Preparation of Biopolymer Fibers by Electrospinning from Room Temperature Ionic Liquids

Gunaranjan Viswanathan,[†] Saravanababu Murugesan,[‡] Victor Pushparaj,[†] Omkaram Nalamasu,[†] Pulickel M. Ajayan,[†] and Robert J. Linhardt*[,§]

Department of Material Science and Engineering, Department of Chemical and Biological Engineering, Department of Biology, and Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, 110 8th Street, Troy, New York 12180, and Pharmaceutical Research Institute, Albany College of Pharmacy, Albany, New York 12208

Received November 2, 2005; Revised Manuscript Received January 1, 2006

Electrospinning is a versatile process used to prepare micro- and nano- sized fibers from various polymers dissolved in volatile solvents. In this report, cellulose and cellulose−heparin composite fibers are prepared from nonvolatile room temperature ionic liquid (RTIL) solvents by electrospinning. RTILs are extracted from the biopolymer fiber after the fiber formation using a cosolvent. Micron to nanometer sized, branched fibers were obtained from 10% (w/w) concentration of polysaccharide biopolymer in RTIL solution with an applied voltage of 15−20 kV. Cellulose−heparin composite fibers showed anticoagulant activity, demonstrating that the bioactivity of heparin remained unaffected even on exposure to a high voltage involved in electrospinning.

Introduction

Electrospinning is a widely used simple technique to prepare micron- to nanometer-sized fibers of various polymers.[1] Electrospun fibers find applications in the making of fiber-reinforced composites, membranes, biosensors, electronic and optical devices, and as enzyme and catalytic supports.[2] The electrospinning technique is useful even in large-scale manufacturing environments such as textile industries.[3] A variety of novel tissue engineering scaffolds have been prepared by electrospinning various synthetic and natural biodegradable polymers.[4] However, the range of the polymers that can be electrospun is still limited by the availability of volatile solvents and their limited capability of dissolving polymers of different types. In this report, we conceive of making electrospun fibers from a relatively novel solvent system: room temperature ionic liquids (RTILs). RTILs have become more important in a wide array of chemical processes owing to their capability of dissolving both polar and nonpolar compounds.[5] Other desirable properties of RTILs include low or zero vapor pressure, low melting point, large liquidus range, high thermal stability, large electrochemical window, and recyclability.[6] Further, the properties of an RTIL can be modified by adjusting the structures of its anion or cation or both, and hence, RTILs are also called designer solvents. RTILs have proven to be a promising solvent system for the reactions involving biopolymers such as enzymes[7] and carbohydrates.[8−10] The successful application of RTILs in electrospinning could increase the number and types of materials from which the fibers can be made.

Electrospinning can be considered as a derivative of the electrospray process, as both use high voltage to form a liquid jet. In the electrospinning process, a polymer solution is held by its surface tension at the end of a capillary. When a sufficiently large electric field is applied, the solution at the tip of the capillary elongates to form a cone because of coupled effects of the electrostatic repulsion within the charged droplet and attraction to a grounded electrode of opposite polarity. As the strength of the electric field is increased, the charge overcomes the surface tension, and a fine jet is ejected from the apex of the cone. Fibers were initially thought to be formed by the splitting of a primary jet into multiple filaments, a process known as "splaying",[11] but recent studies[12] have shown that diameter reduction occurs because of the whipping action of a single jet as it nears the target. This whipping instability, caused by small lateral fluctuations in the centerline of the jet as it travels toward the target, causes high-frequency bending and stretching of the jet, leading to the formation of micron- and nanometer-sized fibers. Typically, electrospinning involves the evaporation of the solvent component of the viscoelastic liquid, resulting in fiber formation. In this report, we have demonstrated that it is possible to electrospin cellulose and cellulose−heparin composite fibers from nonvolatile room temperature ionic liquids (RTILs). Cellulose and heparin polysaccharides were selected as a model system also with potential applications as biomaterials. Since RTILs are low-melting salts having very low vapor pressure, it is impossible to evaporate them. Instead, in this report, the RTIL is removed from cellulose and heparin by dissolution in ethanol cosolvent.

Cellulose, a linear polysaccharide composed of β-(1 → 4)-linked glucose, is known for its excellent biocompatibility and thermal and mechanical properties.[13] The insolubility of cellulose in most conventional organic and aqueous solvents is attributed to its very high crystallinity supported by an extensive hydrogen bonding network. Cellulose fibers have been made by electrospinning from a variety of solvents such as acetone, acetic acid, and dimethylacetamide.[14] The RTIL, 1-butyl-3-methylimidazolium chloride ([bmIm][Cl]) (Figure 1) was reported to dissolve up to 25% (w/w) unmodified cellulose with the aid of microwave irradiation.[15] Heparin is a linear, polydisperse, anionic polysaccharide that plays a vital role in regulating many

* Email: linhar@rpi.edu. Phone: (518) 276−3404. Fax: (518) 276−3405.

[†] Department of Material Science and Engineering, Rensselaer Polytechnic Institute.

[‡] Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute.

[§] Department of Chemical and Biological Engineering, Department of Biology, and Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, and Pharmaceutical Research Institute.

10.1021/bm050837s CCC: $33.50
Published on Web 01/26/2006

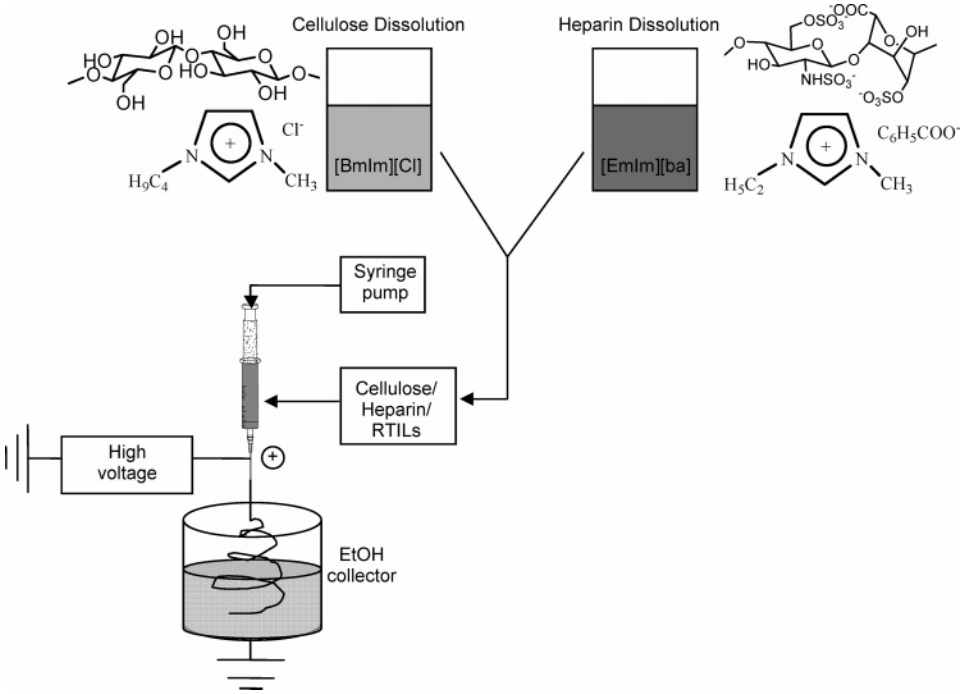

Figure 1. Schematic representation of electrospinning from RTIL solutions.

biological activities.[16] Heparin, the most widely used anticoagulant, has also been extensively investigated to prepare various blood-contacting polymer devices with good blood compatibility.[17−19] Heparin is soluble only in a few organic solvents including dimethylformamide, dimethyl sulfoxide, and formamide. We have recently reported that the RTIL 1-ethyl-3-methylimidazolium benzoate ([emIm][ba]) dissolves up to 2% (w/w) of the imidazolium salt of heparin.[10]

Experimental Section

Preparation of RTIL Solution. Imidazolium salt of heparin was prepared from the pharmaceutical sodium salt form (an extract from porcine intestinal mucosa, average molecular weight (MW_{avg}) = 12 500) by ion exchange chromatography (Dowex cationic (H^+) resin) followed by neutralization with imidazole. Approximately 7 mg of imidazolium heparin was added to ~400 mg of [emIm][ba] and mixed by vortexing and heated to 35 °C for about 20 min to afford a clear, yellow solution. Using the protocol of Swatloski et al., cellulose (MW_{avg} = 5 800 000) was dissolved in the RTIL−[bmIm][Cl].[15] Briefly, a 10% (w/w) cellulose solution was prepared by heating 1 g of [bmIm][Cl] to 70 °C, addition of 100 mg of cellulose, vortex mixing, and microwave irradiation for 4−5 s to afford a clear yellow solution. Both the RTIL solutions (10% (w/w) cellulose in [bmIm][Cl] and 2% (w/w) heparin in [emIm][ba]) were combined and mixed using a vortex for 2 min to afford a clear cellulose-heparin solution.

Electrospinning Method. Both the 10% (w/w) cellulose in [bmIm][Cl] and the cellulose-heparin solution (prepared above) were subjected to electrospinning (Figure 1). A 1 mL sample of polysaccharide RTIL solution was transferred to a syringe attached to a syringe pump, and a voltage of 15−20 kV was applied to the needle of the syringe, with a grounded charge, in the form of an aluminum sheet placed beneath the ethanol collector. The nozzle-to-grounded-target distance was fixed at 15 cm. The flow rate of the syringe pump (0.03−0.05 mL/min) was adjusted in tandem with the applied voltage affording fiber formation. Both of the RTILs selected for this study, [bmIm][Cl] and [emIm][ba], are completely miscible in ethanol, while neither of the polysaccharides are ethanol soluble. Hence, as the fibers formed, the ethanol extractively removed the RTIL solvents, affording pure polysaccharide fibers. The fibers, in the form of a tangled web, were washed with additional ethanol and then dried in vacuo to remove the residual ethanol.

Surface Characterization. A JEOL JSM-6332 FESEM equipped with secondary electron detectors was used at a voltage of 5 kV to study the surface characterization of the fibers. To perform the FESEM analysis, the fibers were first subjected to gold sputtering to form a monolayer of gold on the surface of the fiber to afford a conductive film.

Thromboelastography (TEG). The cellulose−heparin composite fibers were thoroughly washed with water to remove all leachable heparin prior to measuring TEG.[20] Typically, dry fiber (1 mg) was placed in a TEG cup, followed by the addition of 350 μL of human whole blood, and incubated for 5 min. A 10-μL aliquot of 0.01 M $CaCl_2$ was added to recalcify the citrated blood. A coaxially suspended stationary piston was then placed in the cup with a clearance of 1 mm. This pin is suspended by a torsion wire which transduces the torque. The cup is oscillated at an angle of 4° 45′ in either direction every 4.5 s. During the clot formation, fibrin fibrils link the cup to the pin which influences the rotation of the pin, and the disturbance is measured and displayed by a computer. The display, called a thromboelastogram, plots the torque experienced by the pin as a function of time.

Results and Discussion

A 10% (w/w) solution of cellulose dissolved in [bmIm][Cl] and another solution containing cellulose (in [bmIm][Cl]) and heparin (in [emIm][ba]) were prepared and subjected to electrospinning (Figure 1). The fibers formed were directly received in ethanol that can completely dissolve both the RTILs used in the dissolution, but neither of the polysaccharides are ethanol soluble. Hence, as the fibers formed, the ethanol extractively removed the RTIL solvents, affording pure polysaccharide fibers. These fibers were then subjected to further washing with ethanol until there was no residual RTIL found by distillation. The fibers were then dried in vacuo to remove the residual ethanol. Both cellulose and cellulose−heparin composite fibers were made by this approach. Elemental analysis (C, H, N, S) (Galbraith Laboratories, TN) of cellulose fibers showed no N or S, while cellulose−heparin composite fibers showed the

Table 1: Clotting Kinetics Values of Human Whole Blood Treated with Fibers from RTILs

fibers	*R* (min)	*K* (min)	α (deg)	MA (mm)
human whole blood (control—no fibers)	3.8	2.6	61.9	50.0
cellulose fibers	4.8	1.6	63.9	55.7
cellulose-heparin fibers (1 mg)	24.0	16.1	8.6	40.4
cellulose-heparin fibers (1.8 mg)	69.4	43.3	5.1	50.8

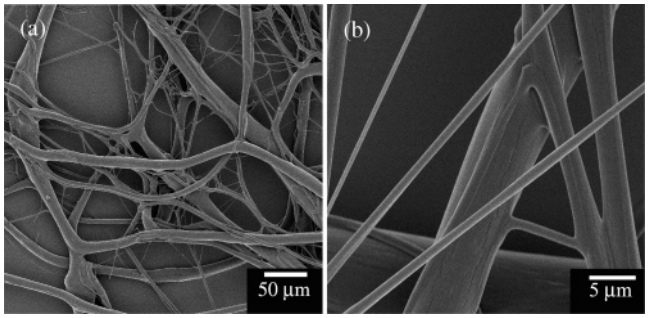

Figure 2. Field emission scanning electron microscope images of cellulose only fibers (a,b) 10% (w/w).

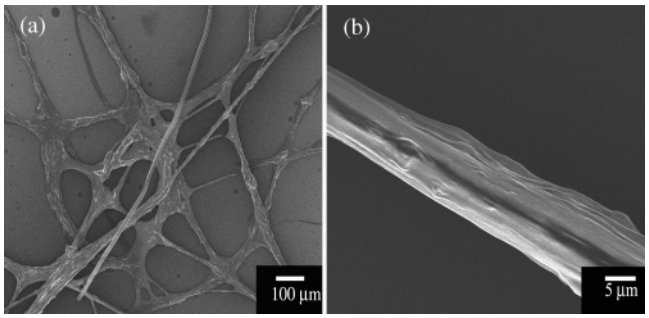

Figure 3. Field emission scanning electron microscope images of cellulose−heparin composite fibers (10% (w/w) cellulose solution, 7% (w/w) heparin−cellulose final concentration).

expected values based on the synthesis described in Figure 1, confirming the absence of RTILs in the dried fibers.

The dried cellulose and cellulose−heparin composite fibers were structurally characterized using field emission scanning electron microscopy (FESEM). The FESEM characterization of the cellulose-only fibers (Figure 2a,b) showed the formation of highly branched, nanometer-to-micron-sized fibers by electrospinning from RTIL solutions. Cellulose fibers were made out of 10% (w/w) cellulose−RTIL solution. FESEM images of the cellulose−heparin composite fibers are shown in Figure 3. The morphology and diameter distribution of electrospun fibers depend on a variety of process parameters, including the solution concentration, surface tension of solvent, applied voltage, and solution feed rate. The high viscosity and nonvolatility of the RTILs limited the fibers formed to mostly micron-sized diameters (see fiber diameter distributions in supplementary information) and also contributed to the interconnected branched structures. The mean fiber size for the cellulose/heparin composite was larger than that for pure cellulose, mainly due to the higher viscosity. Even when pure cellulose was electrospun from RTILs, only a small percent of nanoscale (~500 nm) fibers were observed. By using low-viscosity RTILs and optimization of the spinning parameters, it should be possible to prepare nonbranched nanofibers of cellulose/heparin composites. The surface roughness of the cellulose−heparin composite fibers was also much higher than that of the cellulose-only fibers (Figures 2b and 3b). This difference may be due to the phase separation of cellulose and heparin in the electrospinning process, although other phenomena such as the differential rate of solvent removal and skin formation due to differences in blend composition or the MW or fiber diameter might also contribute to the observed roughness of the composite fibers.

Biological characterization of the cellulose−heparin fibers was performed by measuring the clotting kinetics of human whole blood exposed to these fibers using thromboelastography (TEG).[20] The clotting kinetics values of the human whole blood treated with the cellulose and the cellulose−heparin composite fibers are given in Table 1. In TEG studies, cellulose fibers behave similarly to the control sample (no added fibers). In contrast, cellulose−heparin composite fibers afford a prolonged clotting time (*R*) in a concentration-dependent fashion. The time taken to reach 20 mm of clot (*K*) increased, and the rate of the clot formation (α) decreased. Little or no effect on maximum amplitude (MA) of clot formation was detected. These observations suggest the presence of heparin in an electrospun fiber acted as an anticoagulant slowing clot formation without altering the final amount of clot formed. It is noteworthy that heparin maintained its bioactivity even after an exposure to high voltages (10−20 kV) required in the electrospinning process.

Conclusions

Cellulose and cellulose−heparin composite fibers have been made for the first time by electrospinning from RTILs. The use of RTILs to form fibers, followed by RTIL removal through ethanol extraction, demonstrates an advantage for the high viscosity of RTIL solvents. FESEM images showed the formation of both micron- and nanometer-sized fibers. The cellulose fibers showed a smooth surface, while the cellulose−heparin composite fibers had a rough surface morphology. Heparin, despite being a biological macromolecule, stayed intact and bioactive even with exposure to high voltage during electrospinning. The application of RTIL solutions in fiber formation by electrospinning is expected to delimit the nature of polymer/material from which electrospun fibers can be made. Finally, cellulose−heparin fibers offer promise in the preparation of woven fabrics for use in the construction of artificial vessels with excellent blood compatibility.

Acknowledgment. This work was supported by the NIH funding HL 52622, GM 38060, and HL 62244. Fuming Zhang, Ashavani Kumar, and Anand Ramasubramanian are appreciated for their valuable discussions.

Supporting Information Available. Diameter distribution of cellulose and cellulose−heparin fibers. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Reneker, D. H.; Chun, I. *Nanotechnology* **1996**, *7*, 216−223.
(2) Li, D.; Xia, Y. *Adv. Mater.* **2004**, *16*, 1151−1170.
(3) Schreuder-Gibson, H.; Gibson, P.; Senecal, K.; Sennett, M.; Walker, J.; Yeomans, W.; Ziegler, D.; Tsai, P. P. *J. Adv. Mater.* **2002**, *34*, 44−55.
(4) Nair, L. S.; Bhattacharyya, S.; Laurencin, C. T. *Expert Opin. Biol. Ther.* **2004**, *4*, 659−668.
(5) Sheldon, R. *Chem. Commun.* **2001**, *23*, 2399−2407.
(6) Welton, T. *Chem. Rev.* **1999**, *99*, 2071−2083.

(7) Park, S.; Kazlauskas, R. J. *Curr. Opin. Biotechnol.* **2003**, *14*, 432−437.
(8) Murugesan, S.; Karst, N.; Islam, T.; Wiencek, J. M.; Linhardt, R. J. *Synlett* **2003**, *9*, 1283−1286.
(9) Murugesan, S.; Linhardt, R. J. *Curr. Org. Synth.* **2005**, *2*, 437−451.
(10) Murugesan, S.; Ren, R. X.; Wiencek, J. M.; Linhardt, R. J. *Carbohydr. Polym.* In press.
(11) Doshi, J.; Reneker, D. H. *J. Electrostat.* **1995**, *35*, 151−160.
(12) Hohman, M. M.; Shin, M.; Rutledge, G.; Brenner, M. P. *Phys. Fluids* **2001**, *13*, 2201−2220.
(13) Mannhalter, C. *Sens. Actuators, B* **1993**, *11*, 273−279.
(14) Liu, H.; Hsieh, Y.-L. *J. Polym. Sci., Part B: Polym. Phys.* **2002**, *40*, 2119−2129.
(15) Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. *J. Am. Chem. Soc.* **2002**, *8*, 4974−4975.
(16) Capilla, I.; Linhardt, R. J. *Angew. Chem.* **2002**, *114*, 426−450; *Angew. Chem., Int. Ed.* **2002**, *41*, 390−412.
(17) Denizli, A. *J. Appl. Polym. Sci.* **1999**, *74*, 655−662.
(18) Yang, M. C.; Lin, W. C. *J. Polym. Res.* **2002**, *9*, 201−206.
(19) Marconi, W.; Benvenuti, F.; Piozzi, A. *Biomaterials* **1997**, *18*, 885−890.
(20) Mousa, S. A.; Khurana, S.; Forsythe, M. S. *Asterioscler. Thromb. Vasc. Biol.* **2000**, *20*, 1162−1167.

BM050837S