

Preparation and characterization of bioactive and breathable polyvinyl alcohol nanowebs using a combinational approach

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ABSTRACT: Electrospun polyvinyl alcohol (PVA) nanowebs treated with a mixture of honey and polyhexamethylene biguanides (PHMBs, commercially available as Reputex 20) were prepared and characterized to evaluate their applicability in wound dressing applications. Fourier transform infrared spectroscopy was used to confirm the incorporation of functional moieties from honey and Reputex 20 into electrospun PVA nanowebs. Functionalized PVA nanowebs were characterized by evaluating their antimicrobial properties, moisture vapor transport characteristics (breathability), and tensile properties. PVA nanowebs treated with a mixture of honey and PHMBs have shown good antimicrobial activity. Additionally, functionalized PVA nanowebs have shown adequate breathability characteristics, a much needed attribute in textile materials used in wound dressing applications. Nanowebs fabricated from bio-compatible polymers such as PVA, and functionalized in a combinational fashion, could be used in many different biomedical applications, including wound healing bandages and cell or tissue culture scaffolds.

Application: The pulp and paper industry could use a similar combinational approach to develop multifunctional textiles for a variety of applications according to their needs.

Electrospinning has enabled the production of hierarchical structures such as nanofibers with diameters in the nanoscale range. Because of their unique properties, such as high surface area-to-volume ratio and high porosity, electrospun nanofibers have garnered much attention from the scientific community. Another advantage offered by electrospun nanofibers is that they can be functionalized to impart additional properties and uses in textiles, biomedical and life sciences, filtration, and defense [1-7]. One of the important applications of electrospun nanofibers in the biomedical sector is in wound dressings [8].

An ideal wound dressing must mimic the structural and functional biology of the extracellular matrix (ECM). This encourages the proliferation of epithelial cells and the formation of new tissue [9,10]. In vivo, ECM plays a crucial role during the process of wound healing by providing physical support to cells and facilitating conditions for cell attachment, proliferation, migration, and differentiation [11]. With their small diameter and the random orientation of fibers, electrospun nanofibers mimic the structural complexity of ECM [12]. The high surface area of nanofibers helps incorporate suitable drugs and fluid absorption near the vicinity of a wound [12]. More importantly, the high porosity of nanofibers [9] allows fluid and gas exchange between cells and the external environment and prevents wound desiccation and dehydration

[12]. Additionally, the small size of the pores in nanofibers prevents infiltration of exogenous microorganisms [9,12].

Electrospun nanofibers developed for use as potential wound dressings can be classified in to four categories: 1) passive, 2) interactive, 3) advanced, and 4) bioactive [13]. Passive wound dressings meet the physical (water and gas permeability) and morphological (porosity) requirements of wound dressings. These properties are needed to maintain adequate levels of moisture around the wound and to protect wounded tissues from mechanical trauma. Interactive wound dressings possess value added capabilities, such as limiting bacterial proliferation at the wound site and taking care of morphological and physical requirements. Advanced interactive wound dressings are drug-loaded interactive wound dressings. Bioactive wound dressings are multifunctional systems with adequate mechanical and physico-chemical properties [13].

The objective of our study was to develop a functionalized electrospun bioactive nanoweb for use as potential wound dressing material. Polyvinyl alcohol (PVA) was chosen as the electrospinning polymer because of its proven biocompatibility characteristics [14]. PVA nanofibers were treated with a mixture of honey and Reputex 20 (Lonza; Basel, Switzerland), a commercially available polyhexamethylene biguanide (PHMB) antimicrobial product. Despite the many reported

beneficial properties of honey, such as its antimicrobial activity, etc. [15,16], functionalization of nanowebs with honey to impart antimicrobial activity has been a challenge. Puttamatanon et al. [17] investigated the possibility of developing antimicrobial PVA nanowebs by functionalizing them with honey collected from *Apis dorsata* bees. Although pure honey exhibited antimicrobial properties, honey functionalized PVA nanowebs showed no inhibitory effects on tested microorganisms [17].

Previous studies from our laboratory have suggested that treating PVA nanowebs with honey (used in the present study) enhances their moisture vapor transmission characteristics. However, honey has not imparted any antimicrobial properties to treated PVA nanowebs [18]. On the other hand, PHMBs are proven broad-spectrum antimicrobial agents with a low risk and excellent tolerance profile for use in next-to-wound applications [19-21]. Another advantage with using PHMBs is that the possibility of microorganisms developing resistance to PHMBs is negligible [19]. Our previous studies have suggested that treating PVA nanowebs with a PHMB-based microbiocidal solution (Reputex 20) has imparted antimicrobial properties to PVA nanowebs, but compromised their moisture vapor transmission characteristics [22].

For the current study, we used a combinatorial approach that involved treating PVA nanowebs with a mixture of honey and Reputex 20. Such an approach allowed us to investigate whether or not multiple functionalities could be imparted to electrospun nanowebs for a range of applications. The objective was to develop a functionalized breathable biocidal PVA nanoweb by treating it with a mixture of honey and PHMBs to impart the beneficial properties of both to PVA nanowebs.

EXPERIMENTAL METHODS

Materials

PVA (MW: 89000-98000 Da and $\geq 99\%$ hydrolyzed) and Mueller Hinton agar and broth were procured from Sigma-Aldrich (St. Louis, MO, USA). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were purchased from American Type Culture Collection (Manassas, VA, USA). Reputex 20 microbiocidal solution was obtained from Lonza, Inc. (Basel, Switzerland). The 100% pure, raw, unfiltered honey was obtained from Nature Nate Farms (McKinney, TX, USA).

Preparation of electrospinning solution

A 12% PVA solution in 90:10 deionized water and a Reputex 20-honey mixture used as electrospinning dope was prepared in the following fashion. The concentration of PVA was determined from preliminary experiments. Initially, 2.4 gm of PVA is dissolved in 15 mL of deionized water at 80°C for 3 h with intermittent stirring. A mixture of 2 mL Reputex 20, 2 mL honey, and 1 mL deionized water was prepared separately. Finally, both solutions were mixed using a magnetic stirrer after bringing the PVA solution to room temperature. The PVA solution is brought to room

temperature to minimize the effects of high temperatures on Reputex 20 and honey, which might compromise the properties of the mixture.

Electrospinning setup

A syringe equipped with a 20 gauge needle and containing the dope was loaded onto a PHD 2000 infuse/withdraw pump (Harvard Apparatus; Holliston, MA, USA). We maintained a polymer flow rate of 0.02 mL/min. Nanofibers were spun by charging the needle of the syringe to a voltage of 25 kV using an ES 30P-5W power supply unit (Gamma High Voltage Research; Ormond Beach, FL, USA). An aluminum collector was placed at a distance of 15 cm from the tip of the syringe.

Henceforth in this paper, nanowebs electrospun from PVA dope alone are referred to as native PVA nanowebs. Nanowebs prepared from a solution of PVA in Reputex 20/honey are referred to as treated PVA nanowebs. Treated PVA nanowebs were heat cross-linked [14] at a temperature of 155°C for 30 min to enhance their stability in aqueous conditions, and are referred to as treated/heat cross-linked PVA nanowebs.

Scanning electron microscopy characterization of treated PVA nanowebs

We used a Hitachi S-4300SE/N scanning electron microscope (SEM) (Hitachi; Tokyo, Japan) to characterize the morphology of treated PVA nanowebs. An accelerating voltage of 2 kV was used with no coating on nanowebs. The average fiber diameter was calculated by measuring the diameters of 80 fibers from various locations of the web.

Fourier transform infrared spectroscopy measurements

We used a Bruker Vertex 70 spectrophotometer (Bruker; Billerica, MA, USA) to obtain the attenuated total internal reflection mode Fourier transform infrared (FTIR) spectra of all the samples. The spectrophotometer was equipped with a liquid nitrogen cooled mercury-cadmium-telluride detector. Spectra were recorded in the range of 4000-800 cm^{-1} . An average of 128 interferograms measured at a resolution of 4 cm^{-1} and apodized with a Blackman-Harris 3-term function was used to compute the spectra. Dry air from a Parker Balston Model 75-52 purge gas generator (Parker-Hannifin; Haverhill, MA, USA) was used to continuously purge the spectrophotometer bench and sample compartment. All measurements were carried out at 23°C.

Antimicrobial activity tests

The antibacterial activity of treated and treated/heat cross-linked PVA nanowebs was tested against gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria. Briefly, a loop full of test bacteria is streaked onto fresh Mueller Hinton agar plates. The plates were incubated for 24 h to have an active inoculum for assessing the antibacterial activity of treated PVA nanowebs. A 0.5 McFarland equivalent turbidity standard of the test inoculum was streaked onto fresh agar plates

and incubated with the nanowebs (cut into 6 mm diameter discs) for 24 h. The presence of zones of inhibition indicates antibacterial activity. Nanowebs were sterilized under ultraviolet light (15 min on each side) to avoid any contamination issues. Treated PVA nanowebs were designated as HR (HR1, HR2, and HR3) and treated/heat cross-linked PVA nanowebs were designated as CL (CL1, CL2, and CL3).

Evaluation of moisture vapor transmission rate of treated PVA nanowebs

British Standard evaporative dish method BS 7209:1990 “Specification for water vapor permeable apparel fabrics” was used to evaluate the water vapor permeability of treated PVA nanowebs, a measure of their breathability. The breathability of nanowebs, measured in terms of moisture vapor transmission rate (MVTR) (g/m²/day), was calculated using Eq. (1):

$$MVTR = 24M/At \tag{1}$$

where:

M = loss in mass of the assembly over a given period t (g)

t = time between successive weighing of the assembly (h)

A = the area of the exposed test specimen (0.005413 m²)

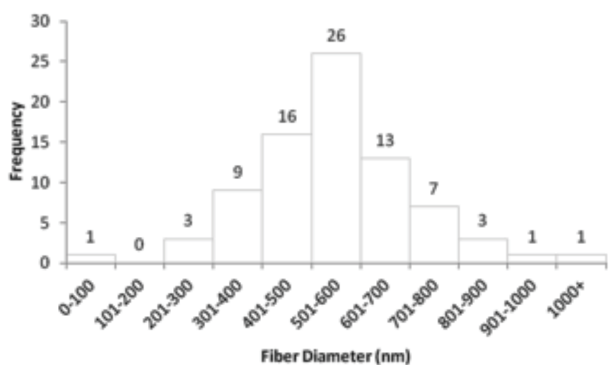
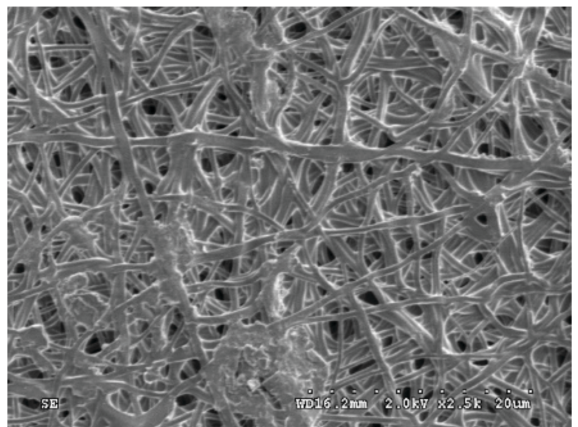
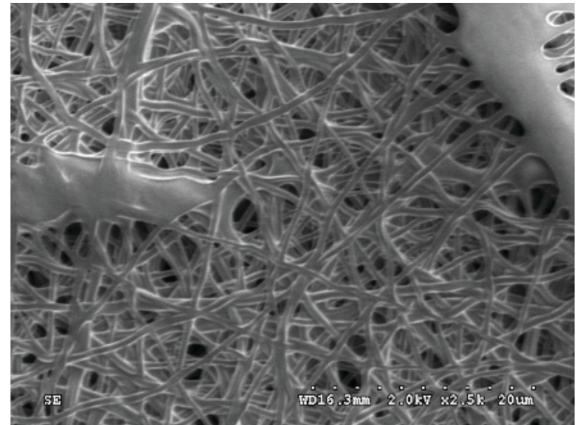
Determination of tensile properties of treated PVA nanowebs

Tensile properties of treated PVA nanowebs were measured with an Instron 5569 tensile tester (Norwood, MA, USA). A 2.5 newton load cell at a crosshead speed of 10 mm/min was used for all the measurements. The maximum load (N), extension at maximum load (mm), and the modulus (MPa) were determined. A gauge length of 2 cm was used. The samples were cut into 1 cm × 3 cm pieces to carry out the tests. A modified version of ASTM D638-10 “Standard test method for tensile properties of plastics” was used to carry out the measurements. This modification was undertaken to suit the nanowebs that were studied.

RESULTS AND DISCUSSION

Morphology of treated PVA nanowebs

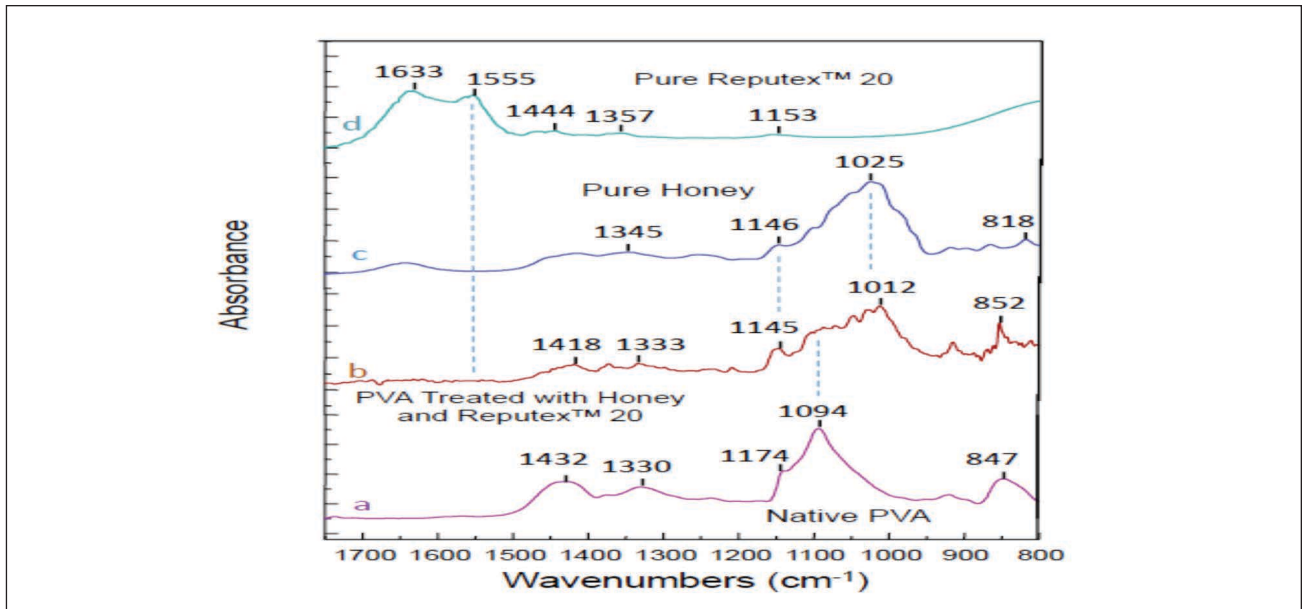
Figure 1 shows the SEM images of treated nanowebs. The average fiber diameter of treated PVA nanowebs was 548 nm (±162 nm). Most of the nanofibers had a diameter of 400-700 nm. The morphology of nanofibers seems to be affected by the presence of honey, which resulted in the formation of bead-like structures. This could be attributed to the increase in the viscosity of electrospinning dope [23] due to honey. Honey is a highly viscous solution, which does increase the viscosity of electrospinning dope. No structural or morphological investigations could be performed on the treated/heat cross-linked PVA nanowebs. The process of heat cross-linking rendered the treated/heat cross-linked PVA nanowebs unsuitable for any further investigations.



1. Scanning electron microscopy images and fiber diameter distribution histogram of treated polyvinyl alcohol (PVA) nanowebs.

FTIR spectra of treated PVA nanowebs

Figures 2 and 3 show the results of infrared spectroscopy measurements comparing native and treated PVA nanowebs. The spectrum of the treated PVA nanowebs (Figs. 2 and 3) contains features common to the honey and Reputex 20 solutions and to the native PVA nanoweb. Peaks appearing at 1012 cm⁻¹ and 2210 cm⁻¹ are not present in native PVA nanowebs. The peak near 1025 cm⁻¹ position is evident in



2. Attenuated total internal reflection spectra of samples in the low energy region of 800-1700 cm^{-1} ; (a) native PVA nanoweb in purple, (b) treated PVA nanoweb in brown, (c) pure honey in blue, and (d) pure Reputex 20 solution in green.

honey and the peak appearing at 2176 cm^{-1} is also common in Reputex 20 spectrum. The spectra demonstrate components of honey and Reputex 20 solutions that become incorporated into the PVA matrix during the electrospinning process (Figs. 2 and 3). The features associated with honey are very distinct. In contrast, bands for Reputex 20 are more difficult to discern. Honey may compete for the binding sites within the PVA matrix much more effectively than Reputex 20.

In the lower energy spectral region, native PVA nanoweb has a strong band near 1094 cm^{-1} associated with C-O vibrations [24-26]. The $1050\text{-}950 \text{ cm}^{-1}$ region in the spectrum of the native PVA nanoweb (Fig. 2a) is relatively featureless. The spectrum of honey has a strong peak near 1025 cm^{-1} that contains weaker features extending toward higher energy characteristic of the C-O stretching and bending vibrations common to honey [27-29]. A peak near 1012 cm^{-1} is identified in the treated PVA nanoweb, which can be attributed to the incorporation of honey within the PVA matrix.

In the higher energy spectral region, a weak band near 2210 cm^{-1} appears in spectra of treated PVA nanoweb (Fig. 2b). A similar band is evident in Reputex 20 spectra at 2176 cm^{-1} , which can be attributed to C≡N stretching of nitrile groups. The band provides additional evidence for integration of functional moieties in Reputex 20 into the PVA matrix.

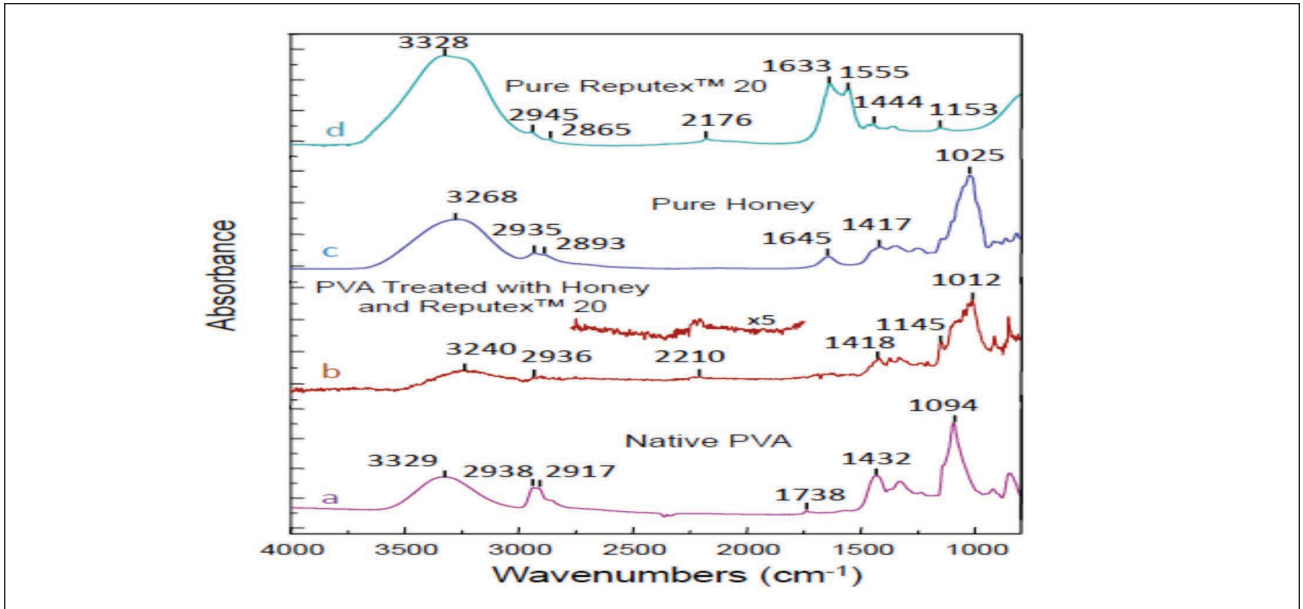
No spectrum was obtained from the treated/heat cross-linked PVA nanoweb. Treated PVA nanoweb became very brittle for examination with an attenuated total reflectance crystal after heat cross-linking.

Antimicrobial activity of treated PVA nanoweb

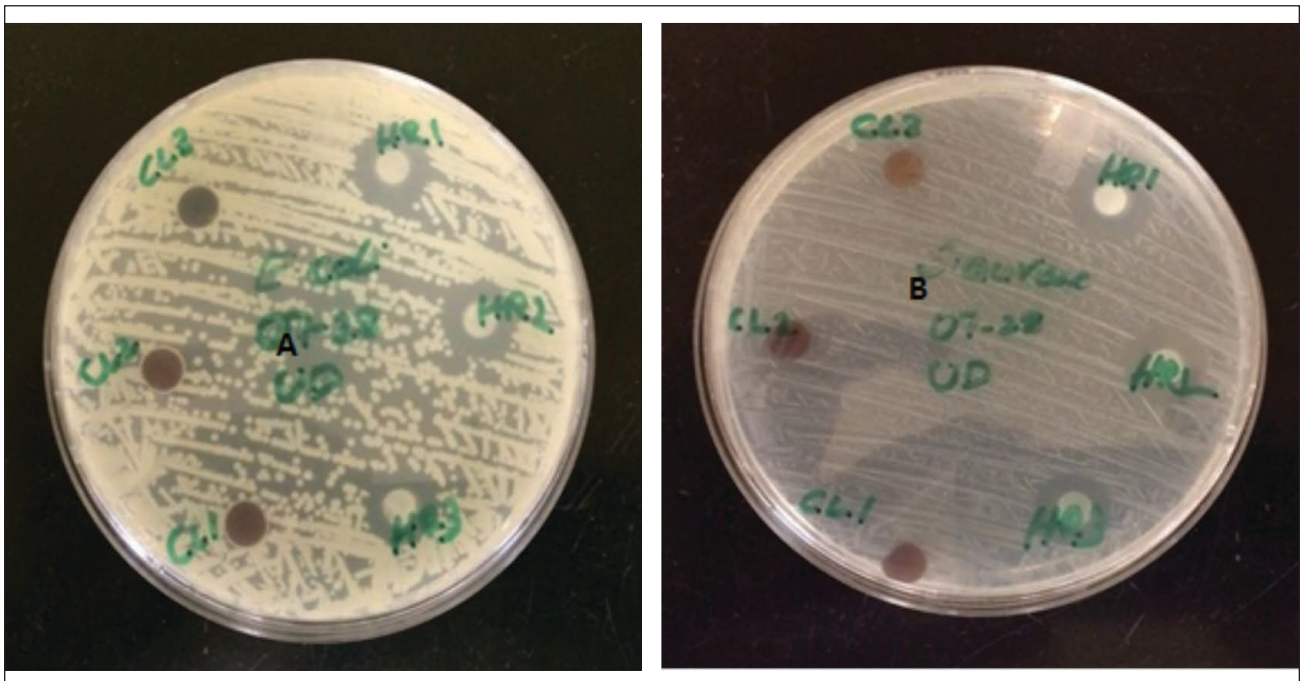
Antimicrobial activity is an important property for wound dressings. Puttamayutanon et al. attempted to functionalize PVA nanoweb with honey from *A. dorsata* bees [17].

Honey functionalized PVA nanoweb had no inhibitory effects on tested microorganisms despite pure honey exhibiting significant antimicrobial activity. Possible reasons this might have resulted are the low concentrations of honey and the failure of the active ingredient to diffuse effectively from nanofibers. Honey is a very viscous solution that needs to be diluted when used in electrospinning dopes. Hence, we added a commercially available microbiocidal solution, Reputex 20, that contains PHMBs to the mixture of PVA solution and honey in this study. Such a combinatorial approach was undertaken in an effort to investigate whether or not the beneficial properties of honey and PHMBs could be imparted to electrospun PVA nanoweb.

Figure 4 presents the results of antimicrobial assays performed with treated PVA nanoweb on *E. coli* and *S. aureus*. The treated PVA nanoweb (Figs. 4a and 4b) have demonstrated antimicrobial activity against *E. coli* and *S. aureus*. Clear zones of inhibition were observed near treated PVA nanoweb (HR1, HR2, and HR3 in Figs. 4a and 4b), indicating that treating with a mixture of PHMBs and honey has functionalized PVA nanoweb. Results from FTIR spectra of treated nanoweb also support this observation wherein incorporation of active moieties (PHMBs) from Reputex 20 into the PVA matrix was observed. The diameter of the zones of inhibition was 13 mm for *S. aureus* and 15 mm for *E. coli*. However, no zones of inhibition were observed around heat cross-linked PVA nanoweb (CL1, CL2, and CL3 in Fig. 4a and 4b). Hence, it can be inferred that the process of heat cross-linking has compromised the antimicrobial properties of functionalized PVA nanoweb. Considering the lack of FTIR spectra from treated/heat cross-linked PVA nanoweb, further investigations are needed to explain this effect.



3. Attenuated total internal reflection spectra of samples in the high energy region of 1000-3500 cm^{-1} ; (a) PVA nanoweb in purple, (b) treated PVA nanoweb in brown, (c) pure honey in blue, and (d) pure Reputex 20 solution in green. The insets in (b) expand the y-axis scales across the wave number regions indicated by a factor of 5.



4. Antimicrobial activity of treated (white) and treated/heat cross-linked (brown) PVA nanoweb; (a) activity of nanoweb on *Escherichia coli*, and (b) activity of nanoweb on *Staphylococcus aureus*.

Breathability of functionalized PVA nanoweb

An often overlooked property of a wound dressing is its breathability, which governs the loss of water and other exudates from the wound. Studies have suggested that breathability of a wound dressing in the range of 2000-2500 $\text{g}/\text{m}^2/\text{day}$ prevents excessive dehydration of the wound and the build-up of wound exudates [8,30]. That is why we have under-

taken experiments to quantify the breathability of functionalized PVA nanoweb.

In our study, the breathability of functionalized PVA nanoweb was found to be 1856.85 $\text{g}/\text{m}^2/\text{day}$ ($n = 5$, $se = 36.32$). Hence, it can be inferred that functionalized PVA nanoweb have demonstrated breathability values suitable for use in wound dressing applications (in the range of

Measure	Value	Standard Error
Maximum load	7.98 newtons	0.97
Extension at maximum load	12.97 mm	2.41
Modulus	88.02 MPa	20.71

I. Tensile properties of functionalized PVA nanowebs (n = 5).

2000-2500 g/m²/day). Such a phenomenon can be explained by the following reasons. First, FTIR spectra have confirmed the integration of components of honey into functionalized PVA nanowebs. This involves the incorporation of carbohydrate functional groups into the PVA matrix, in corroboration with previous studies [29]. This explains the excellent moisture vapor transmission characteristics shown by functionalized PVA nanowebs, as breathability of a substrate is strongly affected by the presence of surface functional groups [31]. In our study, the FTIR spectra suggest the addition of carbohydrate functional groups to the PVA matrix. The 3329 cm⁻¹ band in the spectrum of native PVA nanowebs (Fig. 2a) arises from the O-H stretching vibration of the alcohol group in the polymer [29]. The band is shifted considerably to lower energy (3240 cm⁻¹) in the spectra of the functionalized PVA nanowebs (Fig. 2b). The shift reflects the contribution from hydrogen bonding of O-H groups in PVA and the associating sugars and water molecules hydrating the matrix. Also, the hygroscopic property of honey, which is its ability to absorb moisture from its surrounding humid environment and release it to dry air [32], explains the observed breathability values. Finally, the large fiber diameter of functionalized PVA nanowebs, compared with native PVA nanowebs electrospun under similar conditions [24], as evident from the SEM images (Fig. 1), might also explain the observed breathability values. Large fiber diameters suggest low fiber packing densities that increase the porosity of nanowebs [33]. A low fiber packing density often results in the presence of large pores in the nanoweb that may enhance the process of moisture vapor transmission.

Tensile properties of functionalized PVA nanowebs

Wound dressings must possess certain mechanical properties such as tensile strength, sustainability, flexibility, bendability, and elasticity. Among these, for physically handling, it is the tensile properties that are critical, and so our emphasis was placed on evaluating the tenacity of functionalized PVA nanowebs. **Table I** shows the tenacity results.

Results from FTIR analysis have suggested a sharp peak at 1145 cm⁻¹ in the spectrum of the functionalized PVA web (Fig. 2b). The intensity and sharpness of the band are characteristic of increasing PVA crystallinity, which is also evident in honey and Reputex 20 [24,26]. The growth of the

1145 cm⁻¹ peak in functionalized PVA nanoweb might be the result of increasing organization within the PVA matrix driven by hydrogen bonding between amine and imine groups in Reputex 20 and O-H groups in the PVA polymer, or the result of ordering within the PVA matrix facilitated by interactions between carbohydrates and segments of the PVA polymer. Hence, we can infer that the process of functionalization might result in an increase in the tensile properties of functionalized PVA nanowebs, compared with those of native PVA nanowebs [24].

CONCLUSIONS

Bioactive and breathable electrospun PVA nanowebs were fabricated using a combinatorial approach by functionalizing PVA nanowebs with a mixture of honey and Reputex 20. FTIR analysis confirmed the presence of functional moieties from honey and Reputex 20 in functionalized PVA nanowebs. Functionalized PVA nanowebs showed good moisture vapor transmission characteristics and exhibited excellent antimicrobial activity. The process of functionalization also resulted in an increase in tensile properties of PVA nanowebs. The study highlights the importance of functionalizing electrospun nanowebs with natural and artificial substances to enhance their applicability in biomedical applications such as wound bandages. More importantly, functionalization of nanowebs with a combination of natural and artificial substances may pave the way to the fabrication of nanowebs for a multitude of applications. **TJ**

ACKNOWLEDGEMENTS

Seshadri Ramkumar gratefully acknowledges financial support from The CH Foundation, Lubbock, TX, USA. The authors are very thankful to Nathan H. Sheets, from Nature Nate Farms, for graciously providing the honey used in this study. We sincerely thank Bo Zhao at Texas Tech University (Lubbock, TX, USA) for all her help with SEM imaging of nanowebs. Thanks also to the University Arts & Sciences EOX Imaging Facility, Texas Tech University, instrument funded by NSF MRI 04-511.

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ABOUT THE AUTHORS

We started this work to develop breathable and biocidal nanowebbs for use in biomedical applications such as wound dressings. An often-overlooked aspect in the design and development of wound dressings is their moisture vapor transmission characteristics. Special emphasis was placed on developing a nanoweb that is both breathable and biocidal in nature. More importantly, nanowebbs were developed using 1) a biocompatible polymer polyvinyl alcohol (PVA), 2) honey, and 3) a safe antiseptic, polyhexamethylene biguanides (PHMBs).

To the best of our knowledge, the treatment/functionalization of electrospun nanowebbs with honey and PHMBs has been investigated to a very limited extent. Previous research from our laboratory has focused on treating/functionalizing PVA nanowebbs with honey and PHMBs separately. Treatment with PVA enhanced the moisture vapor transmission characteristics of PVA nanowebbs. Treatment with PHMBs imparted antimicrobial properties to PVA nanowebbs. In the present work, we treated PVA nanowebbs with a mixture of honey and PHMBs in an attempt to see if the beneficial properties from both could be imparted to PVA nanowebbs.

Procuring pure PHMBs is very difficult. Hence, we used Reputex 20, a commercially available PHMB-based product. Considering the diversity in the composition and properties of honey, it again is difficult to decide on the choice of honey. Finally, optimizing the concentrations of honey and PHMBs for use in the study is an equally difficult task. We took a trial-and-error approach to accomplish this objective.

An interesting outcome of our study is that the PVA nanowebbs were found to be both breathable and biocidal in nature. Before the study, we were concerned that the honey or PHMBs might mask the beneficial properties of each other. In simpler terms, would the presence of honey affect the diffusion of PHMBs to the surface, and vice versa? A surprising outcome was that the process of heat cross-linking compromised the properties of functionalized PVA nanowebbs. More importantly, they were found to be very fragile for any further investigations.

The pulp and paper industry could use an approach such as we used to develop multifunctional textiles for a variety of applications according to their needs.

Future research could look into investigating the applicability of these nanowebbs in vivo. This involves testing the nanowebbs near the vicinity of a wound. Also, the use of these nanowebbs as cell and tissue culture scaffolds could be investigated.



Turaga



Singh



Ramkumar



Gibson



Presley



Smith



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