LETTER

Electrospun Composite Nanofiber Fabrics Containing Uniformly Dispersed Antimicrobial Agents As an Innovative Type of Polymeric Materials with Superior Antimicrobial Efficacy

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ABSTRACT Herein we report that electrospun composite nanofiber fabrics containing uniformly dispersed antimicrobial agents and having large surface-to-mass ratios are an innovative type of antimicrobial polymeric materials with durable, nonleachable, and biocompatible characteristics, and more importantly, superior antimicrobial efficacy. Specifically, electrospun cellulose acetate (CA) nanofiber fabrics containing an *N*-halamine antimicrobial agent of bis(*N*-chloro-2,2,6,6-tetramethyl-4-piperidinyl) sebacate (Cl-BTMP) were prepared and evaluated; the results of antimicrobial efficacy indicated that the electrospun composite nanofiber fabrics substantially outperformed the control samples that were solution-cast films containing identical amounts of CA and Cl-BTMP. Additionally, the results of trypan blue assay test suggested that the electrospun composite nanofiber fabrics also had excellent mammal cell viability. The developed electrospun composite nanofiber fabrics with superior antimicrobial efficacy are expected to find vital applications in biomedical, hygienic, and many other fields.

KEYWORDS: electrospinning • nanofiber • antimicrobial efficacy • *N*-halamine • cellulose acetate

Polymeric materials with antimicrobial functionality have attracted growing attentions in control of microbial contaminations and/or infections (1−4), particularly those associated with multidrug-resistant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). Such contaminations and/or infections have caused ~88 000 deaths and ~$4.5 billion in excess healthcare cost annually in the United States alone (1). Cleaning and/or disinfecting of "high-touch/high-risk" areas are recommended to mitigate the contaminations and/or infections; nonetheless, common polymeric materials are susceptible to microbial contaminations with some species being able to survive for 90 days or longer (5−7). Furthermore, the cleaned and/or disinfected areas can be readily and rapidly recontaminated, and thus become sources for cross-contamination and/or cross-infections (4, 8, 9).

The conventionally adopted approach in the preparation of antimicrobial polymeric materials is to impregnate antimicrobial agents as additives into the target polymers (10−15). However, the maximal potential of antimicrobial functionality can hardly be achieved in most cases. This is because (1) the antimicrobial agents often have low solubility/compatibility in the polymers, leading to the aggregations of antimicrobial agents, and (2) the prepared antimicrobial polymeric materials often have low surface-to-mass ratios; both issues reduce the contact between the antimicrobial agents and the targeted microorganisms, resulting in low antimicrobial efficacy.

We explored the technique of electrospinning to successfully address the above two issues through converting antimicrobial polymeric materials into composite nanofiber fabrics. Electrospinning is a material-processing technique that utilizes the electric force to drive the spinning process and to produce fibers with diameters in the submicrometer to nanometer range (16−20). In this study, an antimicrobial agent and a polymer were first dissolved in their common solvents to form a solution (spin dope), which was then electrospun into composite nanofibers that were collected as fabrics (21−27). Our rationale is that during electrospinning, the evaporation of solvents (i.e., the solidification of electrospinning filaments) is extremely fast; more than 99%

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of the solvents are removed during or shortly after bending instability occurs (20); the short solidification time (<0.1 s) likely prohibits the molecules of antimicrobial agents to aggregate and results in the formation of composite nanofibers with antimicrobial agents uniformly dispersed in the polymers.

We selected cellulose acetate (CA) as the polymer because CA fibers have been widely used in biomedical and hygienic applications (such as respirators, face masks, and hospital gowns). We chose a hindered amine-based *N*-halamine, bis(*N*-chloro-2,2,6,6-tetramethyl-4-piperidinyl) sebacate (Cl-BTMP), as the antimicrobial agent because our previous studies revealed that Cl-BTMP had potent antimicrobial activity with good thermal and light stabilities (14, 15). Similar to many other antimicrobial agents, Cl-BTMP has low solubility/compatibility in many polymers including CA; and the antimicrobial polymeric materials containing Cl-BTMP prepared through conventional approaches (such as solution casting) showed low efficacy against many microorganisms. It was our hypothesis that the electrospun composite nanofiber fabrics with uniformly dispersed Cl-BTMP and large specific surface area would significantly outperform the control samples that were solution-cast films containing identical amounts of CA and Cl-BTMP (see the Supporting Information for sample preparations, as well as tests of antimicrobial efficacy and mammal cell viability).

Scanning electron microscopy (SEM) was used to characterize the morphological properties of the samples. As shown in Figure 1, the neat CA film had a smooth surface. For the CA film containing 5 wt % Cl-BTMP, the surface was relatively rough (Figure 1B). After the treatment with ethanol (ethanol can dissolve and remove Cl-BTMP), the neat CA film remained smooth (Figure 1C), while the CA film containing 5 wt % Cl-BTMP (Figure 1D) showed pores with sizes in the micrometer range. This indicated that Cl-BTMP aggregated into separated domains in the film, as schematically illustrated in the left image of the Table of Contents graphic. As shown in images E and F in Figure 1, respectively, the nanofibers electrospun from both the neat CA and the Cl-BTMP containing CA had smooth surfaces; and the fiber diameters were in the range from tens to hundreds of nanometers. After the ethanol treatment, the surface morphologies of both nanofibers remained smooth without microscopically identifiable pores (Figure 1G,H). This suggested that Cl-BTMP did not aggregate in the electrospun nanofibers and predominantly existed as individual molecules and/or molecular clusters, as schematically illustrated in the right image of the Table of Contents graphic. Such a suggestion was further supported by differential scanning calorimetry (DSC) results. As shown in Figure 2, the neat CA film (curve "a") did not undergo noticeable phase transitions in the temperature range of 50−140 °C, whereas the CA film containing 5 wt.% Cl-BTMP (curve "b") showed a melting peak of Cl-BTMP centered at 80.3 °C (14). This confirmed that the Cl-BTMP molecules aggregated into domains and formed crystallites in the CA matrix. In contrast, the DSC

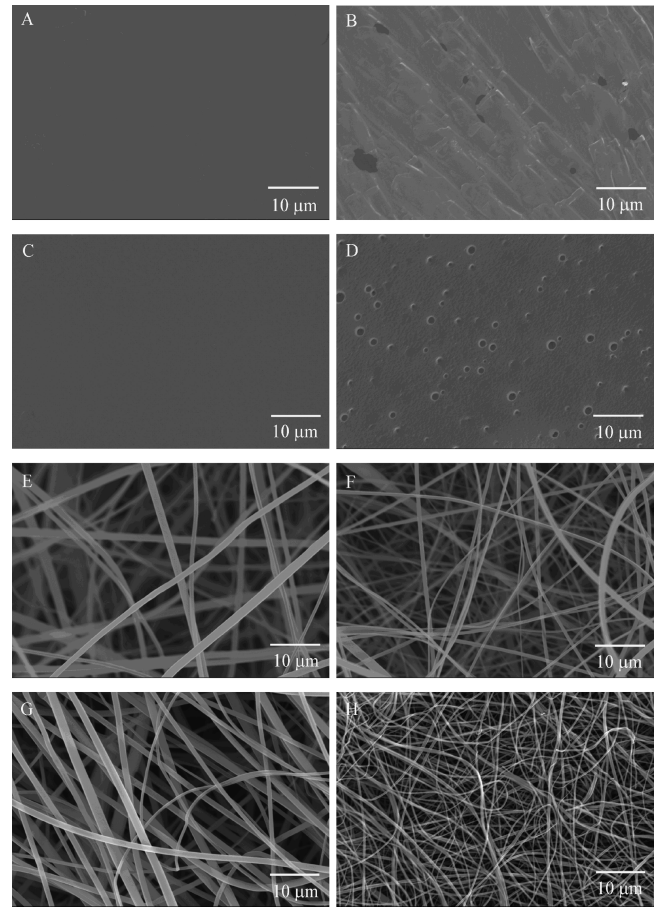

FIGURE 1. Representative SEM images of (A) solution-cast neat CA film, (B) solution-cast CA film with 5 wt % Cl-BTMP, (C) sample "A" after 24 h of ethanol treatment, (D) sample "B" after 24 h of ethanol treatment, (E) electrospun neat CA nanofiber fabric, (F) electrospun CA nanofiber fabric with 5 wt % Cl-BTMP, (G) sample "E" after 24 h of ethanol treatment, and (H) sample "F" after 24 h of ethanol treatment.

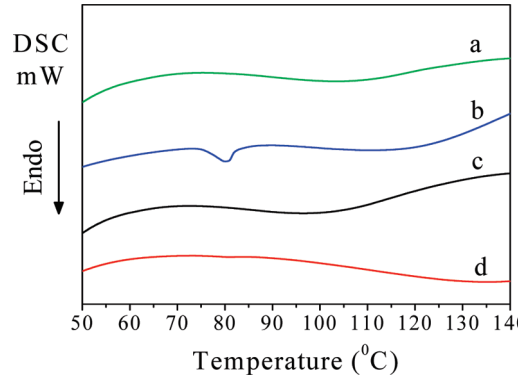

FIGURE 2. DSC curves of (a) solvent -cast CA film, (b) solvent-cast CA film with 5 wt % Cl-BTMP, (c) electrospun CA nanofiber, and (d) electrospun CA nanofiber with 5 wt % Cl-BTMP.

curves of the electrospun neat CA nanofibers (curve "c") and CA nanofibers containing 5 wt % Cl-BTMP (curve "d") were similar; no melting peak of Cl-BTMP could be detected, indicating uniform and/or molecular distribution of Cl-BTMP in the CA matrix.

In the antimicrobial efficacy studies, all of the microbial species were provided by the American Type Culture

Table 1. Comparison of Antimicrobial Efficacies between Solution-Cast CA Films and Electrospun CA Nanofibers with 5 wt % Cl-BTMP[a]

species		log reduction of various microbes after contacting the specimens for different periods of time[b] (min)				
		15	30	60	240	480
S. aureus	film	0	0	0	2 log	2 log
	nanofiber	1 log	4 log	6 log	6 log	6 log
MRSA	film	0	2 log	3 log	3 log	3 log
	nanofiber	6 log	6 log	6 log	6 log	6 log
VRE	film	0	1 log	2 log	3 log	3 log
	nanofiber	6 log	6 log	6 log	6 log	6 log
E. coli	film	0	0	0	2 log	2 log
	nanofiber	3 log	4 log	6 log	6 log	6 log
P. aeruginosa	film	0	0	1 log	3 log	3 log
	nanofiber	1 log	3 log	6 log	6 log	6 log
C. albicans	film	0	2 log	2 log	3 log	3 log
	nanofiber	4 log	6 log	6 log	6 log	6 log

[a] The original microbial concentration was 1×10^8 CFU/mL; the highest antimicrobial efficacy observed was a 6-log reduction of the microorganisms under the test conditions. [b] Each test was repeated 3 times, and the longest contact time of the observed log reduction for the microbes (i.e., the lowest antimicrobial efficacy observed) was reported.

Collection (ATCC, Manassas, VA). *Staphylococcus aureus* (*S. aureus*, ATCC 6538, Gram-positive), *Escherichia coli* (*E. coli*, ATCC 15597, Gram-negative), and *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 10145, Gram-negative) were chosen to represent clinically important nonresistant bacteria (19). MRSA (ATCC BAA-811) and VRE (ATCC 700221) were selected to represent drug-resistant strains because these species have caused serious problems in healthcare and various community settings (1−4). *Candida albicans* (*C. albicans*, ATCC 10231), a diploid fungus, was studied as a representative example of fungi due to its importance in the emerging fungemias of immunocompromised individuals and its role in healthcare-related infections (28). The solution-cast films and electrospun nanofiber fabrics prepared from the neat CA were studied as the control samples; and neither showed any inhibiting effect against the tested microorganisms due to the absence of Cl-BTMP. On the other hand, as summarized in Table 1, the CA film containing 5 wt % Cl-BTMP showed moderate antimicrobial activity (1−3 log of reduction); increasing the content of Cl-BTMP (data not shown) and/or extending contact time up to 480 min only improved the antimicrobial efficacy slightly. Such results were attributed to the aggregation of Cl-BTMP, i.e., the majority of the Cl-BTMP molecules were inside their own domains that were trapped in the CA matrix (the "islands-in-the-sea" morphology); only a small amount of the Cl-BTMP molecules could make contacts with the microorganisms, resulting in low antimicrobial efficacy. It is also noteworthy that the film samples had low surface-to-mass ratios, which further reduced the contact between the Cl-BTMP and the microorganisms. In contrast, the electrospun CA nanofiber fabrics containing 5 wt % Cl-BTMP had much larger specific surface areas and the Cl-BTMP molecules were uniformly dispersed in the CA matrix; therefore, at the same Cl-BTMP level of content, the nanofiber fabrics demonstrated much higher antimicrobial efficacy. The BET surface areas of electrospun nanofiber fabrics and solution cast films (with the same thickness of ~10 μm) were determined by a Micromeritics ASAP 2010 surface area analyzer using N_2 adsorption at 77 K. The results indicated that the BET surface areas of the nanofiber fabrics were approximately 2 orders of magnitude higher than those of the films, and this was consistent with the theoretical calculation based upon the density and dimension of the materials. With 5 wt % Cl-BTMP, the nanofiber fabric demonstrated a 6-log reduction of the nonresistant species after 30−60 min of contact, and a 6-log reduction of MRSA and VRE within 15 min of contact.

Parallel to these studies, zone of inhibition tests revealed that neither the films nor the nanofiber fabrics containing 5 wt % Cl-BTMP generated any noticeable inhibiting zone against the tested microorganisms during the test period of 3 days. These results indicated that both film and nanofiber samples killed microbes mainly by direct contact, and almost no Cl-BTMP leached out of the samples. Such a nonleaching characteristic led to an excellent stability of antimicrobial efficacy: at 21 °C and 50−60% relative humidity, the film and nanofiber samples containing 5 wt % Cl-BTMP were stored for more than 12 months without any significant variation of antimicrobial efficacy against the microbial species. In real applications, the nonleaching characteristic would eliminate the concern of antimicrobial agents entering the surrounding environments to cause undesirable complications.

Another criterion of antimicrobial efficacy is related to the biofilm-controlling effect (28−30). To evaluate this effect, the film and nanofiber samples were immersed individually in *S. aureus* broth at 37 °C for 3 days. As shown in Figure 3A, the surface of the neat CA film was covered with a layer of bacteria, indicating colonization and biofilm formation. Because of the large specific surface area, the bacterial cells colonized even more heavily on the neat CA nanofiber fabric (Figure 3B). Under the same conditions, the surface of the solution-cast CA film containing 5 wt.% Cl-

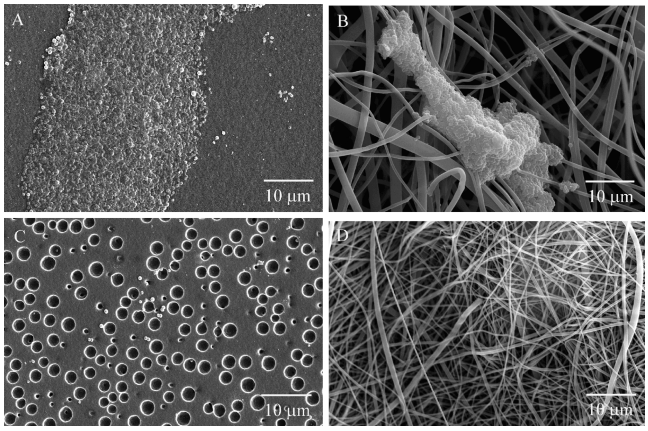

FIGURE 3. Representative SEM images of (A) solution-cast neat CA film, (B) electrospun neat CA nanofibers, (C) solution-cast CA film with 5 wt % Cl-BTMP, and (D) electrospun CA nanofiber fabric with 5 wt % Cl-BTMP, all after incubating with *S. aureus* for 3 days.

Table 2. Results of Cell Viability Test Acquired from the Rat Skin Cell Line CRL-1213

samples	cell viability after 1 h (%)	cell viability after 2 h (%)	cell viability after 6 h (%)
cell-only (control)	91.8 ± 4.7	92.6 ± 7.0	93.8 ± 4.3
electrospun CA nanofiber fabrics	89.7 ± 7.2	88.2 ± 10.2	91.3 ± 7.7
electrospun CA nanofiber fabrics containing 5 wt % Cl-BTMP	86.7 ± 6.2	88.4 ± 8.5	87.5 ± 4.1

BTMP was much cleaner (Figure 3C); only scattered bacteria could be identified (the pores were resulted from the removal of Cl-BTMP domains by the ethanol treatment; see the Supporting Information for experimental details of SEM studies). The biofilm-controlling effect was attributed to the antimicrobial activities of Cl-BTMP; i.e., when the bacteria came into contact with the film, most of them were inactivated, resulting in cleaner surfaces. It might also be possible that bacteria could sense the presence of Cl-BTMP, and they would avoid this surface because it was inappropriate for adhesion (15). For the electrospun CA nanofiber fabric containing 5 wt % Cl-BTMP (Figure 3D), because of the potent antimicrobial efficacy (see Table 1), no adherent bacteria could be identified on the surface and the sample demonstrated superior biofilm-controlling effect.

Additionally, the nanofiber fabrics containing 5 wt % Cl-BTMP also showed excellent mammal cell viability on the rat skin cell line CRL-1213, as summarized in Table 2. In the test of trypan blue assay (31), among all of the CRL-1213 cells exposed to the nanofibers (with or without Cl-BTMP), few had trypan blue-stained nuclei (indicating the death of cells); additionally, when observed by phase-contrast microscopy, the stained cells had the same size and shape as the unstained cells.

In summary, this study revealed that the electrospun composite nanofiber fabrics made of CA and Cl-BTMP were an innovative type of antimicrobial polymeric materials with durable, nonleachable, and biocompatible characteristics, and more importantly, superior antimicrobial efficacy. We envision that the antimicrobial efficacy of many polymeric materials could be substantially enhanced through the processing technique of electrospinning; this is due to the reasons that conventional processing techniques (such as solution casting) often lead to (1) aggregations of antimicrobial agents in polymer matrices and (2) low specific surface areas of the resulting materials, both of which reduce the contact between antimicrobial agents and the targeted microorganisms thus decrease the antimicrobial efficacy of the materials. The problems can be substantially mitigated through electrospinning the polymeric materials into composite nanofiber fabrics containing antimicrobial agents uniformly dispersed in polymer matrices and having large specific surface areas. The electrospun composite nanofiber fabrics are expected to find vital applications in biomedical, hygienic, and many other fields.

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Supporting Information Available: Experimental details including preparation of Cl-BTMP, fabrication of nanofibers and films containing Cl-BTMP, and studies on antiinfective efficacy, zone of inhibition, biofilm-controlling function, and cell viability analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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