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Fabrication, characterization and *in vitro* evaluation of silver-containing arabinoxylan foams as antimicrobial wound dressing

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Abstract

Arabinoxylan ferulate (AXF) foams were fabricated via enzymatic peroxidase/hydrogen peroxide crosslinking reaction followed by freeze-drying and studied as a potential wound dressing material. The AXF foam's rheological, morphological, porous and swelling properties were examined. AXF foams were found to be a viscoelastic material that proved to be highly porous and water absorbent. AXF foams possessed low endotoxin levels and were cytocompatible with fibroblasts. Silver was successfully integrated into AXF foams and slowly released over 48 hours. AXF foams impregnated with silver demonstrated efficacy inhibiting bacterial growth according to a modified Kirby-Bauer disk diffusion susceptibility test. Overall, AXF foams possess appropriate material properties and the silver-loaded AXF foams showed antimicrobial activity, necessary to be a candidate material in wound dressing development.

Keywords

arabinoxylan; silver dressing; wound healing; Kirby-Bauer disk diffusion susceptibility test; polysaccharide

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INTRODUCTION

Wound dressings play an important role in promoting wound healing and preventing infections post-injury.^{1,2} Polysaccharides have been extensively used in development of wound dressings because of their desirable structural features and material properties.^{3–6} In particular, they are natural, highly abundant, biodegradable, and non-toxic. They are an important class of biopolymers and play an important role in forming the extracellular matrix.⁷ Polysaccharides can be crosslinked to form highly absorbent gels to carry and release drugs in a controlled fashion. Development of wound dressings based on polysaccharides such as alginate, chitosan and hyaluronan has been successful in not only protecting the wound site but also providing a naturally derived matrix that influences cells and their signaling factors to enhance the wound healing process. These advanced dressings aim to function beyond covering the wound from the external environment. In addition, polysaccharides have demonstrated an ability to act as a drug delivery scaffold, enabling potential delivery of relevant wound healing therapeutics such as silver.^{8–16}

Arabinoxylan (AX) is a type of polysaccharide composed of a xylose backbone, to which arabinose is attached through O-2 and/or O-3 positions.¹⁷ Some of the arabinose residues are linked to ferulic acid (FA) via ester linkages (Scheme 1). It is the presence of FA in AX that enables AX to form hydrogels via enzymatic reaction.^{18,19} In particular, FA substituents form dimers and trimers that lead to covalently cross-linked AX networks using enzymatic free radical generating agents such as laccase and peroxidase/H₂O₂. In contrast to most polysaccharides, AX enzymatic gelation does not require the use of harsh organic solvents or toxic chemical cross-linking agents. Such property of AX is appealing for the development of biomaterials and drug delivery systems. The applications of polysaccharides related to AX range from packaging materials to prebiotics that regulate gut metabolism.^{20–22} AX gels have been developed as delivery systems to deliver therapeutics such as methylxanthine. Nonetheless, AX application as a wound dressing material has yet to be realized. Wound dressing materials are expected to be absorptive, impermeable to bacteria, and inexpensive. The goal of the study was to fabricate and investigate basic properties of AX in relation to wound dressing development. Antimicrobial properties are critical in preventing infections in wound care, and various types of antimicrobial dressings have been developed.^{23–28} In this study, silver-containing AX hydrogels were fabricated in the presence of peroxidase/hydrogen peroxide and then converted into foams via freeze-drying. These foams were characterized and compared as an antimicrobial wound dressing against a commercialized polysaccharide Alginate dressing in terms of material properties, biocompatibility, and antimicrobial activity.

MATERIALS AND METHODS

Materials

Arabinoxylan ferulate (AXF) (extracted from maize bran) was purchased from Cambridge Biopolymers (Cambridge, UK). Horseradish peroxidase (HRP), hydrogen peroxide (H₂O₂), and silver sulfadiazine were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate buffered saline (PBS) was purchased from EMD Chemicals (Gibbstown, NJ). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Invitrogen (Carlsbad, CA). Fetal

calf serum was purchased from Lonza (Walkersville, MD). Hyclone 0.05% Trypsin was purchased from Thermo Scientific (Logan, UT). Penicillin-Streptomycin was purchased from Life Technologies (Grand Island, NY). 3M™ Tegaderm Alginate and Tegaderm Alginate Ag dressings were purchased from 3M Health Care (St. Paul, MN). LAL chromogenic endotoxin quantitation kit was purchased from Thermo Scientific (Rockford, IL).

Scaffold preparation

To prepare AXF foams, 600 mg of AXF dry powder alone or along with 65 mg of silver sulfadiazine was added to 10 mL of deionized water, stirred, and shaken until dissolved completely. Next, 50 µl of 1 mg/mL HRP solution and 60 µl of (3% w/v) H₂O₂ were each added to the AXF solutions and stirred to initiate enzymatic crosslinking.²⁹ Immediately, the prepared solution was poured into a 100 mm Petri-dish and placed in an ultrasonic bath (1510R-DTH Branson) for 30-minute sonication at room temperature with a fixed frequency of 42 KHz. After sonication, the solution was allowed to sit at room temperature for three hours to cure. After curing, the resulting AXF hydrogel was frozen (−20 °C) and lyophilized overnight to produce the final product. Two batches were prepared. Tegaderm Alginate and Tegaderm Alginate Ag dressings containing 5% silver were used as controls for comparison.

Morphology examination

Prior to SEM imaging, samples were placed on a 1 cm diameter stub. The stub was placed on a specimen holder and platinum sputter coated. SEM images were taken utilizing a JEOL JSM-5610LV scanning electron microscope.

Rheological testing

Rheological testing was performed on samples that were cut into 20 mm diameter disks. Testing was performed on wet samples on a temperature controlled plate of a Discovery Hybrid Rheometer (HR-3, TA Instruments). Samples were immersed in 1 mL of phosphate buffer saline (PBS) for one hour before blot drying. Each set of samples on the plate at 37 °C were subjected to compression and shear stress by a 20 mm diameter parallel plate. An amplitude sweep was performed in order to confirm that all the measurements were conducted within the linear viscoelastic region. Oscillatory frequency sweeps were then carried out with a constant strain of 0.1% in the frequency region of 0.1 – 100 rad/s. These settings were derived from Zuidema et al.³⁰

Swelling studies

AXF samples were cut into 10 mm diameter disks by a biopsy punch and then weighed. Each sample was immersed in 1.5 mL of PBS equilibrated at 37 °C. At pre-determined time points (6, 12, 24, 48, 72, and 168 hours), the swollen foams were taken out, blot dried, and weighed. The swelling kinetics over the period of 168 h was reported. Swelling ratio is defined as the ratio of mass of swollen sample at each time point to the initial mass of dry sample.

Porosity measurements

For determination of the density of the scaffold, a Quantachrome Instruments Ultrapyc 1200e pycnometer Model MUPY-31 was employed. Density analysis was conducted according to manufacturer's protocol using ultrahigh purity Helium gas and a maximum pressure of 3 psig. Prior to each sample run, the pycnometer was calibrated to determine the volume of the sample cell. For each sample run, the sample weight was entered into the instrument's software which output the volume of a holding cell and the holding cell containing the sample. The difference of these volumes and the known mass of the weighed sample were taken to calculate the sample density. This measurement was done for six replicates of the wound dressing material tested.

Mercury porosimetry was performed to evaluate the distribution of porosity within the AXF and Tegaderm Alginate foams. Thermo Scientific Pascal Model 140 porosimeter was used for this measurement. The samples underwent pressurized mercury intrusion according to manufacturer's instrument protocol with the use of a CD3 N dilatometer using the following parameter settings: macular height, 90.5 mm; stem mercury height, 64.5 mm, filling volume, 456 mm³; cone height, 21.0 mm; electrode gap, 5.0 mm; and stem radius, 1.5 mm. The sample's weight and density values obtained by the pycnometer were entered into the porosimeter instrument software (SOLID) prior to mercury filling. The samples were loaded into the dilatometer, filled with mercury, and pressurized to 400 kPa. After mercury intrusion, the sample's pore diameter's size representative distribution data was collected and analyzed. This measurement was done for six replicates of the samples tested.

Permeability measurements

Permeability testing was performed to quantitatively evaluate interconnectivity of the pores within the scaffolds. Tegaderm alginate and the AXF foam samples were obtained using a 10 mm biopsy punch. Permeability measurements were determined via a previously published protocol and apparatus design.³¹ The thickness (**L**) of each sample was recorded prior to testing. Each sample was placed between rubber gaskets within the water tight apparatus. The height (**h**) of the system was adjusted to 0.986 m. Water was allowed to flow through the apparatus and the scaffold. The fluid volume (**V**) and time (**t**) were recorded. Pressure was determined via the equation for pressure $P = \rho gh$ where ρ is the density of water (1 g/cm³ at 25 °C), **g** is the gravitational force (9.8 m/s²), and **h** is the height of the system (0.986 m). The permeability (**P_m**) in Darcy (D) was calculated for each scaffold (n=3) using Darcy's equation of permeability $P_m = \frac{\mu \times V \times L}{A \times t \times p}$ where μ is the known viscosity of water (0.89 cp at 25 °C), **A** is the cross-sectional area of scaffold subjected to the flow of water, **L** is the scaffold thickness, and **p** is the calculated pressure through the system.

Limulus amebocyte lysate (LAL) endotoxin assay

To determine the scaffold endotoxin content, the AXF foams were weighed, sterilized with 1000 ppm peracetic acid for 15 minutes, washed in PBS three times, and air dried for 10 minutes using the protocol developed by Yoganarasimha et al.³² Sterilized AXF foams and unsterile foams were placed into a tissue culture insert inside each well. The inserts containing the foam samples were immersed in PBS for four hours. After immersion, a 50 μ l

aliquot was extracted from the release media and diluted 20-fold with addition of 1 mL of endotoxin free media from the endotoxin assay kit. After dilution, 50 µl aliquots were taken from the diluted samples and added to a 96-well plate. Next, 50 µl of LAL reagent was added to the sample for a 10-minute incubation at 37 °C. Afterwards, 100 µl of chromogenic substrate was added to the reaction mixture and incubated for six minutes at 37 °C to induce a colorimetric reaction based on protease enzyme activity from the endotoxin. A 50 µl aliquot of 25% acetic acid was added to end the reaction. Samples were gently shaken and subjected to spectrophotometric measurement at 405 nm for quantification of endotoxin levels against a standard curve.

AXF foam cytocompatibility assessment

AXF foam samples were sterilized with 1000 ppm peracetic acid for 15 minutes and washed in PBS three times for 10 minutes using the protocol developed by Yoganarasimha et al.³² NIH3T3 fibroblasts were seeded at a density of 50,000 cells at the bottom of each well in a 12-well plate. The sterilized AXF foams were air-dried for 10 minutes and placed into Corning® Transwell® polycarbonate membrane cell culture inserts (pore size 0.4 µm). The inserts loaded with the sample foams were introduced to the wells (above, not contacting the cells), and the samples were completely submerged in the cell culture medium. After incubation for 24 and 72 hours at 37 °C and 5% CO₂, cell viability was assessed using Trypan blue exclusion assay. Tegaderm Alginate foams were tested under the same conditions for comparison. The cells cultured in the absence of scaffold were used as a positive control.

Silver release studies

Silver-containing AXF foams were cut into 10 mm diameter disks and then immersed in 20 mL of 2% nitric acid solution at 37 °C. At pre-determined time points (1, 2, 6, 12, 24, and 48 hours), aliquots of release medium (5 mL each) were taken out and then subjected to inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis for silver quantification. Immediately following each sampling, 5 mL of blank medium was added to maintain its volume. The total mass of ionic silver in the scaffold was approximated as 3% of the scaffolds mass. Silver release from Tegaderm Alginate Ag foam was also tested under the same condition for comparison. Cumulative silver release profiles over the period of 48 hours for both types of dressings (n=8) were reported.

Bacterial growth inhibition assay

A modified Kirby-Bauer disk diffusion susceptibility test was performed to determine bacterial susceptibility based on the size of their growth inhibition zones.³³ *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis* were subject bacteria in this study because they have been most frequently examined in epidemiological studies on patients with post-surgical wound infections.³⁴ Five µL of frozen aliquots of gram-positive bacterial species *Staphylococcus aureus* strain RN450 and *Enterococcus faecalis* strain V583 were cultured in BHI broth media for 18 hours at 37 °C. Gram-negative strain *Pseudomonas aeruginosa* PAO1 was cultured in LB broth media for 18 hours at 37 °C. *S. aureus* and *P. aeruginosa* were cultured in a standard incubator shaking at 200 rpm while *E. faecalis* was

incubated in a 6% oxygen Anoxomat jar (Advanced Instruments, Inc., Norwood, MA). All species were cultured overnight to a concentration of approximately $1-2 \times 10^9$ CFU/mL.

A sterile cotton swab was dipped into each culture medium and spread on plates containing the same medium plus 1.5% (w/v) agar in a 100 mm Petri-dish and allowed to dry for five minutes. Antibiotic tetracycline (30 μ g deposited on a 6 mm diameter filter paper) was used as positive control for *P. aeruginosa* and *E. faecalis* treatments. Erythromycin (15 μ g deposited on a 6 mm diameter filter paper) was used as positive control for *S. aureus* treatment. Silver-containing AXF, Tegaderm Alginate Ag, and AXF foam without silver (negative control) of 6 mm in diameter were placed in the remaining three quadrants on the agar. After 24-hour incubation, top view images of the Petri dishes were acquired using a digital camera and analyzed with image processing software (FOTO/Analyst PC Image, Fotodyne, Inc.). The inhibition zone diameters were measured with a caliper. Disk diffusion susceptibility testing was performed twice on separate occasions under the same conditions. The mean values of the inhibition zone diameters of each treatment were then determined.

Statistical analysis

Statistical analysis was carried out using an unpaired t-test and one way analysis of variance (ANOVA) with post-hoc analysis for subgroup comparison. *P* values less than 0.05 were considered statistically significant.

RESULTS

Rheological properties

We fabricated AXF hydrogels using peroxidase/H₂O₂ enzymatic cross-linking reaction (Scheme 1) and converted them to foams with porous structures using freeze drying. To determine the linear viscoelastic region (LVR), we first performed an amplitude sweep on hydrated AXF foams and Tegaderm Alginate dressings with and without silver. As shown in Fig. 1A and 1C, both AXF foams and silver containing AXF foams had a longer LVR than Tegaderm Alginate foams, indicating that AXF foams were more structurally stable. The frequency sweeps were then measured at a fixed strain of 0.1%. As shown in Fig. 1B and 1D, all the samples display typical hydrogel viscoelastic behavior as their storage moduli (*G'*) are higher than their loss moduli (*G''*), and frequency-independent. Tegaderm Alginate Ag shows slightly higher storage modulus than Tegaderm Alginate while the storage modulus of silver-containing AXF is comparable to the storage modulus of AXF. In the hydrated state, AXF foams have significantly lower storage moduli than Tegaderm Alginate foams. We believe that it is because AXF foams are more highly water absorbent that polymer chains in the foam expanded in a more appreciable manner upon hydration, hence compromising their elasticity and mechanical strength.

Morphology

The AXF foams exhibited a porous, smooth morphological structure (Fig. 2). Also, the pores were in the micro scale and evenly distributed along the surface. Tegaderm Alginate foams possessed a smooth surface with a random entanglement of microfibers. The random

microfiber entanglement mesh in Tegaderm Alginate foams may affect fluid absorption and entrap ionic silver complexes to make significantly slow silver release as shown in our work.

Porosity

The AXF foams are more porous than the Tegaderm Alginate counterparts. The porosity values for Tegaderm Alginate and AXF foams were $36.4 \pm 8.0\%$ and $99.0 \pm 0.5\%$, respectively. Although the porosity of Tegaderm Alginate Ag was lower than Tegaderm Alginate, adding silver to AXF did not result in a significant reduction in porosity (Table 1). The pore distribution in AXF and Tegaderm Alginate foams was heavily skewed by smaller sized pores. In particular, 89.2% of pores in AXF foams had a pore diameter less than $50 \mu\text{m}$. The skew was much more modest in Tegaderm Alginate foams as only 48.6% of pores within the scaffold had pore diameters less than $50 \mu\text{m}$ (Fig. 3). The difference in pore distributions between the Tegaderm Alginate and AXF foams is likely attributed to the adoption of different scaffold fabrication methods. Tegaderm Alginate foams are less densely packed with non-woven ribbon-like fibers. AXF foams are much denser and contain more small pores created from the freeze-drying postprocess.

Permeability

The results from permeability testing revealed that the AXF foams were significantly more permeable than Tegaderm Alginate. The AXF foams exhibited a permeability of $22 \pm 4.4 \text{ D}$, nearly three-fold the permeability of Tegaderm Alginate scaffolds ($7.6 \pm 0.4 \text{ D}$) (Fig. 4). This indicates that the degree of interconnectivity of the pores within the AXF foams is greater than that of Tegaderm Alginate. A high degree of interconnectivity and porous microstructure within AXF foams enabled greater fluid absorption at the wound site as demonstrated in the swelling study.

Swelling behavior

Both Tegaderm Alginate and AXF foam scaffolds have been shown to possess high swelling capacities (Fig. 5). Upon immersion, Tegaderm Alginate absorbed fluid quickly and the mass of the swollen sample increased 10 fold in 1 hour of incubation. The swelling ratio was stable throughout the observation, indicating no further fluid absorption by the dressing. The AXF foam exhibited a significantly higher swelling capacity. The mass of the swollen AXF increased 13 fold in the first hour of incubation. It continued to absorb fluid up to 48 hours and reached a swelling ratio of 20, which was two times higher than that of Tegaderm Alginate. A slight decrease in swelling ratio at later time points may be attributed to the disintegration of the gel network. The highly swelling capacity of AXF foams was consistent with its high degrees of permeability and porosity.

Endotoxin analysis

LAL endotoxin levels for unsterilized and sterilized AXF foams were 5.30 ± 1.10 and $3.42 \pm 0.87 \text{ EU/mg}$, respectively, showing a 35.6% reduction after treatment (Fig. 6). The Food and Drug Administration does not specify a uniform endotoxin level limit for wound dressing materials. However, it is imperative to minimize endotoxins which would otherwise cause symptoms such as fever and septic shock. The reduction of endotoxins after paracetic

acid sterilization helped contribute to AXF foams being less cytotoxic, maintaining baseline cell viability, and proliferation *in vitro*.

Cytocompatibility assessment

Integrating AXF foams into the *in vitro* cell microenvironment did not have a cytotoxic effect on NIH3T3 fibroblasts. Their viability values were 96.6 and 96.4% over a 24 and 72-hour time period, respectively. After exposure to the Tegaderm Alginate foam, fibroblasts were 96.7 and 97.3% viable after 24 and 72 hours, respectively. Untreated fibroblasts had viability values of 95.8 and 96.9% after 24 and 72 hours, respectively (Fig. 7). There were no significant differences in cell viability values between AXF foams, Tegaderm Alginate foams, and untreated test groups. Based on the results, it can be concluded that fibroblasts, which play a prominent role in the proliferative and remodeling stages of wound healing, were not negatively affected after exposure to AXF foams as their viability maintained baseline levels through the 72-hour time period.

Fibroblast proliferation results after exposure to AXF foams were encouraging partly due to their high viability *in vitro*. After incubating at an initial cell seeding density of 50,000 cells per well, fibroblast cell numbers increased to 166,000 and 212,000 after 24 and 72 hours, respectively. Fibroblasts cultured with 3M Tegaderm Alginate foams proliferated to 129,000 and 302,000 cells per well after 24 and 72 hours, respectively (Fig. 8). Meanwhile, non-treated test groups had cell numbers of 205,000 and 289,000 cells per well after 24 and 72 hours, respectively. Cell proliferation has been observed for all treatment groups. However, there were no significant differences among the treatment groups at 24 and 72-hour time points.

Silver release

Silver was released from Tegaderm Alginate Ag foam at a very low rate. Under the conditions we used, ICP-OES analysis results show that there was no appreciable silver release from Tegaderm Alginate Ag dressing. Less than 0.1% of silver was released in 48 hours (Fig. 9). In contrast, a burst release of silver from AXF foams was observed. Nearly 21% of silver was released in the first hour. Following the initial burst release, silver was released more slowly and reached a cumulative release of 26% in 48 hours. This was most likely attributed to initial swelling, bulk degradation, and erosion of the AXF foam in comparison to alginate foams.

Inhibition of bacterial growth

The four treatment groups were assessed for their antimicrobial activity by measuring inhibition zones for each species of bacteria. *P. aeruginosa* was highly susceptible to silver sulfadiazine impregnated AXF foams, which produced an inhibition zone of 22.17 ± 1.53 mm, greater than the inhibition zone of the tetracycline positive control (Fig. 10 and Table 2). The efficacy of silver sulfadiazine was consistent with previous data in the literature showing its applicability in a skin wound model.³⁵ Gram-positive *S. aureus* and *E. faecalis* species were less sensitive to the silver sulfadiazine impregnated-AXF foams as evidenced by smaller inhibition zone diameters of 12.39 ± 1.26 and 12.32 ± 0.81 mm, respectively.

All three species of bacteria were sensitive to AXF foams impregnated with silver sulfadiazine. *P. aeruginosa* was most susceptible to the silver sulfadiazine impregnated AXF foams. For Tegaderm Alginate Ag, bacteria were more resistant with smaller inhibition zones than silver sulfadiazine-impregnated AXF foams. In fact, *E. faecalis* was completely resistant to Tegaderm Alginate Ag. Silver sulfadiazine impregnated AXF foams against *P. aeruginosa* produced irregularly shaped inhibition zones possibly due to the scaffold degradation during incubation. All three positive control treatments achieved clear inhibition of the three species after 24-hour incubation. As expected, none of the bacterial species showed any sensitivity to AXF foams without silver sulfadiazine. The results suggest antimicrobials such as silver sulfadiazine can be integrated into AXF foams for infection prevention.

DISCUSSION

The mercury porosimeter generates a high pressure and may cause pore collapse within the foams. This concern holds true for other types of materials as well. This can happen if a material is pressed against the walls of the dilatometer and essentially compressed via the high pressure of the mercury. To prevent this issue, we developed a small "sample support cage" structure to hold and protect scaffolds/foams/sponges from being pressed against the walls of the dilatometer. The sample support cage was placed within the dilatometer for calibration so its presence during the test was not reflected in the resulting data.

Additionally, the sample support cage was not porous, and therefore it did not interfere with data received during the test. All of the samples were tested using the sample support cage. The foams appeared the same following the porosimeter run as they did before the testing. Following the porosimetry testing where foam samples were filled with mercury, visual observation showed that mercury fell out of foam once it was removed from the pressurized dilatometer, suggesting large pores within the foam sample were kept during the test. The application of the sample support cage made the mercury porosimetry less disruptive to samples by providing or allowing the equal pressurization in three-dimensions of the samples to minimize pore structure collapse.

In future work, crosslinking density will be fine-tuned to modulate mechanical and structural properties of AXF foams in dry and moist environments such as degradation and viscoelasticity. Surface morphology of AXF foams suggested a highly porous structure that enabled fluid absorption and drug release that was greater than in Tegaderm Alginate foams. Nonetheless, future studies should include analysis of the porous structure using micro-CT scanning to help further elucidate foam microstructure in three dimensions. In addition, the porosity of these foams may be better controlled using porogen leaching methods for modulation of silver release rate. In addition, lyophilization conditions such as vacuum pressure and freezing temperature will be further investigated as they may have played a role influencing pore size distribution. Additionally, the results demonstrated the cytocompatibility of AXF foams over the 72-hour time period in comparison to non-treatment and Tegaderm Alginate groups. The mean cell number for AXF foam treated groups was greater at 24 hours than Tegaderm Alginate foams but was not statistically significant. Another insight from the data was the mean cell number of fibroblasts after

exposure to AXF foams was less than Tegaderm Alginate and non-treated groups after 72 hours. However, these differences were not statistically significant.

The samples used in the silver release study were not sterilized with peracetic acid. Sterilization procedures such as peracetic acid treatment may alter silver release kinetics and consequently affect silver antimicrobial activity. In future studies, we would like to use sterilized dressings for animal testing where sterilization becomes critical. At that time, we will systemically examine the effect of sterilization methods on silver release kinetics and antimicrobial activity. Furthermore, viscoelastic properties of the dressing materials are important. We will conduct a systematic investigation of such properties in conjunction with fabrication and sterilization processes in the future work.

Silver loaded in the dressing material may follow one of the two mechanisms to exert antimicrobial activity: it is released to the wound bed to kill pathogens on the wound surface and throughout the wound bed or it is retained in the dressing to annihilate the pathogens in the exudate that is absorbed into the dressing. Our silver release study suggested the latter for the Ag Alginate. Silver release from AXF foam is the combination of both mechanisms, which may be an even better approach for effective control over infections. We will investigate and engineer silver release kinetics by modulating AXF foam structure and silver loading density. AXF foams will be examined *in vivo* to assess its properties that promote wound healing while preventing infections in a living physiological model. Given the flexibility of the foam fabrication and drug loading, combinations of antibiotics and silver will be considered to create an anti-microbial cocktail to prevent or treat infections against both Gram-positive and Gram-negative bacteria.³⁶

CONCLUSIONS

The AXF foams prepared via peroxidase-catalyzed crosslinking reaction in the presence of hydrogen peroxide were found to be highly porous, cytocompatible, and highly absorbent. AXF foams impregnated with silver sulfadiazine demonstrated efficacy inhibiting bacterial growth. Although AXF foams compared favorably to Tegaderm Alginate foams in the aforementioned areas, further optimization and investigation of its structural properties are necessary. *In vitro* tests such as wound healing, *in vivo* drug release, antimicrobial activity, and host immune responses will be conducted to bring this class of biopolymers to be the forefront of wound dressing development.

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FIGURE 1.

Rheological properties of Tegaderm Alginate and AXF foams with or without silver. (A, C) amplitude sweep, (B, D) frequency sweep at a fixed strain of 0.1%.

FIGURE 2.
SEM images of **A)** 3M alginate at 500 μm ; **B)** 3M alginate at 100 μm ; **C)** AXF foam at 500 μm ; **D)** AXF foam at 100 μm .

FIGURE 3.
Pore size distributions of Tegaderm Alginate (A) and AXF foam (B).

FIGURE 4.
Permeability of Tegaderm Alginate and AXF foams.

FIGURE 5.
Swelling kinetics of Tegaderm Alginate and AXF foams.

FIGURE 6. LAL endotoxin content in AXF foams before and after sterilization. (n = 8) (* indicates $p < 0.05$).

FIGURE 7. Cell viability of NIH3T3 fibroblast cells after exposure to Tegaderm Alginate and AXF foams after 24 and 72-hour incubation period (n = 8).

FIGURE 8.

Cell proliferation of NIH3T3 fibroblast cells after exposure to Tegaderm Alginate and AXF foams after 24 and 72-hour incubation period. (n = 8).

FIGURE 9.
Cumulative silver release from Tegaderm Alginate Ag and silver-containing AXF foam (n = 8).

FIGURE 10.

Antimicrobial activity of dressing materials and control groups (Table 2) against *P. aeruginosa* (A), *S. aureus* (B) and *E. faecalis* (C) using a modified Kirby-Bauer disk diffusion susceptibility test.

SCHEME 1.

Enzymatic cross-linking reaction of AXF (A) and chemical structure of alginate (B)

Table 1

Porosities of wound dressing materials.

Dressing Material	Porosity
3M alginate foam	$36.4 \pm 8.0\%$
AXF foam	$99.0 \pm 0.5\%$
3M alginate foam w/silver	$24.1 \pm 6.4\%$
AXF foam w/silver	$96.2 \pm 3.0\%$

Table 2

Sensitivity profiles of bacterial species after treatment.

Species	Treatment	Average inhibition zone (mm)
<i>P. aeruginosa</i>	Positive Control (30 µg Tetracycline)	14.40±5.44
	AXF foam w/ 5% silver sulfadiazine	22.17±1.53
	ALG foam w/ 5% silver	10.04±1.82
	Negative Control (ALG foam)	0
<i>S. aureus</i>	Positive Control (15 µg Erythromycin)	23.02 ±0.59
	AXF foam w/ 5% silver sulfadiazine	12.39 ±1.26
	ALG foam w/ 5% silver	5.75 ±0
	Negative Control (ALG foam)	0
<i>E. faecalis</i>	Positive Control (30 µg Tetracycline)	27.62 ±0.16
	AXF foam w/ 5% silver sulfadiazine	12.32 ±0.81
	ALG foam w/ 5% silver	0
	Negative Control (ALG foam)	0