Chapter 10

Preparation of Biopolymer-Based Materials Using Ionic Liquids for the Biomedical Application

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The insolubility of unmodified biopolymers in most organic solvents has limited the applications of biopolymer-based materials and composites. Because of their inherent biocompatibility and biodegradability, such materials have many potential applications in biomedicine, including for tissue engineering, drug delivery systems, wound treatment, dialysis membranes, and biosensors. Ionic liquids (ILs) are good solvents for polar organic, nonpolar organic, inorganic and polymeric compounds. Biopolymers such as cellulose, chitin/chitosan, silk, and DNA can be fabricated from ILs into films, membranes, fibers, spheres, and molded shapes. Various biopolymer/biopolymer and biopolymer/synthetic polymer composites also can be prepared by co-dissolution of polymers into IL mixtures. Heparin/biopolymer composites are especially of interest in preparing materials with enhanced blood compatibility.

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Introduction

Biopolymers

Bio-based materials have garnered considerable interest recently as they can decrease dependency on fossil fuel. Biopolymers are naturally obtainable macromolecules including polysaccharides, polyphenols, polyesters, polyamides, and proteins, which play an important role in biomedicine with applications in tissue engineering, regenerative medicine, drug-delivery systems, and biosensors. The inherent biocompatibility and biodegradability of these materials make them particularly useful in biomedical applications. For example, tissue engineering requires the seeding of cells into porous polymer matrices that offer channels for host cell migration and that biodegrade into non-toxic products *in vivo* (*1*). Biopolymer nanofibrous mats could be used as particle filters, wound dressings, medical textiles, and for drug delivery (*2*). However, the homogenous modification of biopolymers and preparation of unmodified biopolymer-based materials still remains a challenge. The reason for this challenge is the low solubility of unmodified biopolymers in conventional solvents making the chemical modification and the formation of biopolymer-containing composites difficult. Therefore, new solvents for the dissolution of unmodified biopolymers are of interest in developing various biopolymer-based materials.

Cellulose

Cellulose, a linear polysaccharide of D-glucose residues linked by β-(1→4)-glycosidic bonds, is the most abundant renewable biopolymer on earth. It has excellent thermal and mechanical properties (*3*). Unmodified cellulose offers excellent biocompatibility and is considered a promising material for biomedical applications. For example, woven cellulose pads are used as wound dressings. They are sterilizable, biocompatible, porous, elastic, easy to handle and store, and provide optimal control of wound (*4*). Hollow fibers of cellulose can be used as artificial blood vessels. The blood compatibility of vessels prepared from artificial cellulose fibers has been tested as devices in dog models (*5*). Cellulose has also been applied as a membrane to protect immobilized glucose oxidase in biosensors used to assay glucose in blood (*6*). The development of unmodified cellulose materials with various additives and cellulose composites has been hampered by the difficulty of dissolving cellulose. Cellulose is highly crystalline as a result of an extensive hydrogen bonding network, making it insoluble in most conventional organic and aqueous solvents. Although some unconventional solvents such as *N*-methylmorpholine-*N*-oxide (NMMO), CdO/ethylenediamine (*7*), LiCl/*N*,*N*-dimethylacetamide (DMAc) and near supercritical water (*8*) have been used for dissolving cellulose. Thus, greener and nontoxic solvents need to be developed for the widespread application of unmodified cellulosics (*9*). Chemically modified celluloses such as cellulose acetate, cellulose propionate and cellulose acetate-butyrate are found in a wide range of biomaterials (*10*). They are used for film coatings, dialysis membranes, solid supports, sponges and fibers in

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various biomedical applications. However, the chemical modification of cellulose is complicated by its high degree of crystallinity and the degree of modification for cellulose is often difficult to control due to the heterogeneous reactions.

Chitin

Chitin, a co-polymer of over 50% *N*-acetyl-glucosamine and *N*-glucosamine units, is one of the most abundant polysaccharides with an annual production just second behind cellulose. Chitin has been also successfully used for biomedical applications owing to its biodegradability and biocompatibility. For example, porous chitin matrices were used for cell transplantation applications to regenerate tissue (*11*). Chitin's monomer unit, *N*-acetyl-glucosamine, occurs in hyaluronic acid, an extracellular macromolecule that is important in wound repair (*1*). A chitin membrane, named Vinachitin, has been used to treat deep burns, orthopedic, trauma and ulcer conditions in over 300 patients (*12*). The use of chitin in many applications has also been limited due to its insolubility in most organic solvents. Chitin forms strong intermolecular and intramolecular hydrogen bonds that are difficult to break using common molecular solvents. Although few solvents, including DMAc containing 5% LiCl, methanesulfonic acid, and hexafluoro-2-propanol (HFIP), dissolve chitin, these solvents are often toxic and corrosive, and the resulting chitin solution is unstable (*2*, *13*).

Heparin

Heparin is a mixture of linear anionic polysaccharides having 2-*O*-sulfo-α-iduronic acid and 2-deoxy-2-sulfamino-6-*O*-sulfo-α-D-glucose as its major repeating disaccharide and minor amounts of β-D-glucuronic acid and 2-acetamido-2-deoxy-α-D-glucose. Heparin is widely used as an injectable anticoagulant for acute coronary syndromes (e.g., myocardial infarction, arterial fibrillation, deep-vein thrombosis and pulmonary embolism) (*14–16*). Heparin is administered, either by *intravenous* or *subcutaneous* routes, to maintain blood flow of inpatients on extracorporeal therapy (*17*). Therefore, heparin immobilized to a surface, enhances the blood compatibility of that surface, reducing platelet adhesion, the loss of blood cells, and increasing plasma recalcification time and activated partial thromboplastin time (APTT). Immobilized heparin also inhibits initial contact activation enzymes through an antithrombin-mediated pathway, and thus has enhanced anticoagulant properties (*18*, *19*). Heparinized devices, include currently used macrodevices such as kidney dialyzers in extracorporeal circuits, indwelling catheters and stents as well as implantable nanodevices and nanomachines under development for applications such as drug delivery systems (*20*). Recently, there have been many reports of using heparinization to enhance the surface properties of various polymeric materials for medical applications (*19*, *21–23*). These composites, based on their biological properties and morphology, were proposed in a variety of applications including as blood compatible, hollow fiber, and nanoporous membranes for kidney dialyzers.

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Silk

Silks are spun into fibers by silkworm, spiders, scorpions, mites, and flies. *Bombyx mori* silk worms are the most thoroughly studied silk producers whose silk has been used in biomedical applications as a suture material for repairing wound injuries for centuries because of its biocompatibility, biodegradability, excellent mechanical properties, low inflammatory responses, and good oxygen and water vapor permeability (*2*, *24*, *25*). Recently, the ability to fabricate silk-based materials including films, sponges, mats, and fibers has been of great interest in the manufacturing of biomedical materials, including tissue engineering scaffolds (*26*). However, silks are insoluble in common solvents such as water, dilute acids, and alkali. A single strand of natural cocoon silk fiber from *Bombyx mori* silkworm contains two silk fibroin cores surrounded by a protective, glue-like sericin coating. The actual fibroin cores consist of 391 kDa heavy and 26 kDa light chain. The hydrogen bonded crystalline region of heavy chain is responsible for excellent mechanical properties and difficulty in its dissolution (*24*, *25*). Traditionally, the procedure to make stable silk fibroin solution includes sericin stripping by Na_2CO_3, dissolving in a high concentration of aqueous lithium salt solution, dialysis, lyopholization, and redissolving in HFIP (*24*). HFIP is extremely corrosive and toxic and the procedures to make silk solutions are very laborious. The development of environmental-friendly and efficiently silk dissolving solvent is a major interest in manufacture of silk-based materials for biomedical applications.

Ionic Liquids

Ionic liquids (ILs) are organic salts that usually melt below 100°C. Interest in ILs stems from their potential application as ‘green solvents’ (*27*). Specifically, their non-volatile character and thermal stability make them attractive alternatives to volatile organic solvents. In chemical processes, ILs exhibit excellent physical characteristics including the ability to dissolve polar and nonpolar organic, inorganic, and polymeric compounds. Moreover, the combinations of anions and cations encompassed by ILs are vast. In addition, owing to their associated synthetic flexibility, ILs are referred as ‘designer solvents’ (*28*). Recently, ILs were demonstrated to be good solvents for dissolution and reconstitution of unmodified biopolymers (*29–31*). Major merits of the procedure to make biopolymer-based materials by using ILs are high solubility of biopolymers that cannot be dissolved in general organic solvents, greener procedures using non-volatile solvents, and easy production of composites comprising of various biopolymers and synthetic polymers. In this work, the dissolution of biopolymers in ILs and the preparation of various biopolymer-based materials using ILs will be investigated.

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Dissolution of Biopolymers Using ILs

Polysaccharides are highly complex, chiral organic compounds that are challenging to modify and are insoluble in most common organic solvents. Only certain polysaccharides can be dissolved and/or modified in selected polar solvents (e.g., water, pyridine, formamide, dimethylformamide and dimethylsulfoxide) (*23*, *30*, *32*, *33*). Thus, it is important to investigate new solvent systems capable of dissolving polysaccharides.

The first organic molten salts, *N*-alkylpyridinium salts, as cellulose solvents, were published in 1934. However, this was not considered as commercial solvent for cellulose because of its high melting point (118° C) (*20*, *34*). In 2002, the use of 1-alkyl-3-methylimidazolium salts as solvents for cellulose was reported by Rogers group (*9*). They tested the ability of ILs containing 1-butyl-3-methylimidazolium ([Bmim]) cation with various anions to dissolve cellulose and the most effective anion was found to be the chloride. Cellulose could be dissolved at 25 wt% in [Bmim][Cl] by microwave irradiation and dissolved cellulose can be reconstituted by the addition of an anti-solvent such as water, ethanol, and acetone. These results have opened up new paths for commercially relevant routes of homogeneous cellulose chemistry and for the preparation of various unmodified cellulose composites. NMR studies on the dissolution mechanism of cellulose in [Bmim][Cl] indicates that the $[Cl]^-$ of ILs acts as a hydrogen bond acceptor which interacts with the hydroxyl group of cellulose (*34–36*). Although [Bmim][Cl] has been reported to be chemically stable, side reactions resulting from the abstraction of the proton at position 2, high viscosity, toxicity associated with high reactivity of Cl^-, and biodegradability also need to be considered. Ren et al. synthesized 1-allyl-3-methylimidazolium chloride ([Amim][Cl]) and showed that the solubility of cellulose in [Amim][Cl] was better than [Bmim][Cl] (*37*). Heinze and coworkers reported that 1-butyl-3-methylpyridinium chloride ([Bmpy][Cl]) could dissolve up to 39 wt% Avicel, while [Bmim][Cl] dissolved at 18 wt% Avicel (*34*, *36*). Mikkola et al. used ultrasound irradiation to dissolve cellulose in [Bmim][Cl] and [Amim][Cl] (*38*). The use of high-power ultrasound dramatically intensified the dissolution process and resulted in complete dissolution within a few minutes. Recently, acetate, formate, methyl phosphate, and dicyanamide counter anions of 1-alkyl-3-methylimidazolium salts were reported as good ILs for cellulose dissolution (*39–42*). Among the room temperature ILs with low viscosity suitable for cellulose dissolution are 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]) and formates of allylimidazolium based ILs. The solubility of celluloses in ILs is shown in Table 1.

There are very few results reported on the dissolution of chitin in ILs. Xie et al. showed the dissolution of 10% chitin in [Bmim][Cl], a good solvent for cellulose (*44*). However, the chitin was not fully soluble and still showed some crystallinity in these solutions. The ability to dissolve chitin in [Bmim][Cl] is highly dependent on chitin molecular weight, degree of acetylation, and its origin. Recently, Wu et al. showed the successful dissolution and regeneration of various native chitins in [Bmim][Ac] (*13*). The acetate anion in [Bmim][Ac] is a stronger hydrogen bonding acceptor than the chloride anion in [Bmim][Cl] and thus, can dissolve

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higher concentrations of chitin. The acetate anion of [Bmim][Ac] is believed to strongly interact with the hydrogen bond networks in chitin by depriving the proton of the amino or hydroxyl groups from the carbonyl groups. In contrast, chitosan is more easily dissolved in [Amim][Cl], [Bmim][Cl], [Bmim][Ac], and aqueous [Bmim][BF_4] solution (*45–47*).

Heparin, like cellulose, is soluble in only a very few conventional solvents such as water, dimethylsulfoxide, dimethylformamide and formamide. Therefore, many studies have been conducted to test the solubility of various heparin-like glycosaminoglycans (GAGs) in novel solvents (*19*, *30*, *33*). Linhardt and coworkers first suggested the use of ILs for the dissolution of GAGs. They studied the synthesis and properties of ILs having benzoate as the anion and a cation comprised of alkyl substituted imidazolium, pyridinium or phosphonium moieties (*48*). The aim of this work was to find ILs that could dissolve the sodium or imidazolium salts of heparin. Four different ILs ([Emim][Ba], [Bmim][Ba], [Bmim][PF_6], and [Bmim][BF_4]) were used to study GAG solubility (*48*). A total of eight GAGs, four with sodium and four with imidazolium counterions, were tested in these dissolution studies. [Emim][Ba] showed the best dissolution of GAGs, and the imidazolium salts of GAGs dissolved better than their corresponding sodium salts. Following their initial success of heparin dissolution, [Emim][Ba] was used in the glycosylation of unprotected donors with protected acceptors (*49*) and the fabrication of blood compatible composite membranes (*19*), and the preparation of biopolymer composite fibers by electrospinning (*50*).

Table 1. Solubility of cellulose in ILs[a]

Ionic liquids	*Type of cellulose*	*Solubility*
[Emim][Cl]	Avicel (DP 286)	12% (10°C above mp.) (*43*)
	Spruce sulfite pulp (DP 593)	6% (10°C above mp.) (*43*)
	Cotton linters (DP 1198)	4% (10°C above mp.) (*43*)
	Eucalyptus pulp (DP 569)	>16% (vertical kneader) (*42*)
[Bmim][Cl]	Avicel (DP 286)	18% (80°C) (*36*)
	Spruce sulfite pulp (DP 593)	13% (80°C) (*36*)
	Cotton linters	10% (80°C) (*36*), 10 wt% (ultrasound) (*38*)
	Microcrystalline cellulose (Aldrich, 20 μm)	8 wt% (ultrasound) (*38*)
	Kraft pulp (0.35 mm)	9 wt% (ultrasound) (*38*)
	Eucalyptus pulp (DP 569)	>14% (vertical kneader) (*42*)
	Pulp (DP 1000)	3% (70°C) (*9*), 10% (100°C) (*9*), 25% (microwave) (*9*)
[Hmim][Cl]	Pulp (DP 1000)	5% (100°C) (*9*)

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Table 1. (Continued). Solubility of cellulose in ILs[a]

Ionic liquids	*Type of cellulose*	*Solubility*
[Omim][Cl]	Pulp (DP 1000)	slightly soluble (100°C) (*9*)
[Bdmim][Cl]	Avicel (DP 286)	9% (10°C above mp.) (*43*)
	Spruce sulfite pulp (DP 593)	6% (10°C above mp.) (*43*)
	Cotton linters (DP 1198)	4% (10°C above mp.) (*43*)
	Eucalyptus pulp (DP 569)	>13% (vertical kneader) (*42*)
[Bmpy][Cl]	Avicel (DP 286)	39% (105°C) (*36*)
	Spruce sulfite pulp (DP 593)	37% (105°C) (*36*)
	Cotton linters (DP 1198)	12% (105°C) (*36*)
[BDTA][Cl]	Avicel (DP 286)	5% (60°C) (*36*)
	Spruce sulfite pulp (DP 593)	2% (60°C) (*36*)
	Cotton linters (DP 1198)	1% (60°C) (*36*)
[Amim][Cl]	Microcrystalline cellulose (Aldrich, 20 μm)	2-11 wt% (80-100°C) (*39*), 27 wt% (ultrasound) (*38*)
	Cotton linters	13 wt% (ultrasound) (*38*)
	Kraft pulp (0.35 mm)	8 wt% (ultrasound) (*38*)
	Cellulose	8-15 wt% (80°C) (*41*)
[Bmim][Br]	Pulp (DP 1000)	5-7% (microwave) (*9*)
[Admim][Br]	Avicel (DP 286)	12% (10°C above mp.) (*43*)
	Spruce sulfite pulp (DP 593)	4% (10°C above mp.) (*43*)
	Cotton linters (DP 1198)	4% (10°C above mp.) (*43*)
[Bmim][SCN]	Pulp (DP 1000)	5-7% (microwave) (*9*)
[Emim][Ac]	Eucalyptus pulp (DP 569)	>14% (vertical kneader) (*42*)
[Bmim][Ac]	Eucalyptus pulp (DP 569)	>13% (vertical kneader) (*42*)
[Amim][HCOO]	Microcrystalline cellulose (Aldrich , DP 250)	11-21 wt% (60-85°C) (*39*)
[Emim][(MeO)HPO_2]	Microcrystalline cellulose (Aldrich , DP 250)	4-10 wt% (30-45°C, 30 min) (*40*)
[Emim][(MeO)$MePO_2$]	Microcrystalline cellulose (Aldrich , DP 250)	4-10 wt% (40-55°C, 30 min) (*40*)
[Emim][$(MeO)_2PO_2$]	Microcrystalline cellulose (Aldrich , DP 250)	4-10 wt% (55-65°C, 30 min) (*40*)

[a] Hmim = 1-hexyl-3-methylimidazolium, Omim = 1-octyl-3-methylimidazolium, Bdmim = 1-butly-2,3-dimethylimidazolium, BDTA = benzyldimethyl(tetradecyl)ammonium, Admim = 1-allyl-2,3-dimethylimidazolium.

Table 2. Solubility of biopolymers in ILs

Biopolymer	*Ionic liquids*	*Solubility*
Heparin (imidazolium salt)	[Emim][Ba]	7.0% (35° C) (*48*)
	[Bmim][Ba]	7.0% (35° C) (*48*)
Heparan sulfate (imidazolium salt)	[Emim][Ba]	3.0% (35° C) (*48*)
	[Bmim][Ba]	2.8% (35° C) (*48*)
Chondroitin sulfate (imidazolium salt)	[Emim][Ba]	9.9% (35° C) (*48*)
	[Bmim][Ba]	5.7% (35° C) (*48*)
Hyaluronic acid (imidazolium salt)	[Emim][Ba]	10.0% (35° C) (*48*)
	[Bmim][Ba]	10.0% (35° C) (*48*)
Chitin	[Bmim][Ac]	6% (α-chitin, 110° C) (*13*), 6-7% (low MW β-chitin, 110° C) (*13*),
		3% (high MW β-chitin, 110° C) (*13*)
	[Bmim][Cl]	partially soluble (α-chitin & low MW β-chitin, 110° C) (*13*),
		>10% (110° C) (*44*)
Chitosan	[Bmim][Ac]	12% (110° C) (*13*)
	[Bmim][Cl]	10% (110° C, DAC=5%) (*13*), <10% (110° C, DAC=12%) (*44*)
	[Amim][Cl]	8% (110° C) (*13*)
Cocoon silk	[Emim][Cl]	23.3% (100° C) (*24*)
	[Bmim][Cl]	13.2% (100° C) (*24*)
	[Bdmim][Cl]	8.3% (100° C) (*24*)
	[Bmim][Br]	0.7% (100° C) (*24*)

The dissolution of various proteins such as silks (*bombyx mori* silk, spider silk, and silk-elastin fusion protein), collagen, elastin, and gelatin in ILs was also reported, although accurate solubility data for these proteins are rarely reported (*24*, *25*, *29*, *51*). Interestingly, the cocoon silk was dissolved in [Emim][Cl], [Bdmim][Cl], and [Bmim][Cl]. The silk dissolved solution indicated amorphous structure and no β-sheet which shows crystalline region. The silk ILs solutions could be diluted with water and the silk was regenerated by methanol, acetonitrile, and acetone (*24*). Table 2 shows the solubilities of biopolymers in various ILs.

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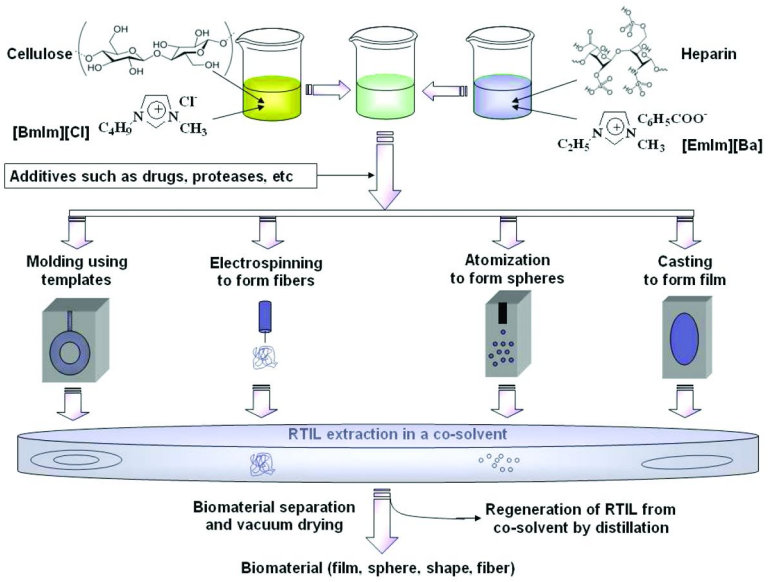

Figure 1. Schematic representation for the preparation of heparin/cellulose composite materials.

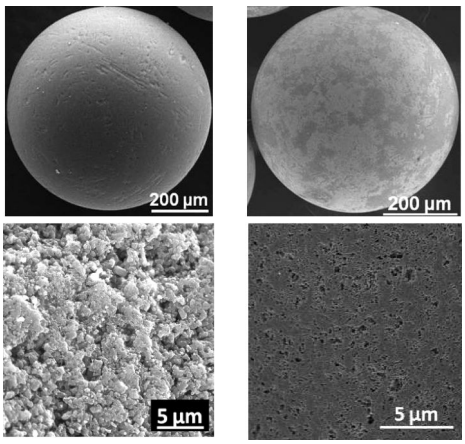

Figure 2. FESEM images: (A, B) uncoated charcoal (C, D) heparin/cellulose/charcoal composite.

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Biopolymer-Based Materials Prepared by Using ILs

Film, Coating, and Membrane

The cellulose dissolution and reconstitution in ILs has great potential importance for the preparation of various biopolymer-based materials. Biopolymer-based film, coating, and biocompatible membrane can be used for biomedical application such as tissue engineering scaffolds, kidney dialysis, biosensor, drug delivery, and implantable devices.

Biopolymer-Based Materials

Rogers and coworkers have extensively explored the use of ILs to dissolve cellulose and reconstitute cellulose composites. Turner et al. used [Bmim][Cl] to encapsulate laccase into cellulose membrane (*52*). The enzyme was entrapped and the material being formed was used in producing a low-leaching bioactive films. A hydrophobic [Bmim][Tf2N] coating resulted in higher enzyme activity, by protecting the enzyme from the high and inactivating concentrations of Cl^- present in [Bmim][Cl]. Turner et al. also reported cellulose/polyamine composite films and beads from [Bmim][Cl] as solid support matrices for biocatalyst immobilization (*53*). They prepared the surface functionalized cellulose composites with primary amine functional groups necessary for chemical bonding between the enzyme and the support resulting in enhanced stability. Poplin et al. developed sensor platforms based on encapsulating a probe molecule within a cellulose matrix (*54*). The [Bmim][Cl] was used to codissolve cellulose and the hydrophobic dye/metal complexant 1-(2-pyridylazo)-2-naphthol, a good extractant and indicator for metal ions. Bagheri et al. developed a surface active cellulose films for covalent attachment of laccase by codissolution of cellulose with polyamidoamine dendrimers in [Bmim][Cl] followed by regeneration with water (*55*). These results indicate that cellulose composites can be used as supports for biosensors, biocatalysis, and novel drug delivery system. Tsioptsias and Panayiotou recently prepared cellulose and cellulose/nanohydroxyapatite composite for tissue engineering scaffolds by a particulate leaching technique, with poly(methyl methacrylate) particles as the porogen (*56*). The materials regenerated from [Bmim][Cl] solution showed different properties from the materials regenerated from *N*,*N*-dimethylacetamide and LiCl solutions. The properties of these new materials make them promising for bone regeneration applications. Additionally, the scaffold fabrication process by using [Bmim][Cl] was fast, inexpensive, and environmentally friendly.

Chitin solution in [Bmim][Ac] gels after being cooled down to room temperature. The [Bmim][Ac] could be extracted away using water or methanol (*13*). Thus, making very easy to prepare transparent soft materials. Additional removal of ILs from hydrogel and drying can also make films, membranes, coatings, and sponges. The regenerated β-chitin showed a transition to crystal structure close to α-chitin, the more stable crystal form. The regenerated chitins are also thermally more stable than the native chitin. The dissolution/regeneration

process of chitin has a great potential for the preparation of chitin-based materials such as dialysis membranes, porous matrices, wound dressings, and other composites for biomedical applications, similar to previously described cellulose-based materials. Chitin-based materials prepared by casting without chemical modification show antibacterial effects, biocompatibility, and biodegradability.

Silk solutions in [Bmim][Cl] were used to cast films and the structures of regenerated films were highly dependent on the anti-solvent. Acetonitrile yields a convoluted surface structure with little crystallinity, while methanol yields a transparent film with a high degree of crystallinity, and water could not be used because of dissolution of silk film (*24*, *25*). Recently, Gupta et al. demonstrated the casting of patterned silk films and these films were found to support normal cell proliferation and differentiation(*26*) . They showed that silk films cast from silk solutions in [Bmim][Cl] did not have any detrimental effect on cell viability and gene expression, indicating that ILs can be used for the fabrication of silk scaffolds for tissue engineering applications.

Blood Compatible Biopolymer Composites

The surfaces of extracorporeal and prosthetic medical devices that come directly into contact with blood or body fluids and tissues must be biocompatible. Such devices should not trigger blood clotting, nor should they induce inflammatory responses when brought into contact with tissues. Blood compatibility is a major factor in biocompatibility because thrombogenesis is induced by surfaces of medical devices that are not blood compatible. Thrombus formation on the surface of an extracorporeal medical device or an implanted biomedical device can result in heart attack, stroke or pulmonary embolism (*19*, *20*). For example, kidney dialysis membranes should have excellent biocompatibility and blood compatibility, without activating the complement and coagulation cascade, triggering the blood clotting (*19*, *57*).

Heparin can improve the blood compatibility of synthetic polymers and biopolymers. Linhardt and coworkers reported novel biopolymer/heparin composites to enhance the blood compatibility of biopolymers, making it unnecessary to chemically couple heparin to these biopolymers. To fabricate these blood compatible composites, they exploited the ability of [Emim][Ba] to dissolve heparin. The mixtures of heparin containing [Emim][Ba] and cellulose containing [Bmim][Cl] were prepared. The resulting solution of cellulose and heparin could be fabricated into cellulose/heparin composite materials in various shapes and forms, including films and membranes, micro- or nanospheres, micro- or nanofibers, or any other shapes molded by using templates (Figure 1) (*19*, *50*, *58*). This simple approach uses IL mixtures to prepare various biopolymer/biopolymer or biopolymer/synthetic polymer composites. Heparin/cellulose composite membranes, prepared by this method, showed uniformly distributed heparin throughout the cellulose matrix. APTT and thromboelastography demonstrated that this composite had excellent blood compatibility when compared to other existing biomaterials prepared through the covalent bonding of heparin. The

composite membrane was also used to test the potential for kidney dialyzers through an equilibrium experiments on urine and bovine serum albumin (BSA). Urea reached equilibrium within 60 h in while BSA did not reach equilibrium even after 45 h. Thus, these membranes show appropriate selectivity for urea (*19*). Recently, Park et al. reported novel cellulose/heparin/charcoal composites by using [Bmim][Cl] and [Emim][Ba] mixture (*58*). Activated charcoal is useful for treating individuals in danger from oral drug overdose of depressants such as alcohol, barbiturates, and benzodiazepines, or stimulants. However, uncoated activated charcoal generally results in thromboresistance when used in direct hemoperfusion. Although various polymers such as modified cellulose, agarose, chitosan, and synthetic copolymers were used to coat or entrap charcoal to increase blood compatibility of charcoal composite (*59*, *60*), the use of charcoal composites in hemoperfusion still requires the transfusion of whole blood or the addition of human serum albumin. To solve this problem, Park et al. prepared blood compatible heparin/cellulose/activated charcoal bead composites to enhance the biocompatibility and blood compatibility of activated charcoal beads while decreasing the size of their active pores (*58*). The FESEM image of heparin/cellulose/charcoal composites showed the smooth, uniformly coated surface with a large number of small, nano-sized pores (Figure 2). The surface morphology indicates that the composite is potentially capable of inhibiting the adsorption of proteins while permitting small drug molecules to adsorb to the underlying charcoal bead. APTT results and adsorption efficiency of phenytoin compared to BSA showed that the coating of activated charcoal with cellulose/heparin are useful for direct hemoperfusion to remove free-diluted and protein-bound toxins of small size or useful as potential oral agents in the cases when strict preservation of large molecules is necessary.

Fibers

Natural biopolymer fibers have been used in a variety of biomedical applications. For example, silk fibers are used as suture thread and absorbent cotton in medical dressings. Fibers and fibrous membranes have a number of potential biomedical applications due to their flexibility, permeability, high liquid retention and high surface area. Biopolymer-based fibers such as cellulose, chitosan, alginate, gelatin and silk fiber have been fabricated and evaluated for biomedical applications.

Dry-Jet Wet Spinning Using ILs

Cellulose fibers are the most widely used biopolymer-based fibers and are renewable, biocompatible and biodegradable. Although NMMO has been used industrially in dry-jet wet spinning processes (*61*), the development of a greener and easily recyclable system using non-toxic and non-volatile solvents would be advantageous. Recently, several groups obtained unmodified cellulose fibers with dry-jet wet spinning process using ILs such as [Emim][Cl], [Bmim][Cl],

[Amim][Cl], [Emim][Ac] and [Bmim][Ac] (*42*, *62*, *63*). Synthetic polymers, such as poly(m-phenylene-isophthalamide) (*64*) and polyacrylonitrile (*65*), were also similarly fabricated into fiber. In a typical dry-jet wet spinning process using ILs as solvents, the spin dope is prepared from cellulose solution in ILs. The spinning is performed by extruding the spin dope across an air gap into coagulation bath. The solvent of coagulation bath is miscible with ILs and immiscible with cellulose. Water and alcohols can be used for such solvent. Hence, as the fiber is formed, the solvent of coagulation bath removes ILs from fiber. In the extracting process, it is possible to draw the fiber to enhance the fiber properties. After that, ILs can be recycled easily from co-solvent of coagulation bath due to its non-volatility. Various biopolymer fibers were also obtained by dry-jet wet spinning using ILs. Silk fiber from [Emim][Cl] solution (*25*) and DNA fiber from [Bmim][BF_4] solution (*66*) were reported. Biopolymer composite fibers such as wool keratin/cellulose with improved mechanical property and cellulose/m-aramid with enhanced antimicrobial activity were also fabricated by using [Bmim][Cl] (*67*, *68*).

Electrospinning Using ILs

The electrospinning has been recognized as a simple and versatile method for producing ultrathin fiber with an extremely high surface area on a sub-cellular scale. In medical field, fabric materials made of electrospun fiber are anticipated to be candidates for tissue engineering scaffolds, in wound dressing and as protective clothing (*2*, *69*). In a typical electrospinning process, a strong electric field is applied to a droplet formed by a solution of polymer and volatile solvent at the tip of a die which acts as one of the electrode. The droplet is deformed as it is charged and forms a jet that is accelerated in the direction of the collector electrode with an evaporation of the solvent. This conventional electrospinning method using volatile solvents can be defined as a dry spinning process (*70*). Because the evaporation of volatile solvents is involved, this process is sensitive to state of the atmosphere such as temperature and humidity (*71*). The recovery of evaporated organic solvent is usually difficult. Especially, in case of the inflammable solvent where ignition by a spark can take place.

Recently, electrospinning methods have used non-volatile ILs as solvents to fabricate ultrathin fibers of unmodified cellulose (*50*). This method can be defined as a dry-jet wet electrospinning process, which forms biopolymer-based ultrathin fibers fabricated by collecting the jet in a grounded coagulation bath. The dry-jet wet electrospinning process with ILs is stable system at atmosphere, no need of gas recovery, and a fire-safety system. Although the high viscosity of the ILs is a presumed disadvantage for electrospinning, nanoscale (about 500 nm) fibers could be observed. The success of this method might result from the jet of highly conductive ILs solution might be subjected to a greater tensile force in the presence of an electric field. Zhang et al. reported that diameter of electrospun fiber decreased with increasing the solution conductivity (*72*). The control of solution properties such as the viscosity, the surface tension and conductivity were also reported using a mixed solvent approach (*73*, *74*). By addition of

dimethylsulfoxide or dimethylformamide as co-solvents to cellulose solution in [Amim][Cl], the viscosity and surface tension could be decreased and the conductivity could be increased. Electrospinnability was improved and thinner fiber was obtained. They also demonstrated that cellulose coagulation could be accomplished using water vapor. The water vapor such as 80% relative humidity of the environment caused the cellulose solidification from the surface of the jet and then fiber form could be obtained without the use of a coagulation bath. The combination of ILs and the dry-jet wet electrospinning can be also applied to synthetic polymers (*75*) and has more potential because ILs are 'designer solvents' that can be modified specifically for this electrospinning process.

Linhardt and coworkers prepared a cellulose/heparin composite fiber by electrospinning using [Bmim][Cl] and [Emim][Ba] mixture (*50*). FESEM images showed the formation of both micron- and nanosized fibers. The cellulose fiber showed a smooth surface, while cellulose/heparin composite fibers had a rough surface morphology (Figure 3). Cellulose/heparin composite fibers showed anticoagulant activity, demonstrating the activity of heparin remained unaffected even on exposure to a high voltage involved in electrospinning. Cellulose/heparin fibers have a great potential for use in the construction of artificial vessels with excellent blood compatibility. Table 3 shows various fiber prepared by spinning from ILs.

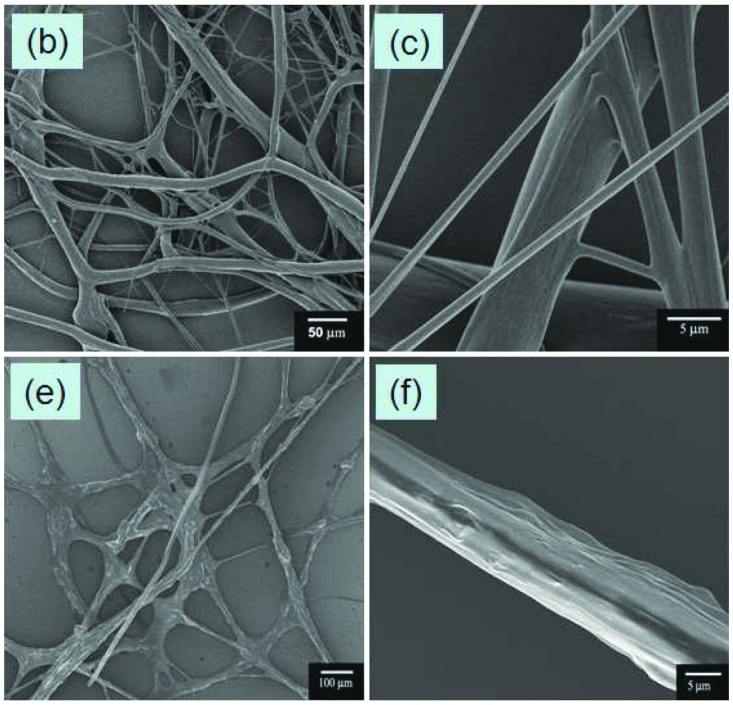

Figure 3. FESEM images: (A, B) cellulose fiber, (C, D) heparin/cellulose composite fiber.

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Electrochemical Device Platforms

The direct electrochemistry of redox proteins recently has gained considerable interests, because it is helpful for understanding the electron-transfer mechanism in biological systems and for constructing biofuel cells and biosensors (*51*, *76*). Various systems have been developed to enhance the direct electron transfer and protein stability, because electroactive centers of redox proteins are embedded in the molecules and proteins are usually unstable. For these purpose, host materials should be able to immobilize proteins well and promote the direct electrochemistry. These materials also should be nontoxic, stable, and biocompatible (*77*, *78*). Biopolymers can provide a favorable microenvironment for redox proteins and enzymes to fabricate excellent biosensors. ILs have also been used in electrochemistry and electroanalysis, because of their high ionic conductivity and wide electrochemical windows (*79*). ILs also allow efficient direct electron transfer of various proteins such as microperoxidase (*80*), hemoglobin, horseradish peroxdiase (*45*), and glucose oxidase (*47*, *81*). Therefore, biopolymer/ILs composite systems might represent unique materials that could open up new opportunities for direct electrochemistry.

Lu et al. proposed a chitosan/[Bmim][BF_4] composite material and the composites were used as immobilization matrices to entrap hemoglobin (Hb) and horseradish peroxidase (HRP) (*45*, *46*). A reagentless HRP biosensor based on chitosan/[Bmim][BF_4] composite showed direct bioelectroanalysis toward H_2O_2. Both biocompatibility of chitosan and inherent conductivity of [Bmim][BF_4] enable the composite material to become an biosensing platform for direct electrochemistry and electrocatalysis of HRP. Wang et al. developed a chitosan/[Bmim][BF_4]/HRP/muti-walled carbon nanotubes (MWNTs) composite (*47*). This composite could form a relative uniform film with unique structure on electrode surface. The composite electrode showed good analytical performance such as low detection limit, good regeneration, and anti-fouling properties for determination of NADH. Sun et al. used [Bmim][PF_6] as binder to fabricate a carbon IL electrode (CILE) (*82*). Hemoglobin was immobilized on the surface of CILE with the sodium alginate (SA), a linear hydrophilic polysaccharide composed of β-mannuronic acids and α-L-guluronic acids, hydrogel film to form a SA/Hb/CILE. The SA/Hb/CILE composite showed good electrocatalytic activity to H_2O_2 and nitrate. They also made SA/SiO_2 nanoparticle/[Bmim][PF_6]/Hb/carbon paste electrode composite. This composite showed dramatically enhanced electrocatalytic activity to the reduction oftrichloroacetic acid, H_2O_2, and oxygen (*83*). Ding et al. developed a composite material based on *N*-butylpyridinium hexafluorophosphate, SA, and graphite to construct a HRP biosensor for the determination of H_2O_2 (*84*). The resulting biosensor not only had economic and disposable property but also showed good detection precision, bioactivity, storage stability, and reproducibility. Yan et al. developed a gelatin/dimethylformamide/[Omim][PF_6] hydrogel film to provide a favorable microenvironment for the direct electrochemistry of HRP at glassy carbon electrode (*51*). The enzyme electrode has good catalytic activity to the reduction of hydrogen peroxide, thermal stability, and reproducibility.

Table 3. Polymer fibers prepared by spinning using ILs[a]

Spinning method	*Polymer*	*Spinning solvent*	*Polymer conc. (wt%)*	*Coagulation solvent*	*Fiber thickness*	*Ref.*
Wet spinning	Cellulose	[Amim][Cl]	4	water	ND[b]	(*62*)
		[Bmim][Cl]	3.5, 13.6	water, ND	1.46, 24.3 dtex[c]	(*42*, *68*)
		[Emim][Cl]	3.8-11.5,15.8	water, ND	1.84 dtex	(*42*, *63*)
		[Bdmim][Cl]	13.2	ND	1.67 dtex	(*42*)
		[Bmim][Ac]	18.9	ND	1.64 dtex	(*42*)
		[Emim][Ac]	19.6	ND	1.76 dtex	(*42*)
	Cellulose/keratin	[Bmim][Cl]	10	methanol	ND	(*67*)
	Cellulose/m-aramid	[Bmim][Cl]	3.5	water	24.3-30.1 dtex	(*68*)
	Cellulose/MWNT	[Amim][Cl]	4	water	ND	(*62*)
	Cellulose/Fe_3O_4	[Emim][Cl]	3.8-11.4	water	ND	(*63*)
	Silk	[Emim][Cl]	10	methanol	150 μm	(*25*)
	DNA	[Bmim][BF_4]	5	water/[Bmim][BF_4]	200 μm	(*66*)
	PMIA	[Bmim][Cl]	14-18	water,water/[Bmim][Cl]	9.2-13.2 dtex	(*64*)
	PAN	[Bmim][Cl]	14-20	water/[Bmim][Cl]	ND	(*65*)
Electrospinning	Cellulose	[Bmim][Cl]	10	ethanol	500 nm	(*50*)
		[Amim][Cl]/DMSO	1-5	water vapor	100-800 nm	(*73*)
		[Amim][Cl]/DMF	2-3.5	water	100-500 nm	(*74*)

Continued on next page.

Table 3. (Continued). Polymer fibers prepared by spinning using ILs[a]

Spinning method	*Polymer*	*Spinning solvent*	*Polymer conc. (wt%)*	*Coagulation solvent*	*Fiber thickness*	*Ref.*
	Cellulose/heparin	[Bmim][Cl]/[Emim][Ba]	7	ethanol	ND	(*50*)
	PMIA	[Bmim][BF_4]	6-8	water	Less than 1 μm	(*75*)

[a] PMIA = poly(m-phenyleneisophthalamide), MWNT = multi walled carbon nanotube, PAN = polyacrylonitrile. [b] no data. [c] dtex = g/(10000 m of fiber).

Prospects

Fabrication of unmodified biopolymer-based materials and composites has been traditionally hampered by the difficulty of dissolving biopolymers due to their highly crystalline nature. ILs have a great potential to dissolve biopolymers and develop biopolymer-based materials, because of their synthetic flexibility by changing the combinations of cation and anion, and green solvent properties such as non-volatility, non-flammability and recyclability. Biopolymers such as cellulose, chitin/chitosan, silk, and gelatin can be easily fabricated into films, membranes, fibers, spheres, and molded shapes by dissolution in ILs and reconstitution in anti-solvent. Biopolymer-based materials with ILs should be useful for the biomedical applications such as tissue engineering scaffolds, wound dressing, drug delivery, implantable devices, and biosensors owing to their inherent biocompatibility and biodegradability. Additionally, heparin/biopolymer composites which can be prepared by using ILs mixtures will be beneficial to enhance the blood compatibility of biopolymer.

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