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Long-term antimicrobial effect of nisin released from electrospun triaxial fiber membranes

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ABSTRACT

Electrospun membranes encapsulating nisin in the core of multi-layer coaxial fibers, with a hydrophobic PCL intermediate layer and a hygroscopic cellulose acetate sheath, have been demonstrated to provide long-term antimicrobial activity combined with a hygroscopic outer layer. Antimicrobial performance has been evaluated using modified versions of the antimicrobial textile test AATCC 100 and AATCC 147 against *Staphylococcus aureus*. The AATCC 147 tests indicate that antimicrobial activity persists up to 7 days. The quantitative analysis from the AATCC 100 test indicates that tri-layer coaxial ("triaxial") electrospun fiber membranes provide >99.99% bacteria kill (4 log kill) for up to five days. This indicates that the nisin-incorporated triaxial fibers have excellent biocidal activities for up to 5 days and then provide biostatic activity for 2 or more days. Compared with other types of electrospun membranes, such as core-sheath coaxial ("coaxial") and single homogenous fibers, triaxial fiber membranes provided more robust and more sustained antimicrobial activity. Single fibers with nisin showed relatively weak activity and only for one day. Coaxial fiber membranes exhibited antimicrobial activity for a long period, but their biocidal activity was much weaker than that of triaxial fiber membranes, and only exhibited >99% bacteria kill (2 log kill) after 1 day of exposure.

Statement of Significance

The increase in drug resistant pathogens has driven the need for alternative treatments that are effective against resistant bacteria and do not contribute to drug resistance. Nisin is an excellent model bacteriocin for antimicrobials because of its size and mode of action, and has been extensively used as FDA-approved food preservatives without any problematic resistance growth in bacteria during past decades. Nisin-containing fibers have been previously reported using conventional electrospinning but sustained antimicrobial effect has not been obtained. Here, we report the encapsulation of nisin into a multi-layered nanofiber construct using triaxial electrospinning in order to obtain a long-term antimicrobial activity. This will be highly beneficial in many applications, such as protective textiles, food packaging and cancer therapy.

1. Introduction

Modern biomedical and pharmaceutical research efforts have developed various antibiotics to successfully treat people with many infectious diseases. However, many treatments employ the use of broad-spectrum antibiotics, which indiscriminately kill most of the existing pathogenic and beneficial bacteria. Resistant strains are allowed to persist and multiply easily because other bacteria strains competing with resistant strains are also killed, resulting in the emergence of antibiotic resistant bacteria. The increase in drug resistant pathogens has driven the need for alternative treatments that are effective against the resistant strains and ideally do not further contribute to drug resistance. Bacteriocins, a group of naturally occurring antimicrobial peptides, have received increasing attention as an alternative to conventional antibiotics [1]. Bacteriocins are toxins produced by most bacteria, which can kill or prohibit the growth of other closely related bacteria in order to compete for nutrients and space in a constrained environment [2]. While conventional antibiotics usually are active against a broad spectrum of bacteria, most bacteriocins, such as colicins

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[3] and lactococcin *G* [4], are only active against closely related bacteria and thus exhibit targeted, narrow spectrum activity. This allows the beneficial bacteria to remain unaffected and decreases the risk of contributing to further antimicrobial resistance. Nisin, a commercially available bacteriocin, is an excellent model for this class of antimicrobials because of its size and mode of action. Nisin is a polycyclic peptide with 34 amino acid residues (molar mass of 3354 g/mol and density of 1.4 g/mL). Nisin is generally active against Gram-positive bacteria [5–8], as well as several Gram-negative species when used in conjunction with a chelating agent [9]. Discovered [10] in 1933, nisin has been extensively used as a naturally-occurring food preservative (approved by FDA in 1988) without leading to any problematic resistance growth in bacteria, such as *S. aureus*, during the past 40 years [11].

Nisin acts on target bacteria by two major steps: (1) passage through cell wall; (2) interaction with lipid II (e.g. binding to lipid II, pore formation on cell membrane), which is essential for biosynthesis on the cell wall. Nisin's mechanism of action (see Fig. 1) involves first binding of the N-terminus to the lipid II complex and forming a pyrophosphate cage that inhibits cell wall synthesis. The C terminal is then responsible for pore-formation. A three amino acid hinge region exists between the N and C domains and allows conformational changes to occur upon contacting a microbe [1,12–14].

The electrospinning technique [15,16], including multi-axial electrospinning [17–21], is a versatile method to produce nanofiber membranes made of various natural and/or synthetic materials, including biopolymers, textile polymers, electrically conducting polymers, and stimuli-responsive polymers. By controlling solution parameters and electrospinning parameters, one can control: (a) fiber diameter, ranging from micro- to nanometer dimensions; (b) fiber composition; (c) fiber morphology (e.g. smooth, wrinkled, porous, etc.), (d) fiber structure. Electrospun membranes have a highly porous non-woven mat configuration providing exceptionally high surface area to volume ratio and excellent breathability. In our previous report using triaxial electrospinning, the effect of operational parameters (e.g. flow rate ratio, solvent selection, nozzle dimensions, etc.) on triaxial fiber membranes has been investigated in order to manipulate the release rate from both fiber core and sheath [18].

Antimicrobial materials whose effects last one week or more will be highly beneficial in many applications, such as protective textiles, biomedical, and food packaging. For example, on the battlefield, soldiers are routinely exposed to potentially pathogenic bacteria on their uniform, but often cannot change their clothes frequently. Previous reports [22–25] for nisin-containing fibers have utilized conventional (homogenous single or blended) polymer electrospinning and the sustained (multiple days) antimicrobial effect of the incorporated nisin has not been obtained. Here, we report the encapsulation of nisin into a multi-layer fiber construct using coaxial electrospinning in order to investigate effects of antimicrobial activity over time due to extended exposure to a damp environment. Encapsulating the biological material in the core polymer fiber may provide antimicrobial protection for an extended period along with a potentially skin-friendly cellulose acetate sheath surface to users [26].

2. Materials and methods

2.1. Materials

Polymers, such as nylon 6, polyvinylpyrrolidone (PVP, Mw = 360), poly(ε-caprolactone) (PCL, Mn = 80 kDa) were purchased from Sigma Aldrich (St. Louis, MO). Pierce BCA protein assay kit and various solvents such as 2,2,2,-trifluoroethanol (TFE, 99.8% purity), dimethylformamide (DMF, 99.9% purity), chloroform (CF, 99.9%) were purchased from Fisher Scientific (Pittsburgh, PA). Nisin powder consisting of 20% nisin and 80% byproducts, such as milk proteins and salts was purchased from ChiHonBio (Lisle, IL). Nutrient broth was purchased from Sigma Aldrich. Mannitol salt broth (Himedia) and bacteriological agar (Acros) were used to prepare mannitol salt agar plates for soft agar overlay studies. Dey-Engley (DE) broth was obtained from BD Falcon. *S. aureus* strain 27,217 was obtained from American Type Culture Collection (ATCC). Biological decontamination tests were performed using a TEMPO system from Biomerieux, and TEMPO supplies were purchased from Biomerieux as well. All materials were used as received with no additional treatment.

2.2. Sample preparation

Three different polymeric solutions were prepared for core, intermediate, and sheath layers. For the core solution, nisin was dissolved into TFE solvent overnight then centrifuged at 5000 rpm (2400*g*) using accuSpin Micro 17 centrifuge (Fisher Scientific) to separate undissolved compounds from the nisin solution. Then, PVP was added to the filtered nisin solution and stirred overnight to obtain a homogeneous nisin/PVP solution. For other solutions, either PCL for the intermediate layer or nylon 6 for the sheath was added to TFE solvent and then homogenized overnight using a rotating stirrer at 20 RPM. Core and intermediate solutions were used as core and sheath solution in coaxial electrospinning, respectively. Specific information on the electrospun fiber membrane samples is contained in Table 1.

For triaxial electrospinning, core, intermediate, and sheath solutions were fed at 0.6, 1.2, and 0.2 mL/hr, respectively. For coaxial

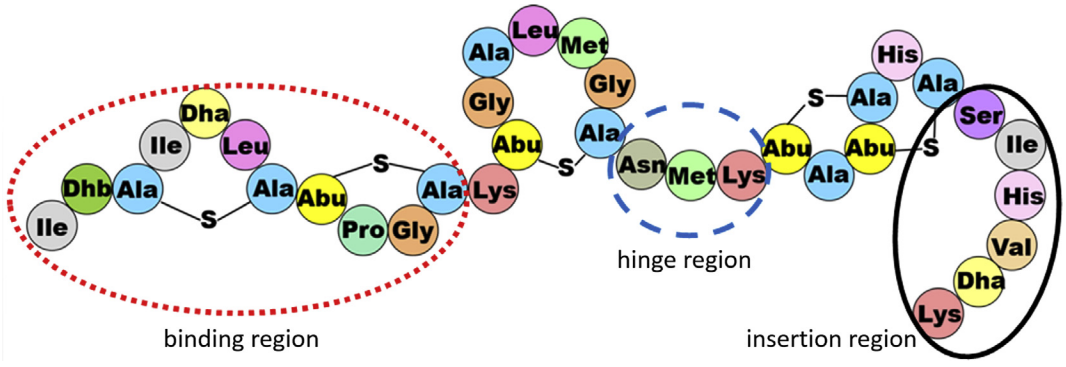

Fig. 1. Nisin molecular structure showing key regions responsible for its antimicrobial activity: binding region to the cell, hinge region to create cage and inhibit cell wall synthesis, and insertion region into the pore.

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Table 1
Description of electrospun membrane samples. All solutions used TFE solvent.

Fiber construct	Solution	Flow rates (mL/hr)	Calculated nisin amount (mg)
Single (Fig. 2d)	PVP 10% + nisin 1%	0.6	~2.5
Single (Fig. 2c)	PVP 10%	0.8	0
Single (Fig. 5b)	PCL 10% + nisin 1%	0.6	~2.4
Single (Fig. 5a)	Nylon6 12% + nisin 1.2%	1.0	~2.3
Single	PCL 10% + nisin 8.6%	1.0	~38.7

Fiber construct	Core solution	Intermediate solution	Sheath solution	Flow rates (mL/hr)	Calculated nisin amount (mg)
Coaxial	PVP 6% + nisin 19%	–	PCL 11%	0.7:1.4 (core: sheath)	~39.3
Triaxial	PVP 6% + nisin 19%	PCL 11%	CA 12%	0.6:1.2:0.25 (core:inter:sheath)	~39.3

electrospinning, the flow rates of 0.6 and 1.2 mL/hr were used for core and sheath solutions, respectively, and the flow rate of 1.0 mL/hr was used for single electrospinning. For all cases, the gap distance between the nozzle and the collector was set to 20 cm and a voltage of 12–14 kV was applied to obtain a stable Taylor cone and liquid jet ejection at the end of the nozzle tip. For comparison, the same amount of nisin powder was encapsulated into all types of fiber membranes by using the same 400 μL volume of nisin solutions. Coaxial and triaxial fiber membranes show encapsulation efficiencies of ~95% and ~87%, respectively, based on comparing the dispensed nisin amount and the weight difference between control and nisin-incorporated membranes. While these values provide a measure of encapsulation efficiency, they may not represent the actual nisin encapsulation efficiency because other materials in the starting nisin powder can be dissolved in the PVP/nisin solution to different concentrations.

2.3. Leaching experiments for AATCC147 and AATCC100

To assess antimicrobial activity over time, leaching of the membranes was allowed for a designated period of time before exposure to bacteria. Membranes were overlaid on 7 mL of sterile mannitol salt soft agar on mannitol salt agar plates and left at room temperature. A 24-h incubation at room temperature is considered one day of leaching. After the appropriate incubation period (up to 7 days), membranes were either inoculated with bacteria or used for a soft agar overlay with bacteria, and then evaluated for antimicrobial activity.

2.4. AATCC147 experiment and soft agar overlay

Both AATCC147 and soft agar overlays were used as qualitative assessments for activity of nisin in electrospun membranes. The AATCC147 method was used for initial screenings. *S. aureus* (ATCC 27217) was grown from glycerol stocks in 10 mL of nutrient broth and incubated on a shaker at 37 °C for approximately 6 h. Once an optical density of 1 had been achieved, a 1:10 dilution of the cells was created into nutrient broth. On mannitol salt agar plates, four lines of diluted *S. aureus* were streaked across the plate about half an inch apart from each other. The membrane was placed in the center of the plate, over the center of the 4 lines and incubated overnight at 37 °C.

For the soft agar overlays, a culture of *S. aureus* (ATCC 27217) was grown from frozen glycerol stocks in 10 mL of nutrient broth and incubated for approximately 6 h on a 37 °C shaker. Once an optical density of 1 had been achieved (approximately 10^7 colony forming units (CFUs)), 20 μL of undiluted culture was added to 7 mL of mannitol salt soft agar. The soft agar mixed with bacteria was poured over a mannitol salt agar plate and allowed to dry before overlaying membranes. After placing the membranes on top of soft agar, the overlay plate was incubated for 16–18 h at 37 °C. The overlay plate was imaged following incubation and inspected for a zone of clearing around the membrane.

2.5. Modified AATCC100 experiment

In this method, electrospun membranes were cut into 1″ × 1″ swatches under sterile conditions to obtain triplicates of each sample. The swatches were overlaid on sterile mannitol salt soft agar (no bacterial inoculation) and incubated at 37 °C for 16 h. The *S. aureus* culture was prepared as describe above and was diluted by a factor of 10 into a nutrient broth. 100 μL of the diluted culture was used to inoculate the membranes. Swatches were inoculated in sterile plastic petri dishes, and then placed in an incubator at 37 °C for 18 h. Swatches were then placed into 5 mL of DE neutralizing broth and vortexed for 5 min to allow bacteria to fall off into solution. Serial dilutions up to 10^{-4} for controls and 10^{-2} for nisin samples were created using 20 mM sodium phosphate buffer (pH 7.2). Solutions were then tested using TEMPO selective *S. aureus* media according to manufacturer's instructions. The TEMPO system has been validated by the Association of Analytical Communities Research Institute as a Performance-Tested Method. TEMPO STA is an automated test for the enumeration of Gram-positive staphylococci (S. aureus) based on the format of the TEMPO Most Probable Number (MPN) procedure (miniaturized card containing 48 wells across 3 different dilutions for the automatic determination of the MPN. The system fills the card, and after 24–27 h incubation at 37 °C, automatically reads the card and calculates the MPN as CFU/mL. TEMPO cards were incubated at 37 °C for 24 h, then read in the TEMPO reader. The portion of bacteria killed were calculated as a log kill and a percent kill from this data.

2.6. Statistical analysis

To determine the fiber diameter, more than 25 measurements were made and then the average diameter with the standard deviation was calculated. For AATCC 100 test results, 3 membrane samples were utilized in each type of membranes and the average value with max/min range bar were plotted in Fig. 7.

3. Results and discussion

3.1. Solvent evaluation

Nisin is known to dissolve in aqueous solvents. However, nisin dissolution in organic solvents, which is needed for fiber formation with the electrospinning technique, is not well studied. Nisin in organic solvents can provide more favorable characteristics for electrospinning, such as polymer host availability, stable encapsulation, and high throughput. To ensure the nisin would be unaffected by organic solvents, the nisin powder, consisting of approximately 20% nisin, 30% milk protein and 50% salts, was dissolved in three organic solvents (ethanol, DMF, and TFE), along with water as a control, at a concentration of 1 mg/mL nisin. After centrifugation, single drops of each solution were placed onto an agar plate containing a *S. aureus* overlay (Fig. 2B). The results show that ethanol was less effective at dissolving the nisin than DMF or

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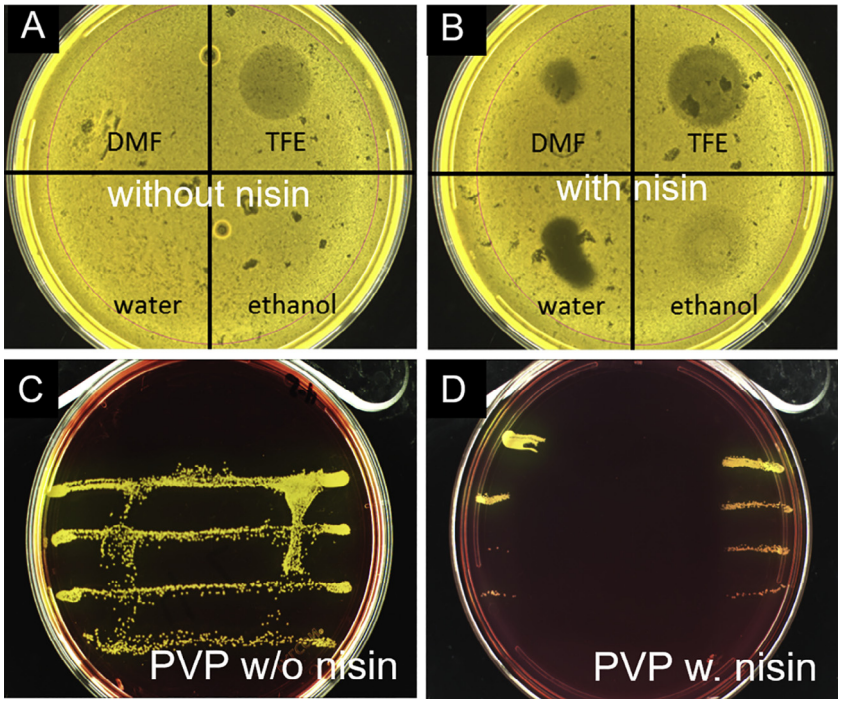

Fig. 2. Images of antimicrobial experiments on agar plates – application of single droplets: (a) pure solvents (water, ethanol, DMF, and TFE); (b) nisin-containing solutions. Electrospun PVP fiber membranes without (c) or (d) with nisin.

TFE, and that all nisin-containing solutions exhibited activity. This trend is consistent with the results of a bicinchoninic acid (BCA) assay (Fig. S2). Pure solvent droplets were also applied onto a separate *S. aureus* overlay to test for growth inhibition due to solvent alone (Fig. 2A). TFE alone also displayed slight antimicrobial activity, which produced some uncertainty regarding which component produces antimicrobial activity if TFE and nisin are present in the fiber membrane.

To resolve this uncertainty, AATCC147 antimicrobial tests were performed with electrospun PVP fiber membranes with and without nisin using TFE as a solvent. As shown in Fig. 2C, PVP-only membranes show no antimicrobial activity, as indicated by the absence of a zone of clearing on the plate. On the other hand, the nisin incorporated PVP membrane shows excellent antimicrobial activity, as shown in Fig. 2D. Because all subsequent membranes were made using TFE, we can be confident that any antimicrobial activity observed is due to the nisin encapsulated in the fiber, and not from residual solvent. Overall, TFE is an excellent nisin solvent, given its appropriate properties for electrospinning, including high vapor pressure, polarity, and ability to dissolve various polymers.

PVP/nisin membranes made using DI water solvent exhibit inferior mechanical properties due to poor electrospinnability, and therefore DI water was not used as a solvent for further investigation.

3.2. Fiber morphology

Since nisin can be dissolved in multiple organic solvents, electrospun fiber membranes have been produced with nisin dissolved in several different solvents. Nisin itself is not electrospinnable due to its relatively low molecular weight. Therefore, either single blended or coaxial electrospinning with TFE solvent were utilized to produce the nisin encapsulated electrospun fiber membranes. After membrane formation, the release of nisin was implemented by immersing them in DI water for 24 h. Fig. 3 shows microscopic images of fiber membranes before and after release of nisin from the fibers. While no obvious change in morphology was found in single blended (containing polymer and nisin) fibers (Fig. 3A), all coaxial and triaxial fibers exhibited dramatic changes, as shown in Fig. 3B–D. Coaxial fibers with a nisin core show flattened fiber morphologies after release of nisin core because of the substantial release of water-soluble core material (PVP/nisin). On the other hand, coaxial fibers with nisin in the sheath (Fig. 3C) show a rougher fiber surface because the sheath material (PVP/nisin) was dissolved into a PBS solution. In this case, the dissolved sheath resulted in inferior mechanical stability, preventing the use of these membranes for overlay experiments.

The structure of coaxial fibers has been confirmed by TEM observations. Coaxial (core-sheath) and triaxial (core-intermediate-sheath) structures were observed as shown in Fig. 4. The detailed structure of triaxial fibers is challenging to illustrate due to the use of similar density polymer materials and the overlapping of three layers.

3.3. Antimicrobial properties

Antimicrobial properties of the nisin-containing fiber membranes were evaluated using soft agar overlay methods with *S. aureus* bacteria, which can cause skin infections [27] and food poisoning [28]. Overlays were done using mannitol salt agar, a selective differential media for *S. aureus*. As bacteria grow, the acid produced from bacteria reacts with the phenol red dye in the media, changing the color from red to yellow. In photographs of the agar plates taken at various times, the yellow color represents bacterial growth while reddish or dark areas represent no bacterial growth.

Although PVP/nisin fiber membranes provide antimicrobial properties (Fig. 2D), they are not suitable for sustained release over a long-term period because PVP is readily dissolved in a damp environment. Therefore, electrospun fiber membranes incorporating nisin into water-insoluble polymers were also investigated.

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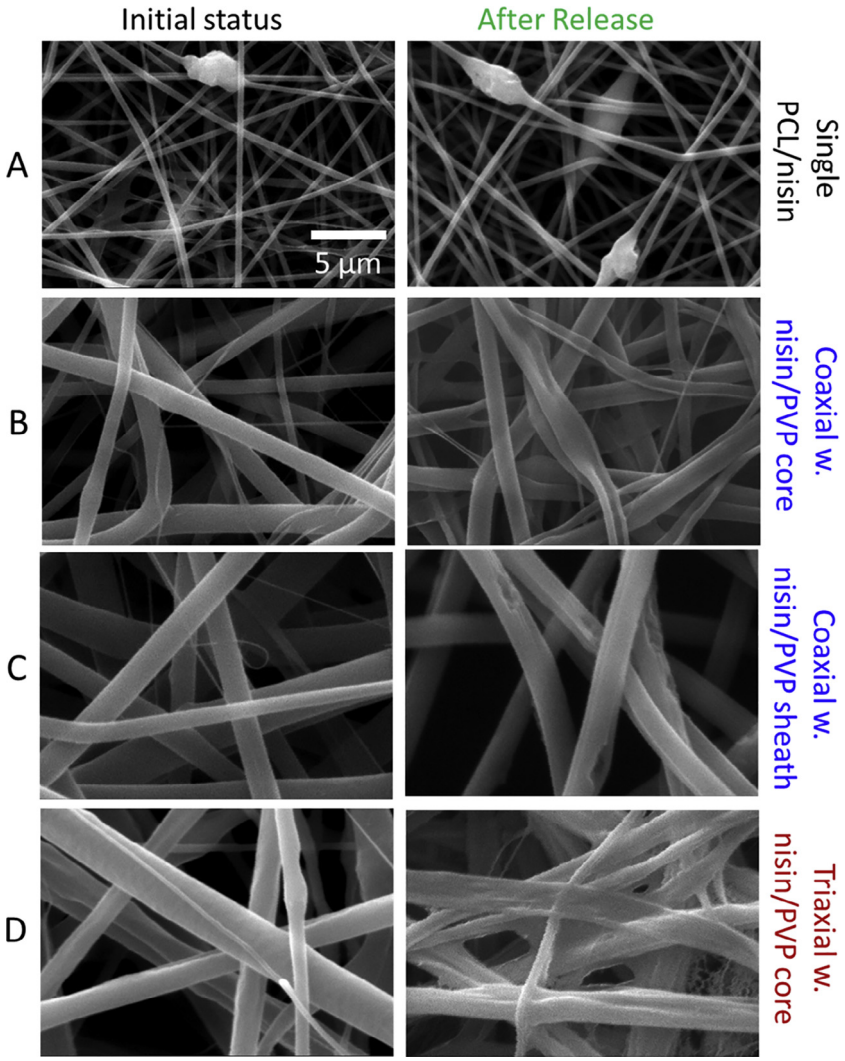

Fig. 3. SEM microphotographs of nisin incorporated electrospun fibers: (a) single PVP/nisin fibers; (b) coaxial fibers with PVP/nisin core and PCL sheath; (c) coaxial fibers with PCL core and PVP/nisin sheath; (d) triaxial fibers with PVP/nisin core, PCL intermediate layer and cellulose acetate sheath. Left and right columns contain images taken before and after releasing of core components, respectively.

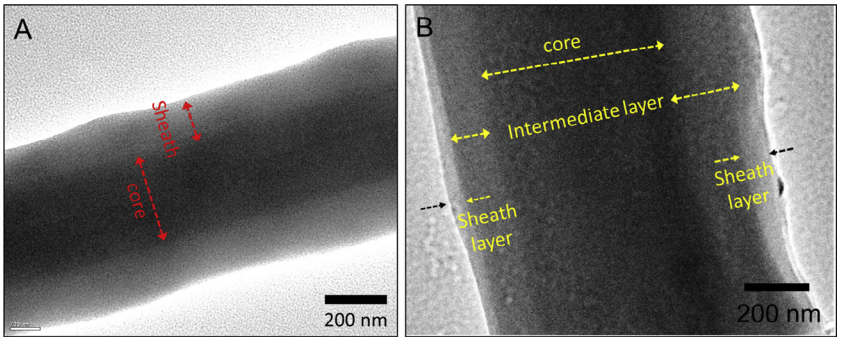

Fig. 4. TEM observation of coaxial fibers: (a) coaxial fibers (PVP/nisin core – PCL sheath); (b) triaxial fibers (PVP/nisin core – PCL intermediate – CA sheath).

As a first step, single electrospinning has been carried out using a blended solution of water-insoluble polymers (nylon 6 and PCL) and nisin. As described above, their antimicrobial activity has been characterized by overlay experiments using *S. aureus* bacteria. As previously described (Fig. 2D), the water-soluble nisin/PVP single blended fiber membrane provides antimicrobial activity but is dissolved immediately in a wet environment. Therefore, water-soluble PVP was replaced with water-insoluble polymers, such as hygroscopic nylon 6 (Fig. 5A) and hydrophobic PCL (Fig. 5B). Interestingly, while these polymers maintain the membrane structure, there was no discernable antimicrobial activity compared to their control samples without nisin. Nisin solubility in organic solvents is not a main reason for lack of activity because nisin/PVP fibers give clear evidence of antimicrobial activity. It is likely that the

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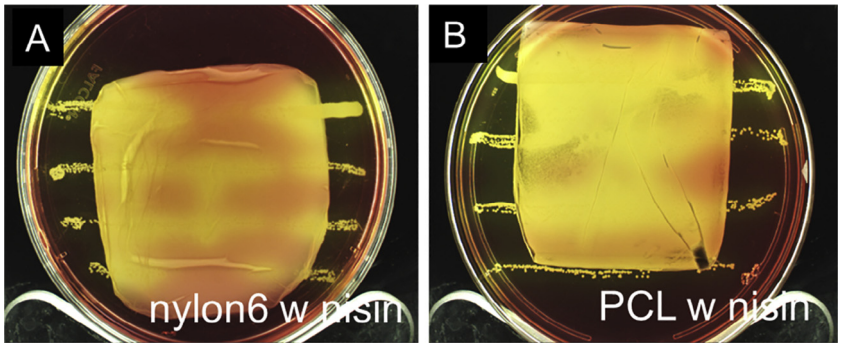

Fig. 5. AATCC147 results from single electrospun nanofibers blending nisin with different host polymer materials: (a) nylon 6 and (b) PCL.

nisin is immobilized within the polymer matrix and does not have a chance to interact with the bacteria. Therefore, the release of water-soluble core through the polymer sheath is necessary to provide good antimicrobial activity. Consequently, nisin incorporated water-insoluble antimicrobial fiber membranes cannot be obtained by a single electrospinning approach.

To resolve this limitation, coaxial electrospinning has been utilized to combine the antimicrobial activity of nisin/PVP fibers with the mechanical robustness of water-insoluble fibers. Fig. 6 compares the antimicrobial activity of several fiber membranes over a 7-day period: (a) PCL/nisin single fiber; (b) coaxial fiber – core: PVP/nisin, sheath: PCL; (c) triaxial fiber – core: PVP/nisin, intermediate: PCL, sheath: cellulose acetate. Interestingly, coaxial fibers with water-soluble nisin/PVP in the core and water-insoluble polymer in the sheath provided very robust antimicrobial activity after one day of exposure and continued to show activity for several days. Since the core material is encapsulated by the polymer sheath, nisin is released at a slower rate for a longer period of time. The sheath thickness plays a main role in the nisin release rate. As shown in Fig. S4, a thinner PCL sheath gives a stronger antimicrobial effect during the first day of exposure because it releases more nisin than a fiber with a thicker sheath. On the other hand, in this case nisin will be released in a shorter time period. As a result, coaxial fibers provide both mechanical stability and excellent antimicrobial activity.

For the triaxial fibers, cellulose acetate was used as a hygroscopic sheath material. Because nisin release is controlled by the hydrophobic intermediate layer, the release time is extended. Single blended fibers and coaxial fibers with a cellulose acetate sheath have been produced for comparison.

As shown in Fig. 6, little to no activity is observed in single fiber (nisin/PCL) samples, even on the first day, whereas both coaxial and triaxial fiber membranes presented obvious antimicrobial activity up to 7 days. Upon closer inspection, it appears that the triaxial membrane exhibited better antimicrobial activity than the coaxial membrane even at day 3, as evidenced by the zone of clear-

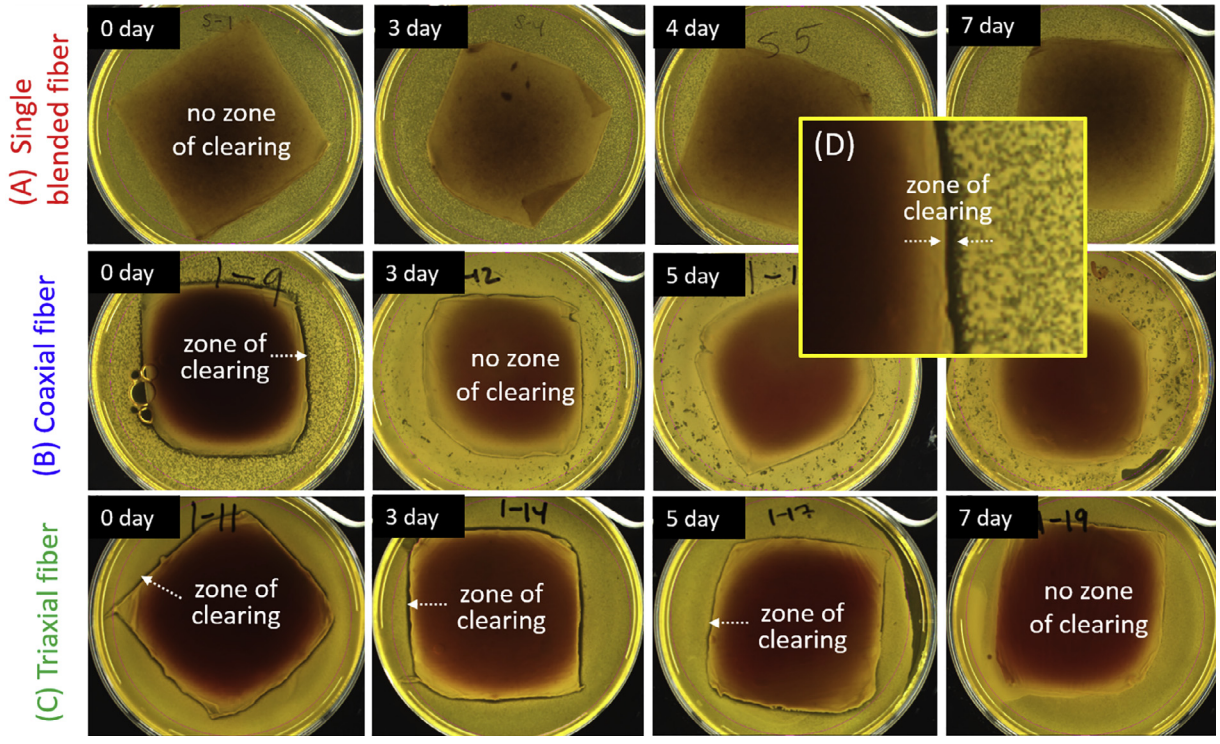

Fig. 6. Qualitative overlay analysis of antimicrobial activity on different types of electrospun membranes: (a) PCL/nisin single blended fibers; (b) coaxial fiber with PVP/nisin core and PCL sheath; (c) triaxial fiber with PVP/nisin core, PCL intermediate, and CA sheath; (d) zone of clearance, observed as a "dark halo" of growth inhibition around the membrane edges.

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ing around the membrane. By day 7, there were no zones of clearing around any of the membrane constructs, although they still showed some biostatic activity directly underneath. A possible reason is that the sheath layer assists the intermediate layer to form a more conformal coating around the core, which can reduce the initial burst release of nisin and enables a sustained release over a longer period.

Although there was a noticeable difference between triaxial and coaxial fiber samples, it is difficult to quantify the difference through the photographs of the overlay experiments shown in Fig. 6. Therefore, the AATCC100 test method was used to analyze the antimicrobial activity quantitatively. As shown in Fig. 7, the triaxial fiber membranes produced the best results with a sustained 4–5 log kill (>99.99–99.999% kill) for 5 days. By comparison, the single fiber and coaxial fiber membranes showed significant biocidal activity only after 0 h leaching and 24 h leaching. These results are consistent with the overlay results, in that the triaxial fiber membrane had sustained antimicrobial activity at least through day 5, whereas the coaxial and single blended fiber membranes did not show antimicrobial activity by day 3. While these two methods both provide information as to whether the nisin is active, AATCC100 provides quantitative biocidal efficacy, whereas overlays merely confirm activity. This is due to the significant differences in the number of bacterial cells each sample is challenged with, and the way that antimicrobial activity is tested. In AATCC100, the sample is challenged by a known number of cells, and then is assessed for biocidal activity by determining the number of cells killed by the active component. When overlays are performed, results are obtained by evaluating the extent of the growth beneath the sample and its immediate surroundings, which is the indicator of antimicrobial activity. However, overlays cannot distinguish whether the activity is biocidal or biostatic because the number of cells exposed to the sample is unknown. Therefore, samples that do not show biocidal activity in the AATCC100 method could still show some antimicrobial activity in the overlay test, as is illustrated in this paper. Based upon the results for membranes with different fiber structures (as described above), it is clear that the triaxial membrane construct is the most versatile for burst and/or sustained release of bacteriocins for antimicrobial effects up to 7 days, depending on the application.

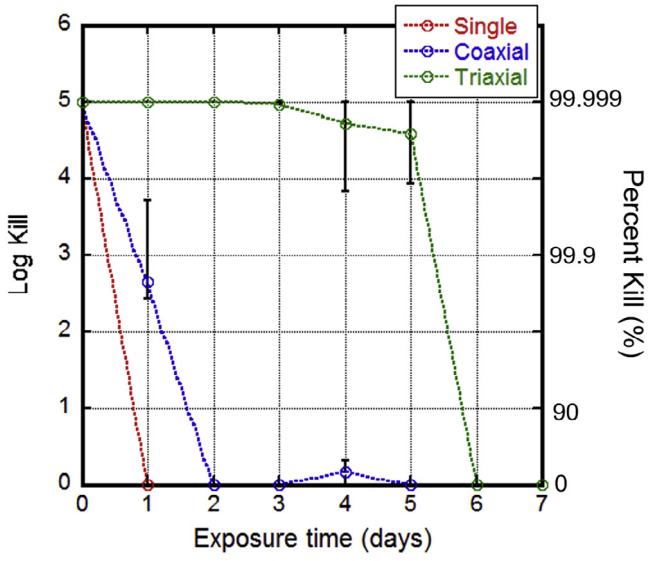

Fig. 7. Quantitative analysis (AATCC100) of antimicrobial activity on electrospun membranes: bacteria log kill value vs. exposure days.

4. Conclusions

The formation of electrospun nisin-containing membranes is very attractive for various applications, such as protective textiles, wound dressing, and food packaging Nisin incorporated/encapsulated fiber membranes have been successfully produced by different electrospinning approaches such as single blended, coaxial and triaxial electrospinning. Single blended nisin fibers with water-insoluble host polymers do not provide any antimicrobial activity because nisin molecules are immobilized within the polymer matrix. Multi-axial fibers with PVP/nisin core and polymer intermediate/sheath provide excellent antimicrobial activity. Coaxial fiber membranes show antimicrobial activity of ~5 log kill on the first day. Their efficacy decays after one day of leaching (<3 log - kill), which suggests that they are useful for applications requiring burst releases of nisin. Triaxial fibers with a PVP/nisin core, hydrophobic intermediate, and hygroscopic sheath layer have provided excellent antimicrobial activity of >4 log kill for longer term (>5 day) exposures. This triaxial fiber system has been demonstrated to provide antimicrobial protection for up to seven days of exposure in a simulated damp environment. It is also shown that the nisin-encapsulated triaxial fibers show excellent antimicrobial activities to other Gram-positive bacteria, such as *Bacillus anthracis Sterne* and *Micrococcus luteus* as shown in Supplementary Fig. S5. Based on our nisin model, other antimicrobial peptides can be incorporated into the triaxial fiber system in order to provide a long-term antimicrobial effect on targeted pathogens. Our membranes retained integrity even with daily manipulation, over the course of the extended antimicrobial activity experiments. The current mechanical strength of the membranes may not be sufficient to be used as a stand-alone material in textiles because PCL and cellulose acetate were used as intermediate and sheath fiber layers. However, the mechanical strength can be readily improved by incorporating textile-oriented materials (such as Kevlar) into the intermediate layer, or by adding a mechanically robust layer on top of our membranes. Future investigation on the electrospinning of stimuli-responsive polymers could be very interesting because it would provide textiles that could trigger antimicrobial release only when necessary, resulting in the potential for antimicrobial activity much beyond 7 days.

Disclosures

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2017.02.029.

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