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Antimicrobial effects of positively charged, conductive electrospun polymer fibers

Somdatta Bhattacharya^a, Domyoung Kim^a, Sneha Gopal^a, Aaron Tice^b, Kening Lang^c, Jonathan S. Dordick^a, Joel L. Plawsky^{a,*}, Robert J. Linhardt^{a,c,*}

^aHoward P. Isermann Department of Chemical and Biological Engineering and Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180

^bDepartment of Mechanical, Aerospace and Nuclear Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA

^cDepartment of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA

Abstract

In recent years, electrospun polymer fibers have gained attention for various antibacterial applications. In this work, the effect of positively charged polymer fiber mats as antibacterial gauze is studied using electrospun poly(caprolactone) and polyaniline nanofibers. Chloroxylenol, an established anti-microbial agent is used for the first time as a secondary dopant to polyaniline during the electrospinning process to make the surface of the polyaniline fiber positively charged. Both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* are used to investigate the antibacterial activity of the positively charged and uncharged polymer surfaces. The results surprisingly show that the polyaniline surface can inhibit the growth of both bacteria even when chloroxylenol is used below its minimum inhibitory concentration. This study provides new

*Corresponding authors J.L. Plawsky, plawsky@rpi.edu Phone: (518)276-6049; R.J. Linhardt, linhar@rpi.edu Phone: (518) 276-3404.

Author Contributions

Somdatta Bhattacharya led the research and drafted the manuscript. Domyoung Kim, Sneha Gopal, Aaron Tice, and Kening Lang contributed performed various characterization studies. Jonathan S. Dordick, Joel L. Plawsky, and Robert J. Linhardt provided supervision and funding for this research and assisted in the revisions and proofing of the manuscript.

Credit author Statement

Conceptualization, SB, DK, RJL and JLP conceptualized this study

Investigation, SB, DK, SG, AT and KL conducted experiments

Resources, RJL and JSD provided the resources needed for these studies

Writing - Original Draft, SB and RJL drafted this manuscript

Writing - Review & Editing, SB, RJL, JLP and JSD revised and edited this manuscript

Supervision, RJL, JLP and JSD supervised this study.

Project administration, RJL, JLP and JSD administered this project.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

insights allowing the better understanding of dopant-based, intrinsically conducting polymer surfaces for use as antibacterial fiber mats.

Keywords

Antibacterial materials; Electrospinning; Nanofibers; Structural modifications; Chloroxylenol; Secondary doping; Charged-polymers

1. Introduction

The leading cause of mortality and morbidity worldwide is infectious diseases [1–3], claiming 15 million deaths per year [2,4]. Treatment of injuries, from a simple cut to a deep wound, often require the heavy use of antibiotics, applied either orally or by injection. Unfortunately, the systemic use of antibiotics often leads to unwanted side effects that prove harmful to the patient [5–7]. Thus, new approaches for controlled and localized release of antibiotics and antimicrobials have been investigated to prevent unwanted complications and the overuse of these critical pharmaceuticals [7]. Electrospinning is an exceptionally simple and versatile method to fabricate non-woven fiber structures that resemble the extracellular matrix (ECM). Antimicrobial loaded, electrospun fiber mats, using biocompatible polymers, can be used alone or in conjunction with other antibacterial agents to combat such deadly infections. Extensive research is ongoing, aimed at investigating electrospun fibers as drug delivery systems. Various approaches, such as electrospinning multilayer fibers as vehicles for controlled diffusional release [8,9], adding nanoparticles to facilitate drug adsorption [10,11] altering polymer or drug hydrophilicity [12,13] to modify polymer-drug interactions [14] and mixing different types of polymers of varying molecular structure [15], hydrophilicity [16], and degradation rates [7], have been investigated. However, these methods are generally either too complicated to be economically undertaken, are ineffective, or have uncertain cytotoxicity.

The effect of electrical conductivity, as well as surface charge, on antibacterial activity is a relatively new and an intriguing area of research. Thus far, studies have examined fabricating conductive antibacterial implants using approaches including Cu-based graphene sheets [17], graphene oxide layers [18], silver incorporated poly(aniline) nanofibers [19], carbon nanotubes and glycidyl methacrylate functionalized, quaternized, chitosan cryogels [20]. These studies suggest an important relationship between the conductivity of materials like graphene, graphene oxide, and silver, and the destruction of the bacterial cell membrane. However all these methods involve the use of nanoparticles and other materials that are inherently toxic to humans and to the environment [21,22]. In this report, we use the slow and localized release of a common and well-known antimicrobial agent, chloroxylenol (4-chloro-3,5-dimethylphenol), encapsulated in polymer fibers to address these issues.

Polyaniline (PANI) is a well-studied, intrinsically conducting polymer that is a highly stable material and easy to synthesize [23,24]. It has also been successfully established as an antimicrobial agent on conjugation with several organics and non-organics [25–27]. The emeraldine salt of PANI, formed by using primary dopants, such as camphorsulfonic acid, has been shown to exhibit higher conductivity when solvents such as *m*-cresol and *p*-cresol

are added as secondary dopants [28], The use of such dopants transforms the backbone of PANI into a highly positively charged domain. We speculated that the positively charged backbone might render PANI a useful, antimicrobial material. We selected the nontoxic, chloroxylenol, to replace the more toxic *m*-cresol or *p*-cresol as a secondary dopant to polyaniline to enhance the expected antimicrobial activity of PANI. Our hypothesis was that chloroxylenol, with a pKa value of 9.7, has similar electron donating groups as cresol and that the chlorine present in chloroxylenol should enhance electrophilic aromatic interactions with the PANI backbone structure, making it a highly efficient secondary dopant. PANI develops positive charge in the form of quaternary ammonium cations due to the interaction of the poly(aniline) chain with secondary dopants. The antibacterial effects of polymer chains with anion-exchange groups, such as quaternary ammonium compounds, have been explained in the literature by two mechanisms [29–31], One mechanism suggests that the positively charged polymer chains displace the divalent cations like Ca^{2+} and Mg^{2+} that are responsible for holding together the lipopolysaccharide network of the outer membrane of Gram-negative bacteria [32]. Another mechanism suggests that positively charged polymer chains penetrate the bacterial inner membrane, causing cell leakage and eventually bacterial inactivation [33], In the case of Gram-negative bacteria, such as like *Escherichia coli*, either mechanism would be lethal.

To the best of our knowledge, chloroxylenol has not yet been studied as a secondary dopant of poly(aniline), and the findings reported herein may offer new insights into both the antibacterial action of conductive and positively charged fiber films made entirely out of biocompatible polymers. This study designs PANI-poly(ethylene oxide) (PEO) “smart” antibacterial fibrous mat mesh implants for biomedical applications. It also examines the use of biocompatible and biodegradable polycaprolactone (PCL) with chloroxylenol as non-conductive (neutral or negatively charged) nanofiber mesh implants for biomedical applications.

2. Material and Methods

Chemicals included polyaniline (PANI, emeraldine base, $M_w = 65,000$) and poly (ethylene oxide) (PEO, $M_w = 2,000,000$ g/mol), poly(ϵ -caprolactone) (PCL, $M_w = 14,000$ g/mol), (+)-camphor-10-sulfonic acid (CSA), chloroxylenol and chloroform (HPLC grade) were purchased from Sigma-Aldrich, St. Louis, MO. All materials were used without further purification.

2.1 PANI-based materials and solutions

All processing was done at room temperature. Four different solutions were made for electrospinning fiber mats.

2.1.1. PANI-PEO solution – in 10 mL chloroform, 0.087 g CSA was added and kept under magnetic stirring for 4 h. PANI (0.063 g) was added to the homogenous solution and left under magnetic stirring overnight. PEO (0.05 g) was added the next day and left for 24 h to make certain that it completely dissolved.

2.1.2. PANI-chloroxylenol-PEO (PC1) solution - 0.5 g chloroxylenol was added to the PANI-PEO solution, when the CSA was added, to ensure that the chloroxylenol dissolved completely in the chloroform before being mixed with the polymer.

2.1.3. PCL solution - 1 g PCL was added to 10 mL chloroform and left under magnetic stirring for 24 h.

2.1.4. (PCL)-chloroxylenol solution (PC2) - 0.5 g chloroxylenol was added along with 1 g PCL to 10 mL chloroform solution and left for 24 h under magnetic stirring.

2.2. Electrospinning nanofibers

A schematic of the electrospinning process and a photograph of the apparatus are shown in Figure 1. The rotating mandrel with separation rods (US Provisional Patent: 62/845,535) was covered with aluminum foil and the distance between the rods was adjusted to optimize the fiber forming conditions. The spinneret was connected to the positive lead of a high voltage supply (ES 50P-5W, Gamma High Voltage Research Inc.). A mono-axial spinneret (MECC, Ogori, Fukuoka, Japan) was fitted with a blunt tip aluminum needle (23 Gauge) that has an outer diameter of 0.64 mm and connected to the high voltage supply. The needle tip to collector surface distance was varied from 15–18 cm and charging voltages of 15 – 20 kV were used. The four different solutions were delivered to the spinneret by a syringe pump (NE-1000, New Era Pump System Inc., Wantagh, New York, USA) and the flow rate of fluid was kept fixed at 1 mL/h. Highly aligned fibers were formed perpendicular to the orientation of the aluminum rods. The temperature and the humidity in the electrospinning box was constantly monitored using a digital humidity and temperature monitor (AcuRite®) and was maintained at $20\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ and 16%, respectively.

2.3. Strains and culture conditions

Staphylococcus aureus (ATCC 33807) was purchased from ATCC (Manassas, VA). *E. coli* K-12 (Bacterial strain #49761) was purchased from Addgene (Watertown, MA). The strains used in this study were cultivated in brain heart infusion (BHI) agar or in broth (Becton Dickinson, Franklin Lakes, NJ) for one day at $37\text{ }^{\circ}\text{C}$.

2.4. Determination of minimum inhibitory concentration (MIC)

MIC values were determined separately for chloroxylenol. Cultures (3 ml of 5×10^5 cells/ml) were added to test tubes and different amounts of chloroxylenol (using serial dilution) were added to these cultures to obtain a mass range of 0.1 to 1 mg of chloroxylenol. Cultures were incubated at 37°C for 1 day to determine MIC values (the lowest concentrations preventing bacterial growth).

2.5. Bactericidal activity

A colony-forming unit (CFU) is a unit used to count the number of viable bacteria cells in a sample (10^9 CFU/ml or 10^7 CFU/ml) treated with fibers excluding bacteria and debris on plates. It represents a viable bacterial cell in solution that when grown on solid media yield and individual cell colony we can count. The number of colony forming units per unit volume of diluted and plated solution can be used to determine the number of viable cells

per ml in the solution. The dose-dependent antimicrobial activity of the fibers was determined by treating 100 μ L of a microbial suspension containing 10^9 CFU/mL with 0 to 1 mg PANI-PEO fibers (both control and PCI fibers) and 10^7 CFU/mL with PCL fibers (both control and PC2 fibers) in 24-well plates for 1 h. After 1 h of incubation, 30 μ L aliquots from the mixture were plated on the BHI agar and then the surviving colonies were counted after overnight incubation. The activity of fibers with chloroxyleneol-based antimicrobial was calculated by comparison with the colony numbers for fibers without chloro-xyleneol-based antimicrobials and control in phosphate buffered saline (PBS).

2.6. Cell cytotoxicity assay

HepG2 cells (Passage 10), a human hepatocellular carcinoma cell line was cultured in Eagle's Minimum Essential Medium supplemented with 10% FBS and 1% Penstrep. Cells were seeded at a density of 40,000 cells/well in a 96-well plate and maintained in an incubator at 37 °C and 5% CO₂. After 18 h, they were exposed to both PCL fibers and PC2 fibers. The fiber masses tested were 0 mg, 0.1 mg, 0.3 mg, 0.5 mg, 0.8 mg and 1 mg per well. A positive control without any fibers and a negative control where the cells were treated with 0.05% saponin were also included in the experiment. The cells were treated with the fibers for 24 h after which the fibers were removed. The viability of the cells was then assessed using a WST-1 cell proliferation kit. 10 μ L of a mixture of WST-1 developer reagent and electron mediator reagent per well was added to each sample. The plate was incubated for 45 min at 37 °C and 5% CO₂ following which the absorbance of the samples was measured at 450 nm. The viability of the cells was calculated using the following formula

$$\text{Viability} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{negative Control}}}{\text{Abs}_{\text{positive control}} - \text{Abs}_{\text{negative Control}}}$$

2.7. Microstructure Examination

The thermal stability and the decomposition properties of PC1 fiber and its constituents (pure PEO powder, pure PANI powder, pure CSA powder, and pure chloroxyleneol powder) as well as PC2 fiber and its constituents (PCL powder and chloroxyleneol) were analyzed through thermogravimetric analysis. A computer-controlled TGA-Q50 apparatus (New Castle, Delaware, USA) was used to determine the thermal degradation of the fibers and its constituents. The samples were heated from room temperature to 1000 °C at a constant heating rate of 1 °C /min under constant nitrogen flow. The average decomposition temperatures and the shift in decomposition peaks were determined by TA Instruments Universal Analysis software V4.7A. Furthermore, TA Instruments defines the detection limit of TGA to 0.1% by mass of the sample. A Carl Zeiss Supra field emission scanning electron microscope (Hillsboro, USA—resolution at 1 kV—2.5 nm) was used to investigate the morphology of the different fibers. The average fiber diameters were calculated using NIH ImageJ software (National Institute of Health, MD, USA). The diameters of nearly 3000 individual fibers from 10 identical electrospinning experiments were employed in this fiber diameter analysis. The crystallinity of the fibers with and without chloroxyleneol was studied using a Bruker D8-DISCOVER X-ray diffractometer and compared with the crystal

structure of pure chloroxylenol. The X-ray diffraction (XRD) pattern analysis was performed using Bruker's DIFFRAC.EVA software. Fourier-transform infrared (FT-IR) spectra of the fiber sets was carried out using a Perkin Elmer Spectrum One FFTR Spectrometer using KBr pellets.

The current-voltage characteristics of electrospun fiber mats were measured using a Keithley 4200 IV/CV Meter. The method relies on a linear array of four equally spaced tips that are pressed onto the surface of the material. A voltage sweep from -0.005V to $+0.005\text{V}$ was imposed through the two outer probes and the current was measured across the two inner probes. The resistivity was measured by Eq. (1):

$$\rho = (V/I) \times A/t \quad \text{Eq. (1)}$$

where ρ is resistivity, V is the voltage measured, I is the current passed, t is the thickness of the fiber mat, which was measured using a profilometer (Bruker Optical Profilometer), and A is the area of the fiber mat (distance between two probes multiplied with the width of the mat). The conductivity of the mat was calculated by Eq. (2):

$$\sigma = 1/\rho \quad \text{Eq. (2)}$$

where σ is conductivity.

A Shimadzu QP5050A mass spectrometer with a GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) was used for the analyses. The separation of the volatile components of the mixtures was performed on a ZB-5MSi $30\text{ m} \times 0.25\text{ mm}$ column, with pore size of 25 pm (Phenomenex, USA) using the following temperature gradient: initial temperature of $80\text{ }^{\circ}\text{C}$, hold at $80\text{ }^{\circ}\text{C}$ for 5 min , followed by a linear gradient to $325\text{ }^{\circ}\text{C}$ at $40\text{ }^{\circ}\text{C/min}$, hold at $325\text{ }^{\circ}\text{C}$ for 0.87 min with a total analyses time of 12 min . Samples ($2\text{ }\mu\text{L}$) were injected into the injector port using a Shimadzu AOC-20i/20s autosampler. The injector port was held at $250\text{ }^{\circ}\text{C}$. The detector temperature was $250\text{ }^{\circ}\text{C}$. The instrument was operated in a split mode ($20:1$), helium was used as a carrier gas at flow rate of 64 L/min . The mass spectrometer was operated in electron ionization mode with selected ion monitoring of molecular ions at $m/z\ 156$ and $m/z\ 158$. The instrument control, data acquisition and data processing were performed using Shimadzu GCMS solutions software (version 1.20)

3. Results and Discussion

A previously developed wet-dry electrospinning technique was employed to produce the PANI-PEO, PCL, PC1, and PC2 nanofibers in a one-step process. In wet-dry electrospinning, the polymers are dissolved in an organic solvent or solvent mixture. The solvent rapidly evaporates from the exiting fluid jet to form a polymer filament. This filament is then collected on a rotating mandrel as a fiber mat. In the current study, we used two different sets of polymers to understand the impact of positively charged polymer surfaces on their antibacterial activity. In the case of PANI-PEO fibers, we electrospun one group of fibers without the secondary dopant, chloroxylenol, and these are referred to as control PANI-PEO fibers. Fig. 2a shows a photograph of the electrospun control PANI-PEO fiber mat. The average diameter of the control PANI-PEO fibers is $600\text{ nm} \pm 70\text{ nm}$ (Fig. 2b and c). The

fibers electrospun with the secondary dopant, chloroxylenol, are referred to as PC1. The role of secondary dopants was first demonstrated by McDiarmid and coworkers [28] who demonstrated that secondary dopant enhanced the conductivity of emeraldine salt of PANI by several orders of magnitude when compared to fibers prepared with only a primary dopant. A plethora of secondary dopants for PANI have been studied but we selected a unique secondary dopant, chloroxylenol, having both the required electron donating groups as well as antimicrobial properties. In the preparation of PC1 fibers, the primary dopant (camphorsulfonic acid) and secondary dopant (chloroxylenol) were co-dissolved to ensure that they were completely dispersed within the PANI-PEO. PC1 fibers (Fig. 2d) have a darker green color compared to PANI-PEO control fibers. The average fiber diameter obtained for PANI-PEO electrospun with CSA/chloroxylenol dopants was $1.3 \mu\text{m} \pm 0.14 \mu\text{m}$ ($n = 5$) (Fig. 2e and f). Chloroxylenol secondary dopant for PANI-PEO fibers resulted in a conductivity of $3.2 \text{ S/cm} \pm 0.23 \text{ S/cm}$ that is unexpectedly higher than the conductivity achieved using *m*-cresol as a secondary dopant.

Poly(ϵ -caprolactone) (PCL) was electrospun with chloroxylenol to prepare a white antibacterial fiber gauze that was neutral or had a weak negative charge (Fig. 3). Again, two groups of PCL fibers were prepared one containing chloroxylenol (PC2) and the other without chloroxylenol referred to as control PCL fibers. The control PCL fibers had an average diameter of $130 \text{ nm} \pm 50 \text{ nm}$ ($n = 5$) (Fig. 3a) and the SEM image as well as the average diameter distribution of these fibers is shown in Fig. 3b and c. PCL is an insulating polymer and adding chloroxylenol results in no enhancement of conductivity. A photograph of the PC2 fibers is shown in Fig. 3d. The average diameter of the PC2 fibers was $230 \text{ nm} \pm 30 \text{ nm}$ ($n = 5$) (Fig. 3e and f).

TGA analysis shows the different decomposition temperatures for PC1 and PC2 fibers. The decomposition temperatures of the different constituent elements of the fibers are shown in Fig. 4a. The normalized weight derivatives of these decomposition peaks are shown in Fig. 4b. Pure PEO powder, represented by black line in Fig. 4a and b, had a decomposition temperature of 360°C . There are two major stages of weight losses for the pure PANI powder, represented by the red line in Fig. 4a and b. The first weight loss at the lower temperature is associated with the loss of water. The second thermal degradation region between 500 and 700°C is related to the structural degradation of the polymer. Pure CSA powder degrades at 200°C and is shown by the blue line in Fig. 4a and b. At 120°C the now liquid chloroxylenol is volatilized by nitrogen flow (represented by the green line in Fig. 4a and b). The PC1 fibers, represented by pink line in Fig. 4a and b, show four major weight loss peaks. The peak at about 100°C is attributed to moisture loss and the sharp peak at about 120°C is assigned to the presence of chloroxylenol in the fiber. The shift in the peak is probably due to a reaction with the PANI/CSA backbone. The peaks at 200°C and 380°C are attributed to the presence of CSA and PEO in the fibers. Thermogravimetric analysis and normalized derivatized weight of PC2 fibers are shown in Fig. 4c and d. The weight loss peak of the chloroxylenol was observed at 120°C . The weight loss peak of pure PCL powder was at 400°C (Fig. 4c and d). The PC2 fibers show two sharp weight loss peaks. The one at 120°C is attributed to the volatilization by nitrogen flow of chloroxylenol that is present in the PC2 fibers. The second weight loss peak at 400°C is assigned to the decomposition of PCL.

XRD spectra of PC1 fibers showed distinct diffraction peaks at $2\theta = 19^\circ$, represented by peak #3, and 25° , represented by peak #4 (Fig. 5a). These peaks are ascribed to periodicity parallel and perpendicular to the PANI chains, respectively. The additional peaks (#1, 2, 5, and 6) in the PC1 fiber were due to the doping effect of chloroxylenol with the PANI chains. FTIR of the PANI-PEO and PC1 is shown in Fig. 5b. The N-H stretching of aromatic amines corresponds to the peak at 3443 cm^{-1} . The peak at 2885 cm^{-1} is assigned to the CH_2 asymmetric stretching of the PEO structure. The absorption peaks at 1568 and 1480 cm^{-1} are attributed to C-C stretching of the quinoid and benzenoid rings of PANI, respectively. C-N stretching of PANI is assigned to 1291 and 1242 cm^{-1} . The peak at 1125 cm^{-1} represents the benzenoid-quinoid-benzenoid stretching of emeraldine salt of PANI. The sharpness of the peak numbers 4, 5, 6 and 7 increased which confirmed to the formation of a stretched PANI structure in the PC1 fiber.

XRD of PCL fibers shows distinct diffraction peaks at $2\theta = 21^\circ$ (corresponding to peak #1) and 24° (corresponding to peak #2) but no crystalline peaks were observed for chloroxylenol (Fig. 5c). FTIR of PCL fibers showed typical bands in the near IR region (Fig. 5d). The peak at 2936 cm^{-1} is attributed to the presence of methylene groups. A carboxylic acid bend band (C-O-H) was observed at 1462 cm^{-1} (in-plane) and at 930 cm^{-1} (out-of-plane). Bands observed at $1300\text{--}1000\text{ cm}^{-1}$ were ascribed to C-O stretching. The sharp band at 730 cm^{-1} corresponds to the scissor-like bending of the methylene groups. In the PC2 fibers, an additional sharp peak observed at 1700 cm^{-1} corresponds to the aromatic C=C bending. The peak at 1191 cm^{-1} is assigned to the C- CH_3 asymmetric stretch.

The electrical conductivity of aligned, electrospun-blended fibers of PC1, PC2, PANI-PEO (control 1) and PCL (control 2) was confirmed using a four-point-probe conductivity measurement and Van der Pauw geometry. The conductivities for PC1 and PC2 samples were $3.1 \pm 0.23\text{ S/cm}$ and $1.5 \pm 0.37 \times 10^{-7}\text{ S/cm}$, respectively (Table 1). The blended PC1 fibers show at least six-orders of magnitude higher conductivities than the control and PCL fibers tested.

We challenged the fibers with two typical pathogenic bacteria, Gram-negative *E. coli* K-12 and Gram-positive *S. aureus* to evaluate the antibacterial activity of the PC1 and PC2. In the contact-killing assay the control (PANI-PEO, PCL) and PC1, PC2 samples of 0.1 mg to 1 mg were challenged with a bacterial suspension (10^7 or 10^9 CFU/mL) and bacterial killing was assessed using agar plate counting. The dose-dependent antibacterial activities of the fibers are shown in Fig. 6. After 1 h contact at room temperature in PBS, PC1 showed a significant log reduction of *E. coli*, and *S. aureus* but the control samples (PANI-PEO) resulted an only slight reduction, as expected since PANI has known antimicrobial properties [34,35]. When the amount of PC1 was increased the PC1 fibers were able to completely kill *E. coli* and *S. aureus* at 1.0 mg and 0.5 mg/mL , respectively (Fig. 6a,b).

Next, we examined the antimicrobial activity of PC2 fibers. When 1 mg PC2 fibers was added to $\sim 10^7\text{ CFU/mL}$ *E. coli* and *S. aureus*, a complete 7-log killing of *E. coli* and 4.5 log killing of *S. aureus* were observed (Fig. 6c,d). The control sample (PCL) did not exhibit any reduction in viable bacteria even at 1 mg/mL . As shown above, 1 mg of PC1 and PC2 fibers was able to completely kill *E. coli*, however, PC1 fibers showed complete killing of *S.*

aureus at 0.5 mg and PC2 fibers was much less effective against *S. aureus* even when using 1 mg of fibers. The results for the two fibers were clearly different with PC1 preferentially killing *S. aureus* and PC2 preferentially killing *E. coli*.

We speculate that the positively charged backbone of PC1 along with the antimicrobial activity of chloroxylenol both play a role in effectively killing both *E. coli* and *S. aureus*. The MIC of chloroxylenol alone was found out to be 0.5 mg/mL (SI Fig. 1). In our experiments, we have used 0.071 mg/mL, which is lower than the MIC of chloroxylenol to determine the effect of different polymer surfaces on the fiber. The viability of *E. coli* and *S. aureus* depends on the charge of the polymer surface to which it is exposed (Fig. 6). On PC2 fibers *S. aureus* showed higher resistance to the polymer surface than *E. coli*. We suggest that this difference is due to differences in Gram-positive and Gram-negative bacteria and how they interact with positively charged surfaces [36]. Corroborating previous studies on conducting polymers, we see the same antibacterial effects with PC1 and PC2 fibers. PC2 fibers were effective in killing *E. coli* at 1 mg because of the rapid release of chloroxylenol as demonstrated in Figure 7. However, 1 mg of PC2 fibers was ineffective in completely killing *S. aureus* because of the absence of a positively charged surface.

The release rate of chloroxylenol from fibers was assessed by GCMS and was carried out using 0.5 mg of fibers in 6 vials containing 1 mL of DI water to assess the release rate of chloroxylenol. Aliquots (30 μ L) were collected from each vial at different time points and the release profile was analyzed by GCMS. The profile of chloroxylenol from PC1 and PC2 is shown in (Fig. 7). Chloroxylenol releases faster from the PC2 fibers than the PC1 fibers. The final concentration of the drug determined by GCMS was 70 μ g/mL, which represents the total amount of chloroxylenol calculated to be present within the fiber (see SI). This is substantially lower than the MIC.

CONCLUSIONS

A conductive, positively charged polymer surface was compared with a neutral or slightly negatively charged polymer surface in the study contact antimicrobial activity. The positively charged surface of the PANI-PEO polymer combined with the release of chloroxylenol resulted in potent antimicrobial activity makes these polymer fiber mats extremely effective against both Gram-positive and Gram-negative bacteria. The encapsulation of the antimicrobial, chloroxylenol, resulted in a localized release of this agent increasing its effectiveness and reducing the possibility of unwanted side effects. The results obtained in this study suggest that it is possible to fine-tune the surface properties of fiber mats comprised of different biocompatible polymers to obtain useful antimicrobial properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Highlights

- PANI/PEO and PCL nanofibers were electrospun.
- Highly aligned nanofibers were and collected on a specialized mandrel.
- PANI/PEO nanofibers entrapped chlorosulfonic acid and chloroxylenol and were electrically conductive.
- The PANI/PEO and PCL nanofibers containing chloroxylenol were antimicrobial.

Fig. 1. Wet-dry monoaxial electrospinning process and the method to produce polymer fibers entrapping chloroxylenol (a) Schematic of the process (b) photograph of the process.

Fig. 2.

(a) Photograph of control PANI-PEO fibers, (b) SEM of control PANI-PEO fibers (c) Diameter distribution of control PANI-PEO fibers, (d) Photograph of PC1 fibers, (e) SEM of PC1 fibers, (f) Diameter distribution of PC1 fibers.

Fig. 3.

(a) Photograph of control PCL fibers, (b) SEM of control PCL fibers (c) Diameter distribution of control PCL fibers, (d) Photograph of PC2 fibers, (e) SEM of PC2 fibers, (f) Diameter distribution of PC2 fibers.

Fig. 4.

(a, b) TGA and derivatized weights of PC1 fiber set and its constituents, (c, d) TGA and derivatized weights of PC2 fiber set and its constituents.

Fig. 5.
(a, c) XRD of PC1 and PC2 fibers, respectively, (b, d) FTIR of PC1 and PC2 fibers respectively.

Fig. 6. Antimicrobial activities of fibers. (a) CFU assay showing dose-dependent killing of *E. coli* with PANI-PEO (red) or PC1 (green); (b) CFU assay showing dose-dependent killing of *S. aureus* with PANI-PEO (red) or PC1 (green); (c) CFU assay showing dose-dependent killing of *E. coli* with PCL (red) or PC2 (green); (d) CFU assay showing dose-dependent killing of *S. aureus* with PCL (red) or PC2 (green). Controls with PBS are incubated. Bacterial killing was determined at 1 h based on a viable plate assay. ** Indicates that no colonies were detected. The values were plotted with error bars representing standard deviation from triplicate independent assays.

Fig. 7.
Chloroxylenol release profile from the fibers: PC1 (blue curve), PC2 (red curve)

Table 1.

Conductivity of PANI-PEO blended nanofibers spun from various solvents.

Solvent used in electrospinning	Conductivity (S/cm) (n = 5)
PANI-PEO fibers (control 1)	$9.8 \pm 0.51 \times 10^{-6}$
PC1 fibers	3.1 ± 0.23
PCL fibers (control 2)	$6.5 \pm 0.72 \times 10^{-8}$
PC2 fibers	$1.5 \pm 0.37 \times 10^{-7}$