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〈Research Paper〉

Electro-spun Antimicrobial Acrylic Fiber

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Abstract— Antimicrobial fibers were prepared by an electro-spinning method. Polystyrene hydantoin (PSH) was employed as an antimicrobial precursor to produce an electro-spun antimicrobial acrylic fiber. Increasing the surface area of hydrophobic antimicrobial-fibers provides enhanced antimicrobial efficacy. The biocidal activity of electro-spun acrylic fibers could be rendered through chlorine bleach treatment, and the antimicrobial effectiveness against gram-positive and gram-negative bacteria was investigated. In addition, scanning electron microscopy (SEM) demonstrated the feature of the electro-spun fibers.

Keywords: *N-Halamine, antimicrobial, biocidal, electro-spinning, acrylic fiber*

1. Introduction

As interest on medical textiles and biomaterials for healthcare increases, antimicrobial activity of fibers and polymers can be one of the important properties. The major chemicals used for the research are quaternary ammonium compounds[1,2], chitosan[3,4], metal and metal salts[5,6], photo-catalysts (titanium dioxide)[7,8], and *N*-halamines.

N-halamines are compounds which have at least one nitrogen-halogen covalent bond within molecular structure. After repeated exposure to microorganisms and antimicrobial actions, *N*-halamines can be recharged through simple exposure to diluted household bleach or halogen releasing agents; thus, the biocidal properties of *N*-halamines may be retained indefinitely[9]. The antimicrobial mechanism of *N*-halamines against microorganisms is that the oxidative halogen transfers from the *N*-halamine to a reacting site on the microorganism.

The reaction directly inhibits and inactivates metabolism, and destruction of the microorganisms is promoted[10,11]. Various *N*-halamine compounds have been used as an effective antimicrobial compounds for treating cotton, polyester, sand, paint, etc[12,13].

In our previous work[14], polyacrylonitrile (PAN)/ *N*-halamine precursor, polystyrene hydantoin (PSH) composite fibers, which contained antimicrobial activity, were prepared by dry-jet wet spinning. However, the antimicrobial function of the PAN-based fiber occurred only on the surface of the fiber, caused by the hydrophobicity of the PAN fiber. In addition, the wet-spinning process had difficulty in preparing very thin fibers, e.g. micro- or nano-sized fibers.

Electro-spinning is a well-known method for manufacturing of micro- or nano-sized polymers and fibers. In the electro-spinning process, sufficient high voltage was used to overcome the surface tension of the polymer solution at the end of a capillary. A charged jet is ejected with an increase of electrostatic forces. As the solvent evaporates, the polymers are collected on a metal grid (counter electrode) as a nano-fiber mat.

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Nano-structured materials from electro-spinning can have application field such as filtration[15], tissue engineering[16], drug release systems[17], and protective clothes[18].

In this study, electro-spun fibers which contain an *N*-halamine polymer will be prepared, and the characteristics and antimicrobial properties of the fibers will be described.

2. Experimental

2.1 Materials

Polystyrene hydantoin (PSH) used was synthesized by a reported procedure[19] in a molecular weight range of 800 ~ 5000. The PAN obtained from Solutia Inc. (St. Louis, MO) was a fiber-forming acrylonitrile copolymer. Unless otherwise noted, all chemicals and reagents were purchased from Aldrich Chemical Company (Milwaukee, WI) and were used as received. Bacterial cultures of *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* O157:H7 ATCC 43895 employed were from the American Type Culture Collection (Rockville, MD), and Trypticase soy agar from Difco Laboratories (Detroit, MI).

2.2 Electro-spinning and instrument

The PAN for electro-spinning (10% wt. PAN) was added into dimethyl formamide (DMF) and stirred 2 days at room temperature. Upon the complete dissolution of PAN in DMF, 12% wt.

PSH was added into the polymer solution and it was continuously stirred until a homogeneous solution was obtained. The PAN/PSH polymer solution was spun from an 18 gauge needle. The voltage applied was 20 kV. The distance of the spinneret-to-metal grid was fixed at 20 cm. The flow rate of the polymer solution was 5 mL/h.

The scanning electron microscope (SEM) investigation was conducted with a ZEISS EVO 50. Samples were coated with gold under argon purge before examination.

2.3 Chlorination and titration

The chlorinated nonwovens were prepared with a commercial 6% sodium hypochlorite solution, diluted to 3300 ppm of the commercial strength with distilled water, at pH 7 to produce biocidal materials. Following soaking the nonwoven in the solution at ambient temperature for 60 min, the samples were rinsed with a large excess of distilled water and were dried at 45 °C for 2 h to remove any unbonded chlorine. An iodometric/thiosulfate titration procedure was applied to measure the oxidative chlorine content. The $[Cl^+]$% in the sample was calculated with the following equation:

$$[Cl^+]\% = (V \times N \times 35.45) / (W \times 2 \times 10)$$

where $[Cl^+]$% is the wt% of oxidative chlorine on the sample, V is equal to the volume of the titrant (sodium thiosulfate solution) in mL, N is equal to the normality of the titrant, and W is the weight of the sample (g). The constants referred are 35.45 for molecular weight of Cl, 2 for the change in oxidation state of Cl during titration, and 10 to normalize the units in numerator and denominator to give % Cl.

2.4 Antimicrobial test

Staphylococcus aureus (ATCC 6538) and *Escherichia coli* O157:H7 (ATCC 43895) were employed as gram-positive and gram-negative bacteria, respectively, using a modified AATCC Test Method 100-1999. Bacterial suspensions (25 μL), which were prepared with pH 7 of 100 mM phosphate buffer, were deposited on one inch square nonwoven swatches. A second swatch was covered over the first to ensure contact between the suspension and the nonwoven. After contact times of 5, 30, and 60 min, each sample was transferred to 5 mL sterile 0.02 N sodium thiosulfate solution to quench the chlorine residue and rinse off survived bacteria. Serial dilutions of the quenched samples were obtained using pH 7 phosphate buffer, and they were plated onto Trypticase soy agar (Difco Laboratories, Detroit, MI). After incubation at 37 °C for 24 h, the plates were counted to determine the numbers of viable bacteria.

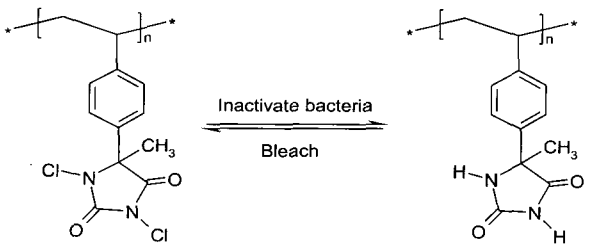


Fig. 1. The scheme of disinfection of chlorinated polystyrene hydantoin (PSH).

3. Results and Discussion

3.1 Properties of the electro-spun PAN/PSH composite fibers

In our previous research[14], antimicrobial acrylic fiber, which contained PSH, was produced by a dry-jet wet spinning method. Due to the limited surface area (6.86 x 10^{-2} m^2/g at 21 denier), sufficient antibacterial efficacies (6.5 log reduction against gram-positive *Staphylococcus aureus* bacteria in 30 min) were obtained only at relatively high concentrations of PSH (8% wt.). We assumed that Cl^+ (oxidative chlorine), which inactivates bacteria, could be released from *N*-halamines on the surface of the fiber. Since, at ambient temperature, i.e. below the Tg (glass transition temperature), the chain of the acrylic fiber could not have enough flexibility, the chlorination of *N*-halamine occurred only on the outer part of the acrylic fiber. Therefore, increased surface area of the antimicrobial acrylic fiber should enhance the biocidal efficacy.

Unlike dry-jet wet spun fibers, the electro-spun fibers would be micro- or nano-sized. Hence, electro-spun fibers possess a sufficient surface-area. Table 1 indicates that the diameter of the composite fiber was decreased dramatically during electro-spinning.

Therefore, surface area was enhanced, which supports the increased oxidative chlorine content to inactivate bacteria. The predicted value of oxidative chlorine content in chlorinated PAN/PSH composite fibers at 1 denier was 0.50 [Cl^+]%.

However, the actual chlorine content of electro-spun fibers was relatively lower (0.60 [Cl^+]% at 0.001 denier). Even though the theoretical surface area of the electro-spun PAN/PSH fiber is much higher than that of the dry-jet wet spun acrylic fiber, the chlorine content of the electro-spun acrylic fiber did not reach the expected amount. It is presumed that the PAN/PSH acrylic fiber has hydrophobic and rigid characteristics, and the entangled parts of the electro-spun fiber could be bonded. Hence, on the entangled parts, PSH has difficulty to be converted to *N*-halamine and it could be one of factors for the relatively lower oxidative chlorine.

Fig. 2 shows the SEM images of the electro-spun fibers. The structure of the nonwoven formed an even fiber structure when the length between a metal grid and the edge of the spinneret (syringe) was 20 cm at 20 kV with 0.5 mL/h as the extrusion rate. The average size of the fiber was around 0.7 μm with a dense entanglement and accompanying fiber fusion or bonding. It should be mentioned that although electro-spinning provides increased surface area and unique properties, much entanglement and bonding could make the hydrophobic fiber possess less surface area than might have been anticipated.

3.2 Antibacterial efficacy against gram-positive and gram-negative microorganisms

Through our experience for antimicrobial efficacy against bacteria, in case of *N*-halamine materials,

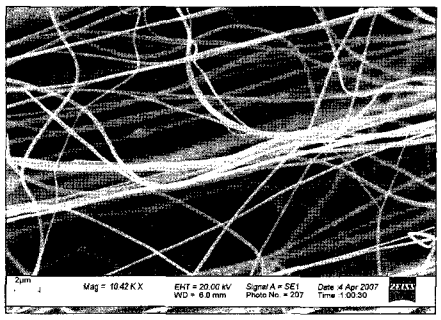
Table 1. The size and chlorine content of the PAN/PSH composite fiber(PAN/PSH, 100/12, w/w)

Spinning method	Diameter of the fiber (μm)	Denier(g/9000 m)	[Cl^+]%	Surface area(m^2/g)
Electro-spinning	~ 0.7	~ 0.001	0.60	989.60 x 10^{-2}
Dry-jet wet spinning [a]	22	1	0.50 [b]	31.10 x 10^{-2}
	100	21	0.11 [a]	6.86 x 10^{-2}

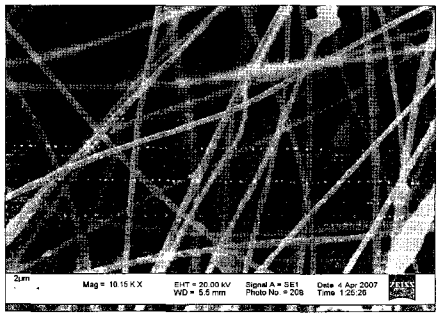
[a] Previous data[14]. [b] Predicted value.

about 0.10% of oxidative chlorine is a sufficient amount to inactivate bacteria[14].

Therefore, the content of oxidative chlorine, 0.60 [Cl^+]% of the electro-spun fiber should be sufficient to kill bacteria. Table 2 demonstrates the biocidal efficacies against *Staphylococcus aureus* and *Escherichia coli* O157:H7, gram-positive and gram-negative bacteria, respectively. The antimicrobial acrylic fibers inactivated both gram-positive and gram-negative bacteria within 30 min with 6 log reductions.



(a)



(b)

Fig. 2. SEM traces of (a) PAN fiber, (b) PAN/PSH fiber.

Because the dissociation constant of an imide structure is higher than for an amide structure[20], the amide halamine is less sensitive than the imide halamine in inactivation rate (Fig. 3). We presume that the adjacent alkyl groups of the *N*-halamine may stabilize the *N*-halamine through an inductive effect in the amide structure, while the delocalization caused by adjacent carbonyl groups of the imide structure make the imide halamine more reactive than the amide halamine.

Consequently, imide halamines provide rapid inactivation of bacteria, and amide halamines retain biocidal efficacy for longer time periods than do imide halamines.

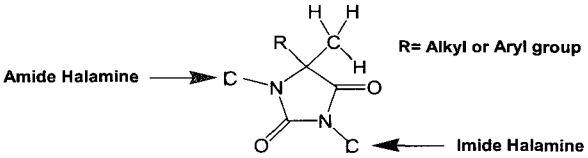


Fig. 3. N-halamine structure of a hydantoin moiety.

4. Conclusions

An antimcirobial electro-spun acrylic fiber was prepared. Dimethyl formamide (DMF) was employed as a solvent for polyacrylonitrile (PAN)/ polystyrene hydantoin (PSH) composite fiber.

When the length between the needle of a syringe and a metal grid was 20 cm at 20 kV with 5 mL/h flow rate, the electro-spun fiber resulted in an optimal nonwoven structure. It was shown that the enhanced surface area of the electro-spun antimicrobial acrylic fiber contained

Table 2. Biocidal efficacy against microorganisms

Samples [a]	Contact time	*E. coli* O157:H7 [b]		*Staphylococcus aureus* [c]	
		Bacterial reduction		Bacterial reduction	
		% reduction	Log reduction	% reduction	Log reduction
Unchlorinated	5 min	31.63	0.2	88.28	0.9
	30 min	45.31	0.3	89.11	1.0
	60 min	50.32	0.3	89.53	1.0
Chlorinated	5 min	97.68	1.6	92.00	1.1
	30 min	100	6.2	100	6.2
	60 min	100	6.2	100	6.2

[a] PAN/PSH (100/12, w/w) nonwovens were used.

[b] Total bacteria: 1.47 x 10^6 cfu/sample.

[c] Total bacteria: 1.60 x 10^6 cfu/sample.

over 0.60% of oxidative chlorine content, which is sufficient to inactivate bacteria. In addition, the diameter of electro-spun fibers was around 0.7 μm. Bacterial effectiveness indicated an inactivation of gram-positive and gram-negative bacteria within 30 min with 6 log reductions.

The electro-spun antimicrobial acrylic fiber (nonwoven) should be applicable to public health-care areas.

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