1500 cP), failure of the spinning jet due to a high viscosity. In all the chitosan samples, homogeneous fiber networks were formed in the viscosity range of 800−1000 cP. The amino groups of chitosan form TFA salts in solution, and under this condition, the crystalline structure of chitosan was completely destroyed.[24] Assuming that the conductivity and surface tension values are almost constant throughout the chitosan solutions tested in area B, viscosity is the critical parameter for the successful preparation of the homogeneous fiber network.

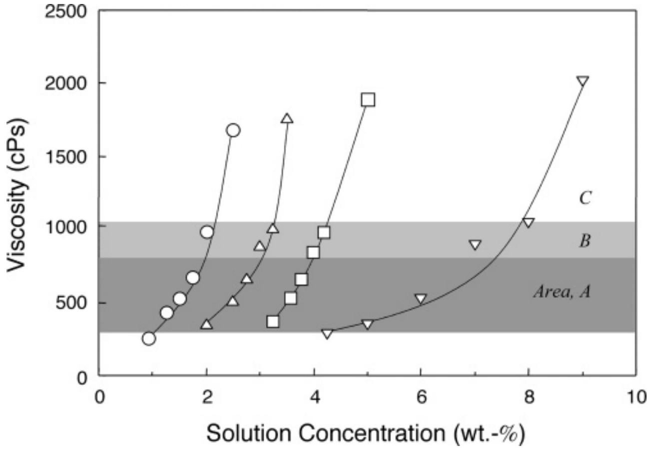

Figure 1. Relationship between concentrations and viscosity values of the chitosan/TFA solutions: circles, chitosan1000; triangles, chiotsan500; squires, chitosan100; inverted triangles, chitosan10; area A, coexistence of fibers and beads; area B, deposition of homogeneous fiber network; area C, failure of the spinning jet due to high viscosity.

Second, the diameters of the electrospun chitosan fibers were measured on the basis of the SEM photographs, and the average diameters were represented with respect to the solution concentration of chitosan. Figure 2 represents the morphologies of the electrospun chitosan fibers. Relatively homogeneous fiber networks were obtained under the optimized conditions for chitosan10 (Figure 2a), chitosan100 (Figure 2b), chitosan500 (Figure 2c), and chitosan1000 (Figure 2d) at 8.0, 4.25, 3.25, and 2.0 wt %, respectively. Under these solution concentrations, the average diameters of the electrospun fibers were 200 ± 24 (chitosan10), 103 ± 16 (chitosan100), 83 ± 11 (chiotsan500), and 60 ± 22 nm (chiotsan1000). Figure 3 shows the field-emission-SEM images for the detailed observation of the morphology of the single chitosan fiber. The chitosan500 nanofiber was found to be frequently interconnected by fusing the attaching points of two individual fibers, while the chitosan100 nanofiber possesses a more homogeneous network with less frequency of the interconnections.

Third, the relationship between the fiber diameter and the solution concentration was examined (Figure 4). As the solution concentration decreased, the average fiber diameter linearly decreased for all of the tested chitosan samples. Apparently, the higher molecular weight chitosan produces the thinner fibers at the lower concentrations. The ranges of the diameter distributions become narrow for the higher molecular weight samples. These results indicate that the higher molecular weight chitosans were successfully fabricated into thinner and homogeneous fibers via the electrospinning process.

^{13}C NMR spectra of chitosan in TFA−CD_3COOD were identical to that in CD_3COOD−H_2O, suggesting that the dissolving states of chitosans in both solvents were similar.[24] This fact further implies that the chain interactions between chitosan molecules are retained in TFA. One of a recent rheological study on chitosan aqueous solution has been reported as $C_e = 5.0$ g/L in 0.5 M AcOH for $M_v = 110 \times 10^{-4}$ (degree of deacetylation, 0.93), and the addition of the NaCl increased the C_e to 7.5 g/L.[25] This indicates the chain interaction of chitosan was reduced in the presence of the neutral salt.

The C_e value of the present chitosan samples in TFA has not yet been determined. Provided that the C_e value in TFA is $C_e = 5.0$ g/L [25] and that the semidilute entangled exponent is 1.5 for a cationic polyelectrolyte,[17] the relationship between the fiber diameter and the normalized concentration could be discussed for the results of chitosan100, since the M_v of chitosan100 is the nearest to that reported in ref 25. As indicated in Figure 1, the homogeneous electrospun fibers were obtained at $C = 4$

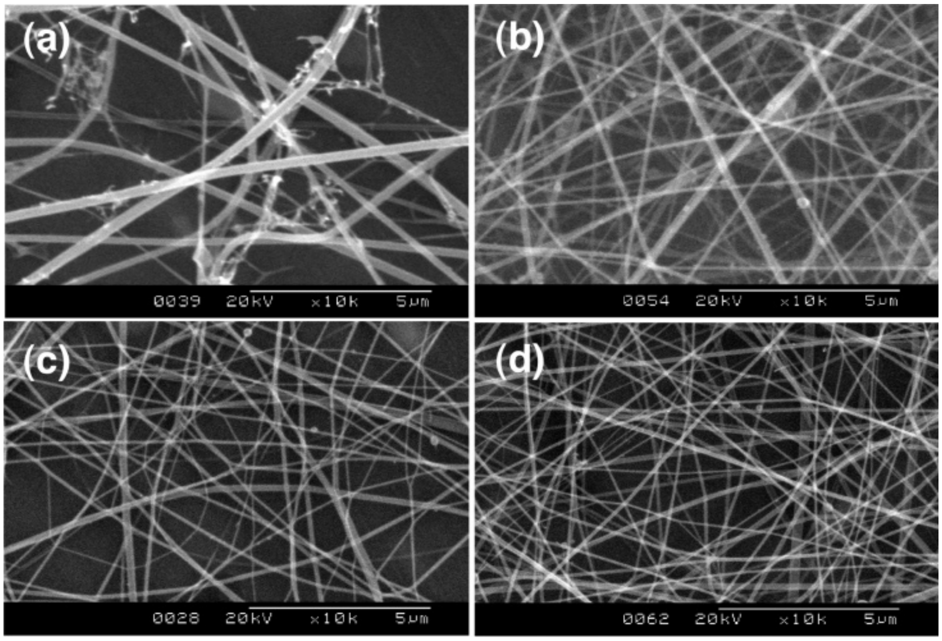

Figure 2. SEM phtographs of the electrospun chitosan fibers: (a) chitosan10 at 8.0 wt %; (b) chiotsan100 at 4.25 wt %; (c) chitosan500 at 3.25 wt %; (d) chitosan1000 at 2.0 wt %. Scale bars: 5 μm (magnification, × 10 000).

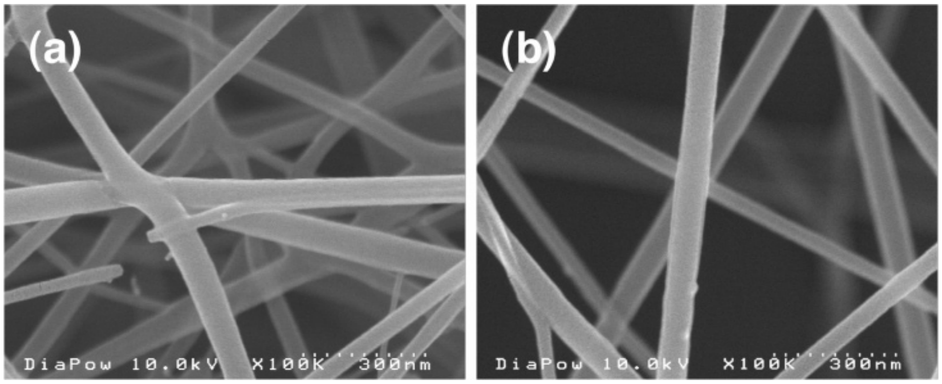

Figure 3. Structural details of the electrospun chitosan nanofibers: (a) chitosan500 at 3.25 wt %; (b) chitosan1000 at 2.0 wt %. Scale bars: 300 nm (magnification, ×100 000). Samples were spattered with platinum−palladium (thickness, approximately 3 nm).

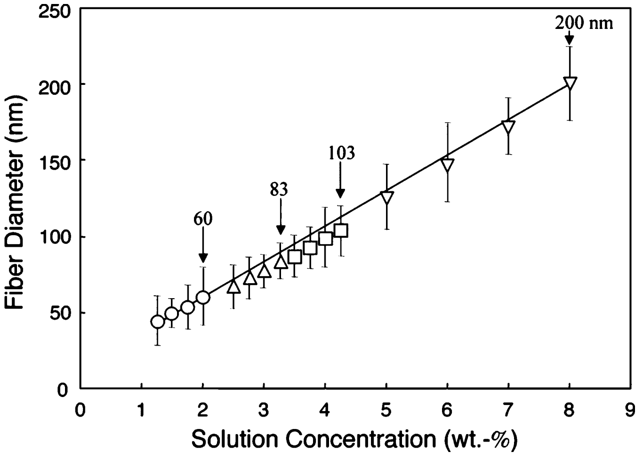

Figure 4. Relationship between the concentrations of the prespun chitosan/TFA solutions and the mean diameters of the electrospun chitosan fibers: circles, chitosan1000; triangles, chiotsan500; squires, chitosan100; inverted triangles, chitosan10. Numbers with arrows indicate the mean fiber diameter at the optimized spinning condition, giving the relatively homogeneous fiber networks.

and 4.25 wt %. These concentrations were higher than C_e. According to Long et al.,[15] by combining the exponent of the semidilute entangled region and an empirical equation by Baumgarten,[21] the following relation can be drawn: D (nm) = $8.05(C/C_e)^{1.2}$. Although further rheological data are required, agreements toward this equation could be obtained by fitting the C_e values, as 3.7 g/L for chitosan1000, 4.5 g/L for chitosan500, and 5.4 g/L for chitosan10. The validity of the present equation will be confirmed and discussed during our ongoing study.

There have been several studies on the electrospinning of chitosan, including pure chitosan[20,26] and blended systems.[27−29] As for the pure chitosan system, two direct spinning methods from chitosan solutions were reported; one is our TFA solvent system,[20] and the other is the concentrated acetic acid solvent system.[26] Jang et al. described that among the three kinds of chitosan samples, $M_w \times 10^{-4} = 3.0$, 10.6, and 39.8, only the second sample can be electrospun to give beads-free thin fibers with pump-feeding of the chitosan/aqueous 90% acetic acid solution.[26] On the other hand, here we succeeded in the electrospinning of chitosan having a broad range of molecular weights ($M_v \times 10^{-4} = 21-180$) without the pump-feeding, indicating a significant advantage of TFA as the practical spinning solvent.

As for the electrospinning system of the blended chitosan, Li and Hsieh have achieved the preparation of an ultrathin chitosan ($M_w \times 10^{-4} = 160$)/poly(vinyl alcohol) blend fiber having an average diameter of 20 nm, which is the thinnest chitosan fiber ever reported.[28] Their results clearly supported that, in the electrospinning of chitosan from aqueous acid solution, the introduction of a component suppressing the strong intermolecular interaction of the chitosan chain is required for the successful preparation of chitosan nanofibers, as we described earlier using poly(vinyl alcohol).[20] In this sense, TFA in our present electrospinning system is not only a volatile solvent but also an agent destroying the crystalline structure of chiotsan.

Conclusion

We first described the universal preparative method of the chitosan nanofiber via electrospinning. The stable production of nanoscaled homogeneous fiber networks was achieved using chitosan100, chitosan500, and chitosan1000. The linear correlation between the fiber diameter and the solution concentration was expected to be a useful direction for the nanofiber production from polysaccharides.

Application of the pure chitosan nanofibers in medical technology has been recently reported as a guided bone regeneration material[30] and as a scaffold for regenerating nerve tissue.[31] Material performance of chitosan nanofibers in the medical fields is very promising. Our technology for effective production of the chitosan nanofiber will contribute to the advancement of this field.

Acknowledgment. This work was supported by Grants-in-aid for the 21st Century COE Program by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References and Notes

(1) Doshi, J.; Reneker, D. H. Electrospinning Process and Applications of Electrospun Fibers. *J. Electrostat.* **1995**, *35* (2 and 3), 151−160.

(2) Fong, H.; Reneker, D. H. Electrospinning and the Formation of Nanofibers. In *Structure Formation in Polymeric Fibers*; Salem, D. R., Ed.; Hanser Gardner: Munich, Germany, 2001; pp 225−246.

(3) Koombhongse, S.; Liu, W.; Reneker, D. H. Flat Polymer Ribbons and Other Shapes by Electrospinning. *J. Polym. Sci., Polym. Phys. Ed.* **2001**, *39* (21), 2598−2606.

(4) Reneker, D. H.; Chun, I. Nanometer Diameter Fibers of Polymer, Produced by Electrospinning. *Nanotechnology* **1996**, *7* (3), 216−223.

(5) Yarin, A. L.; Koombhongse, S.; Reneker, D. H. Taylor Cone and Jetting from Liquid Droplets in Electrospinning of Nanofibers. *J. Appl. Phys.* **2001**, *90* (9), 4836−4846.

(6) Yarin, A. L.; Koombhongse, S.; Reneker, D. H. Bending Instability in Electrospinning of Nanofibers. *J. Appl. Phys*. **2001**, *89* (5), 3018−3026.

(7) Huang, Z.-M.; Zhang, Y. Z.; Kotaki, M.; Ramakrishna, S. A Review on Polymer Nanofibers by Electrospinning and Their Applications in Nanocomposites. *Compos. Sci. Technol.* **2003**, *63* (15), 2223−2253.

(8) Lyons, J.; Ko, F. K. Nanofibers. *Encycl. Nanosci. Nanotechnol.* **2004**, *6*, 727−738.

(9) Chase, G. G.; Reneker, D. H. Nanofibers in Filter Media. *Fluid/Part. Sep. J.* **2004**, *16* (2), 105−117.

(10) Ma, Z.; Kotaki, M.; Ramakrishna, S. Electrospun Cellulose Nanofiber as Affinity Membrane. *J. Membr. Sci*. **2005**, *265* (1−2), 115−123.

(11) Muzzarelli, R. A. A. *Chitin*; Pergamon: Oxford, U.K., 1977.

(12) Sakurai, K.; Shibano, T.; Kimura, K.; Takahashi, T. Crystal Structure of Chitosan. II. Molecular Packing in Unit Cell of Crystal. *Sen'i Gakkaishi* **1985**, *41* (9), T361−T368.

(13) Samuels, R. J. Solid State Characterization of the Structure of Chitosan Films. *J. Polym. Sci., Polym. Phys. Ed.* **1981**, *19* (7), 1081−105.

(14) Titoba, E.; Belavtseva, E.; Gamzazade, A.; Sklyar, A.; Pavlova, S.; Rogozhin, S. Study of Structure Formation of Chitosan in Solutions by Transmission Electron Microscopy. *Acta Polym.* **1986**, *37*, 121−124.

(15) McKee, M. G.; Wilkes, G. L.; Colby, R. H.; Long, T. E., Correlations of Solution Rheology with Electrospun Fiber Formation of Linear and Branched Polyesters. *Macromolecules* **2004**, *37*, 1760−1767.

(16) McKee, M. G.; Elkins, C. L.; Long, T. E. Influence of Self-Complementary Hydrogen Bonding on Solution Rheology/Electrospinning Relationships. *Polymer* **2004**, *45* (26), 8705−8715.

(17) Mckee, M. G.; Hunley, M. T.; Layman, J. M.; Long, T. E. Solution Rheological Behavior and Electrospinning of Cationic Polyelectrolytes. *Macromolecules* **2006**, *39*, 575−583.

(18) Shenoy, S. L.; Bates, W. D.; Frisch, H. L.; Wnek, G. E. Role of Chain Entanglements on Fiber Formation during Electrospinning of Polymer Solutions: Good Solvent, Non-Specific Polymer−Polymer Interaction Limit. *Polymer* **2005**, *46* (10), 3372−3384.

(19) Yu, J. H.; Fridrikh, S. V.; Rutledge, G. C. The Role of Elasticity in the Formation of Electrospun Fibers. *Polymer* **2006**, *47* (13), 4789−4797.

(20) Ohkawa, K.; Cha, D.; Kim, H.; Nishida, A.; Yamamoto, H. Electrospinning of Chitosan. *Macromol. Rapid Commun*. **2004**, *25* (18), 1600−1605.

(21) Baumgarten, P. K. Electrostatic Spinning of Acrylic Microfibers. *J. Colloid Interface Sci.* **1971**, *36* (1), 71−79.

(22) Fridrikh, S. V.; Yu, J. H.; Brenner, M. P.; Rutledge, G. C. Controlling the Fiber Diameter during Electrospinning. *Phys. Rev. Lett.* **2003**, *90* (14), 144502/1−144502/4.

(23) Roberts, G. A. F.; Domsy, J. G. Determination of the Viscometric Constants for Chitosan. *Int. J. Biol. Macromol*. **1982**, *4*, 374−377.

(24) Hasegawa, M.; Isogai, A.; Onabe, F.; Usuda, M. Dissolving States of Cellulose and Chitosan in Trifluoroacetic Acid. *J. Appl. Polym. Sci.* **1992**, *45* (10), 1857−1863.

(25) Cho, J.; Heuzey, M.-C.; Bégin, A.; Carreau, P. J. Viscoelastic Properties of Chitosan Solutions: Effect of Concentration and Ionic Strength. *J. Food Eng.* **2006**, *74* (4), 500−515.

(26) Geng, X.; Kwon, O.-H.; Jang, J. Electrospinning of Chitosan Dissolved in Concentrated Acetic Acid Solution. *Biomaterials* **2005**, *26* (27), 5427−5432.

(27) Bhattarai, N.; Edmondson, D.; Veiseh, O.; Matsen, F. A.; Zhang, M. Electrospun Chitosan-Based Nanofibers and Their Cellular Compatibility. *Biomaterials* **2005**, *26* (31), 6176−6184.

(28) Li, L.; Hsieh, Y.-L., Chitosan Bicomponent Nanofibers and Nanoporous Fibers. *Carbohydr. Res.* **2006**, *341* (3), 374−381.

(29) Min, B.-M.; Lee, S. W.; Lim, J. N.; You, Y.; Lee, T. S.; Kang, P. H.; Park, W. H. Chitin and Chitosan Nanofibers: Electrospinning of Chitin and Deacetylation of Chitin Nanofibers. *Polymer* **2004**, *45* (21), 7137−7142.

(30) Shin, S. Y.; Park, H. N.; Kim, H. Y.; Lee, M. H.; Choi, Y. S.; Park, Y. J.; Lee, Y. M.; Ku, Y.; Rhyu, I. C.; Han, S. B.; Lee, S. J.; Chung, C. P. Biological Evaluation of Chitosan Nanofiber Membrane for Guided Bone Regeneration, *J. Periodontol.* **2005**, *76* (10), 1778−1784.

(31) Itoh, S.; Wang, W.; Matsuda, A.; Shinomiya, K.; Hata, Y.; Tanaka, J. Efficacy of Chitosan Nanofiber Mesh as a Scaffold for Regenerating Nerve Tissue. *Kichin, Kitosan, Kenkyu* **2006**, *12* (2), 194−195.

BM0604395