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ANTIMICROBIAL WOUND DRESSINGS FOR FULL-THICKNESS INFECTED BURN WOUNDS

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ABSTRACT—Infection of wounds delays healing, increases treatment costs, and leads to major complications. Current methods to manage such infections include antibiotic ointments and antimicrobial wound dressings, both of which have significant drawbacks, including frequent reapplication and contribution to antimicrobial resistance. In this work, we developed wound dressings fabricated with a medical-grade polyurethane coating composed of natural plant secondary metabolites, cinnamaldehyde, and alpha-terpineol. Our wound dressings are easy to change and do not adhere to the wound bed. They kill gram-positive and -negative microbes in infected wounds due to the Food and Drug Administration–approved for human consumption components. The wound dressings were fabricated by dip coating. Antimicrobial efficacy was determined by quantifying the bacteria colonies after a 24 h of immersion. Wound healing and bacterial reduction were assessed in an *in vivo* full-thickness porcine burn model. Our antimicrobial wound dressings showed a > 5-log reduction (99.999%) of different gram-positive and gram-negative bacteria, while maintaining absorbency. In the *in vivo* porcine burn model, our wound dressings were superior to bacitracin in decreasing bacterial burden during daily changes, without interfering with wound healing. Additionally, the dressings had a significantly lower adhesion to the wound bed. Our antimicrobial wound dressings reduced the burden of clinically relevant bacteria more than commercial antimicrobial wound dressings. In an *in vivo* infected burn wound model, our coatings performed as well or better than bacitracin. We anticipate that our wound dressings would be useful for the treatment of various types of acute and chronic wounds.

KEYWORDS—MRSA; *E. coli*; *P. aeruginosa*; pig model; alpha-terpineol; cinnamaldehyde; essential oils; polyurethane

ABBREVIATIONS—AT; alpha-terpineol; CMA; cinnamaldehyde

INTRODUCTION

Skin wounds continue to pose a significant challenge to public health worldwide. Acute wounds, represented by open or closed surgical wounds, traumatic wounds, or burns that heal uneventfully (1), led to over 4.9 million emergency department visits in 2020 (2,3). Many of these wounds fail to heal with infections being the most common complication (3–5). Chronic wounds include acute wounds that fail to heal and other etiologies, such as decubitus ulcers, diabetic wounds, and venous ulcers. In general, within the US, there are over 8.2 million people with wounds, representing nearly 2.5% of the general population (6). Healthcare costs associated with caring for wounds is approaching $100 billion (6). Skin wounds ultimately represent a violation of a natural barrier to infection, which brings one area of the body that is naturally colonized with bacteria in contact with one that is normally sterile (7). The infection disrupts and delays wound healing, which in turn leads to increased pain, inflammation, time to wound closure, exposure to systemic antibiotics, risks of severe complications, and hospital length of stay. All of these factors increase the overall costs associated with treating wounds. With increasing age, prevalence of diabetes and obesity, and need for surgical therapies, the burden related to skin wounds and associated infections can only be expected to increase.

For the last several decades, there has been ongoing research and development on different antimicrobial therapies directed

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Competing interests: The University of Michigan has applied for a patent based on this technology. A startup company HygraTek LLC has licensed this technology from the University of Michigan. A.T. has equity and has been a paid consultant, for HygraTek LLC.

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specifically for the treatment and prevention of wound infections. Current therapies include solid wound dressings; gels, creams, and ointments; and wound surface washes. Among these three, solid wound dressings can be the longest lasting and require minimal treatment effort by the patient. All these treatment modalities have utilized different antimicrobial actives such as metals and metal oxides (e.g., silver, copper, zinc oxide), positively charged ammonium ions (e.g., benzalkonium chloride), traditional disinfectants (e.g., betadine, chlorhexidine), and small molecule antibiotics (e.g., bacitracin and neomycin). While showing varied antimicrobial effectiveness (8–12), many of these approaches suffer from drawbacks such as host toxicity (13–17) and delayed wound healing (10,15). For example, bacitracin and silver sulfadiazine creams are two of the most common topical antibiotic and antimicrobial ointments. However, bacitracin is ineffective against gram-negative bacteria and some strains of gram-positive bacteria such as MRSA USA300, and silver sulfadiazine can impair re-epithelialization, cause leukopenia, and result in hyperpigmentation making it unsuitable for visually prominent areas (18–24). Similarly, most commercially available antimicrobial solid wound dressings rely on ionic silver or nanocrystalline silver as a biocide (21). While silver is a highly effective antimicrobial, its widespread use is raising concerns about host toxicity and the development of silver resistant organisms (22,25,26). Additionally, most antimicrobial bandages easily accessible to consumers use benzalkonium chloride. Because benzalkonium chloride is often used in other antimicrobial consumer products such as soaps, sanitizer wipes, liquid body washes, etc., there is also concern about their widespread use leading to resistant microbial phenotypes (27). Overall, most current antimicrobial therapies provide only modest clinical benefit, and the increased cost of such therapies is oftentimes not justified. Thus, there is an urgent need for low-cost, nontoxic, and effective antimicrobial solid wound dressings.

Recently, there has been an increased interest in essential oils (EOs) and their natural antibacterial, antifungal, and antiviral properties as an alternative to traditional antimicrobials. Multiple studies have investigated the effect of EOs on wound healing in *in vivo* animal models and found that treatment of wounds with EOs increases wound contraction and closure (28–33). Other studies have fabricated antimicrobial wound dressings by including EOs into electrospun polymer mats (34–37). There are two main drawbacks to this method of fabrication and EO incorporation. First, because EO are mixtures of highly volatile organic compounds, they are likely to evaporate over a short period of time (several minutes) resulting in the loss of antimicrobial efficacy of the wound dressing. Second, the composition of the EO varies based on plant harvest time, growing conditions, and location, among other factors (38,39). Therefore, the antimicrobial efficacy of the fabricated wound dressing may vary from batch to batch. To overcome the variability of EO, in recent work, we identified a singular monoterpenoid, alpha-terpineol (AT). AT is a component of multiple EOs, such as tea tree oil, and has previously shown excellent antimicrobial activity against a variety of gram-positive and gram-negative bacteria (22). Additionally, the hydroxy group on AT can be reacted with isocyanate groups to form polyurethane bonds. By chemically reacting a fraction of the added AT within a polyurethane network, we showed that it was possible to stabilize and control the release of the remaining unreacted AT. In this manner, the nonporous, solid polyurethane coating incorporating AT could display broad-spectrum, instant and persistent antimicrobial efficacy (i.e., the surfaces could kill a variety of pathogens in a matter of seconds and maintain their antimicrobial effectiveness over several months) (40). Here, we build on our previous work and discuss the fabrication and application of porous, absorptive, antimicrobial wound dressings that feature two different EO components (AT and cinnamaldehyde, CMA) embedded within a medical grade polyurethane coating to reduce bacterial burden in a full-thickness burn infection model.

MATERIALS AND METHODS

Materials

Medical grade Baymedix® AP501 and AR602 were purchased from Covestro. Alpha terpineol (≥96% fragrance grade) and cinnamaldehyde (≥95% fragrance grade) were obtained from Sigma Aldrich. Bismuth neodecanoate (70% in neodecanoic acid) was purchased from Gelest. Large mirasorb gauze sponge (10.1 cm × 10.1 cm) was purchased from Johnson & Johnson. LB broth (Lennox), LB agar, tryptic soy broth and tryptic soy agar were purchased from Fisher Scientific. Bacterial strains *E. coli* (UT189), methicillin-resistant *S. aureus* (COL), and *P. aeruginosa* (ATCC® 27853™) were purchased from the American Type Culture Collection (ATCC).

Antimicrobial gauze coating fabrication

The Baymedix® + 60 wt% AT (BM + 60%AT) solution was prepared by combining the Baymedix® AP501, Baymedix® AR602, and AT in a 9.22 g:30.78 g:60 g weight ratio, respectively. The solution was mixed with a Vortex-genie 2 until thoroughly combined and 150 μL of a 50% weight solution of bismuth neodecanoate (70% in neodecanoic acid) in ethanol was added. For formulations with AT and CMA, the overall 60 wt% was maintained, while changing the ratios of AT and CMA. The polymer solution was then used to immediately coat the gauze. First, the gauze was dipped into the polymer solution and then fed through a rolling mill machine (Seattle Findings) to remove excess until there was 15.5 mg of coating per cm^2 of dressing. Prior to use in experiments, the coated gauze dressings were exposed to ultraviolet radiation (245 nm wavelength) for 30 min on each side to sterilize.

Water absorption capacity

The initial weight of the dressings was measured, and then the dressings were submerged in water for 24 h at room temperature and humidity. The dressings were removed from the water, the excess water was allowed to drip off for 24 h, and the weights were measured again.

Tensile test

A texture analyzer (Model: TA-XT plus, manufacturer: Stable Micro Systems) was used for mechanical property measurements. Samples of the uncoated and coated wound dressings were cut into rectangles with dimensions of 1 cm × 10 cm. The samples were measured for tensile modulus, elongation, and strength at a strain rate of 5 mm per minute. At least four replicates were measured per sample.

Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) was performed on a Thermo Scientific Nicolet 6700 fitted with the diamond Smart Orbit ATR sampling accessory across a range of wavenumbers that spanned 300 cm^{-1} to 4,000 cm^{-1} to analyze the NCO peak at 2270 cm^{-1}.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed on a Discovery TGA 5500 (TA Instruments) under nitrogen flow with a flow rate of 25 mL/min. The temperature was ramped at a constant rate of 50°C/min up to 100°C and kept at an isotherm for 100 min. The % weight loss over time was recorded.

Bacterial culture

E. coli (UT189) was cultured in LB broth or on LB agar. Methicillin-resistant *S. aureus* (COL), *S. aureus,* and *P. aeruginosa* (PA27853) were cultured using

tryptic soy broth supplemented with 1% glucose (TSBG) or on tryptic soy agar. Bacterial strains stored as glycerol stocks at −80°C, were struck onto agar plates, incubated at 37°C for 36 h, and then stored at 4°C for no more than 1 week until needed for experiments. To create working cultures for inoculations, an overnight culture created from a single colony was diluted in a 1:20 ratio with LB or TSBG broth and allowed to grow until it reached an optical density of 0.5 at 600 nm measured with a Biochrom ULTROSPEC 2100® UV-Visible spectrophotometer.

In vitro antimicrobial efficacy

Uncoated gauze (4 ply), coated gauze (4 ply and 12 ply), Silverlon® island dressings (2 cm × 1 cm; Silverlon® ID), Silverlon® wound packing strips (2 cm × 1 cm), and 0.5 g of bacitracin ointment were immersed in 50 mL centrifuge tubes containing a bacteria culture with ~10^6 colony forming units per ml (CFU/mL) and incubated for 24 h at 37°C. After incubation 10 μL of the bacteria culture was transferred to 90 μL of 1X PBS in a 96 well plate and serially diluted an additional 9 times. Then 10 μL of each dilution was plated on agar and incubated for 24–48 h for viable colony enumeration.

Skin irritation tests

The skin irritation index was independently conducted by NAMSA (Northwood, OH), in accordance with ISO 10993-10 (41). In brief, the test involved the application of 25 × 25 mm sections of the uncoated or coated gauze to the skin of a rabbit for 23–24 h. This was followed by dermal observations at 1, 24, 48, and 72 h after removal of the wound dressings. The degree of irritation was scored from 0 to 4. A score of 0.0 indicates no erythema and no edema observed on the skin of the animals.

In vivo full-thickness burn infection model

The study was performed in accordance with the University of Michigan's Institute Animal Care and Usage Committee approved protocol (PRO00008154). A total of seven female, 30–35 kg, Yorkshire mix pigs were used in this study.

The model was based on the model in Mironov *et al.* (2020) with adaptations based on Branski *et al.* (2008) (42,43).

Anesthesia and analgesia

The pigs were acclimated to the facility for one week and fasted overnight before the procedure. For burn procedures, wound biopsies, and dressing changes, anesthesia was induced by an intramuscular injection of Telazol® (2.0–8.0 mg/kg) and xylazine (1.0–3.0 mg/kg). Anesthesia was maintained with isoflurane (1.0%–3.0%) delivered by face mask. While under anesthesia but prior to burn procedures, buprenorphine patches (30 mcg/h) were placed on the dorsum at the base of the neck and a loading dose of injectable buprenorphine (0.05–0.01 mg/kg, IM) was given. Prior to biopsy procedures, a single dose of buprenorphine was given.

Burn wound procedure and bacterial inoculation

Hair was removed using a chemical removal product and skin was prepped with three alternating applications of chlorhexidine surgical scrub and 70% alcohol. A 5 × 5 cm, 150 g copper bar heated in a 200°C oil bath was used to create the burn wounds. The hot copper bar was kept in contact with the skin for 40 s with 5 N of force to ensure reproducible full-thickness burns. A total of 6 burns were created on the back of each pig. Wounds were placed 2.0 cm lateral to midline, with at least 2.0 cm between adjacent wounds. The day the burn wounds were created is considered day −1. The day after the burn wounds were created (day 0) the wounds were inoculated topically with 100 μL of bacterial culture containing *S. aureus* spread evenly across the wound. To make the working bacteria culture for inoculations, bacteria were grown to log phase and then resuspended in saline at a concentration of 10^7 CFU/mL.

Dressing changes and wound assessment

For the control group (n = 18 wounds; 3 pigs), petroleum ointment (vehicle for bacitracin) was applied followed by a gauze bandage secured with Tegaderm (3 M), then cotton padding covered with a self-adherent bandage (Coban) and finally a jacket. In the experimental group (4 pigs), 3 wounds/pig on each pig received bacitracin ointment instead of petroleum ointment (12 wounds total), and the other 3 wounds/pig received either BM + 60%AT coated gauze alone (6 wounds total) or the BM + 60%AT coated gauze and petroleum (6 wounds total). Since wounds heal at different rates in the cranial caudal axis, all three wounds for a given treatment were on the same side to account for this variability. However, the side of the animal for the given treatment was randomized. All wounds in the experimental group were also covered with a Tegaderm, cotton padding, self-adherent bandage, and a jacket. On days 1, 2, 3, 4, 7, 14, and 21 the dressings were changed, and the wounds were photographed for area measurement. Wound area was determined from high resolution photographs taken at each dressing change using ImageJ. Specifically, the boundary of the raw wound bed was detected using an edge detection algorithm. This represents the area of epithelialization remaining. The pixel area of the bounded wound was then converted to cm^2 using in image scale bar. The removed dressings were saved for bacteria quantification. On days 7, 14, and 21 two punch biopsies (3 mm diameter) were taken from the wound bed for histology and quantitative culture, respectively. On day 28 the animals were euthanized with an IV injection of pentobarbital, and the final wound dressings, dimensions, photos, and punch biopsies were collected. Wound samples (6 cm × 6 cm) for *ex vivo* skin adhesion testing were also taken at this time.

Histopathological evaluation

Wound biopsy samples for histopathological evaluation were processed by the University of Michigan Unit for Laboratory Animal Medicine (ULAM) *In Vitro* Animal Core (IVAC). Briefly, samples were fixed in 10% formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin for histopathological evaluation. Sections were read and scored by a blinded certified veterinary pathologist according to the semi-quantitative scored rubric in Table S1, http://links.lww.com/SHK/C35.

Quantitative culture of wound tissue and dressings

To account for differences in tissue sample recovery from the biopsy, all tissue samples were weighed in sterile weigh boats prior to processing. This allowed normalization of colony counts to the mass of wound tissue recovered. Tissue samples were then submerged in PBS and homogenized (IKA T18 Ultra-Turrax homogenizer) at 14,000 rpm for 10–15 s. Whole dressings were submerged in 30 mL PBS followed by sonication (Fisher Scientific Ultrasonic Bath, 5.7 L) for 20 min to liberate individual bacterial cells from the dressings. Dressings were then vigorously vortexed for 1 min. Supernatants of both dressings and tissue were serially diluted and plated on to tryptic soy agar, then incubated at 37°C for 24–36 h prior to colony enumeration.

Ex vivo skin adhesion test

A 6 cm × 6 cm section of skin containing a wound was excised on day 28 and secured to a flat platform. Unused wound dressings were sandwiched between the skin and a glass slide, and a 500 g load was applied to simulate a dressing wrapped wound under compression. The load was removed after 1 h. Each dressing was then peeled at an angle of 180° using a force gauge at a controlled velocity of 74 μm/s. The peak force measurements were recorded.

Statistics

Statistical analysis was performed using GraphPad Prism version 9.2.0 (Graphpad, La Jolla, CA). Comparisons between dressing types in the histopathological evaluation were made with Kruskal-Wallis followed by Dunn's multiple comparison test. Significance was set to $P < 0.05$.

RESULTS AND DISCUSSION

Material selection

Polyurethanes were first used for biomedical applications in the 1950s and remain popular today due to their excellent mechanical properties, durability, biocompatibility, and processability (44). The prepolymer isocyanate (Baymedix® AP501) and polyol (Baymedix® AR 602) from Covestro were selected as the base components for our polyurethane-based antimicrobial coatings because they meet the standards for use in medical devices (ISO10993-1). Note that Baymedix® AP501 has an aliphatic NCO-terminated structure which provides greater flexibility than if using an aromatic diisocyanate.

Fabrication

Fabrication of the antimicrobial coated gauze dressing was performed in a simple two-step process that requires no specialized equipment. First, the antimicrobial polymer coating is made by mixing the antimicrobial component (AT and / or CMA) with the Baymedix® AP501 (prepolymer isocyanate), Baymedix® AR602 (prepolymer polyol), and a catalyst. Then the gauze is

dipped in this coating solution and fed through a roll mill to remove the excess polymer solution. Thermogravimetric The reaction between AT and Baymedix® AP501 was confirmed using FTIR. The isocyanate (NCO) peak at 2273 cm^{-1} in Figure 1C decreases over time, indicating it is reacting with the hydroxyl group on AT. TGA was performed to determine the amount of reacted AT *vs* unreacted AT (Fig. S1, http://links.lww.com/SHK/C32). Results showed that in the BM + 60%AT samples 3.7% of the AT is bonded to the polyurethane polymer chains.

Swelling and mechanical properties

Wounds produce exudate, an aqueous mixture of nutrients, electrolytes, growth factors, leukocytes, inflammatory mediators, and enzymes (45). Exudate retention is important for wound healing, as it keeps the wound bed moist, provides healing factors, and promotes migration of cells. However, excess exudate at the wound site can cause maceration and delay wound healing (46). Thus, tunable absorbency is a critical feature for wound dressings. To quantify dressing absorbency, we evaluated the swelling in water of the different medical gauzes coated with our various antimicrobial coatings. As seen in Figure 2A, as the total coating weight of the BM + 60%AT coated gauze increases, the percentage of swelling (amount of absorbed water) decreases. At a coating weight of 15.5 mg/cm^2 the swelling was approximately 131%. As a comparison, the uncoated gauze swelled by ~500%.

Tensile strength is another important feature for wound dressings. Therefore, tensile strength, elongation, and Young's modulus of the different coated medical gauzes were also quantified. The BM + 60%AT coated 4-ply gauze (coating weight = 15.5 mg/cm^2) had an increased modulus, elongation, and strength compared to a 4-ply uncoated gauze (Fig. 2B).

Effect of coating thickness on antimicrobial efficacy

To determine if the coating thickness on the gauze influenced antimicrobial efficacy, we tested gauze dressings with a thin (11 mg/cm^2) and a thick (32 mg/cm^2) BM+ 60%AT coating against *E. coli*, methicillin-resistant *S. areus* (MRSA), and *P. aeruginosa* (see Fig. 2C). Both coating thicknesses reduced the CFU/mL by 6-log for *E. coli*; however, the reduction in MRSA was less for the thinner coating (4-log reduction), than the thicker coating (6-log reduction). The increased reduction of the thicker coating against MRSA is likely because AT has a higher minimum inhibitory concentration (MIC) for MRSA than *E. coli* (31). These results indicated a slight advantage in applying a thicker coating weight, and therefore the total amount of AT embedded in the wound dressings. The decreased antimicrobial efficacy for both the thin and thick coated gauze against *P. aeruginosa* is somewhat expected, as *P. aeruginosa* is a notoriously difficult bacteria to eliminate and it is resistant to many antibiotics. This resistance is attributed to the low permeability of the cell wall, a large genetic bank of resistance mechanisms, and its ability to acquire new resistance genes from plasmids and bacteriophages (47). To improve the antimicrobial efficacy of the coated gauze against *P. aeruginosa*, other EO antimicrobial components can be added to the coating. Previously, we showed that coatings containing a mixture of AT and cinnamaldehyde (CMA) are effective against *E. coli*, MRSA, and *P. aeruginosa* (40).

Antimicrobial efficacy comparison with bacitracin and commercial silver based wound dressings

To compare the antimicrobial efficacy of our polyurethane coatings with clinically utilized dressings, we created 4 variations of the coated gauze dressings. These included: **1)** BM + 60%AT on 4-ply gauze **2)** BM + 60%AT on 12-ply gauze **3)** BM + 60% (0.5AT + 0.5CMA) on 4-ply gauze, and **4)** BM + 60% (0.05AT + 0.95CMA) on a 4-ply gauze. All variations had a 15.5 mg/cm^2 coating weight. We compared the performance of these developed wound dressings against Silverlon® wound packing strips (WPS), Silverlon® island dressings (ID), and bacitracin ointment. Silverlon® dressings are a nylon fiber substrate coated with approximately 1% silver oxide. Bacitracin ointment is a common antibiotic used for burn wounds, but it is only effective against Gram-positive bacteria (18). For the uncoated 4-ply gauze there was a 5-log increase in CFU/mL compared to the initial inoculum concentration for *E. coli*, MRSA, and *P. aeruginosa* as seen in Figure 2, D–F. This is expected as there is no antimicrobial component to kill the bacteria. The bacitracin ointment, Silverlon® WPS and ID also had an increase in CFU/mL for all three bacteria

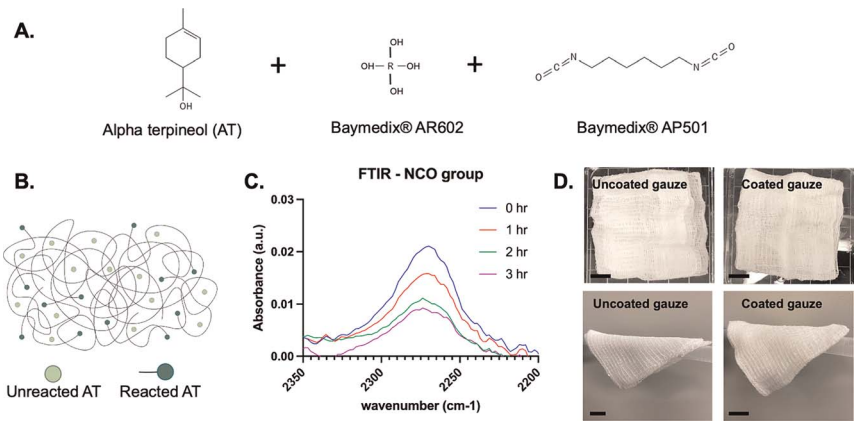

FIG. 1. **Fabrication of antimicrobial wound dressings.** A, Chemical structures of the different components in the BM + 60%AT coating. A, A schematic depicting the structure of BM + 60%AT. A fraction of AT remains unreacted and trapped between the polymer chains, while some AT reacts with the -NCO groups of the Baymedix® AP501 to form a urethane bond. C, FTIR data showing the -NCO peak decreasing as it reacts with the -OH groups on AT and Baymedix® AR602 over the course of 3 h. D, Photographs of an uncoated gauze (10.1 cm × 10.1 cm) and a gauze coated with BM + 60%AT (10.1 cm × 10.1 cm) lying flat and draped over a pipette tip to demonstrate the flexibility of the coated gauze. Scale bar = 1 cm. BM + 60%AT, Baymedix® + 60 wt% alpha-terpineol; FTIR, Fourier-transform infrared spectroscopy.

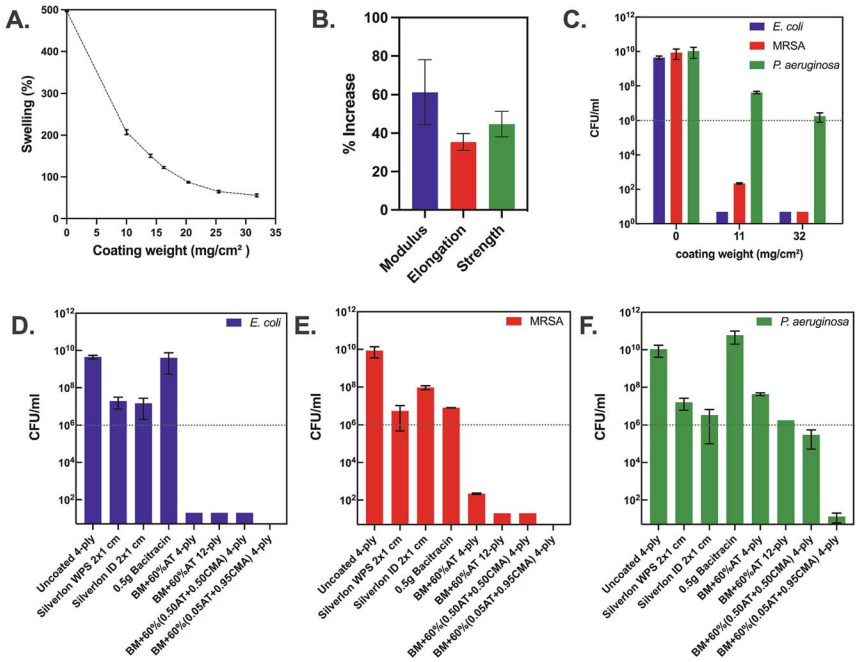

Fig. 2. **Characterization of the antimicrobial wound dressings.** A, Percent swelling *vs* coating weight of BM + 60%AT, n = 3. As the coating weight increases, the swelling percentage decreases. B, Percent increase in modulus, elongation, and strength of 4-ply gauze coating with BM + 60%AT (15.5 mg/cm^2), n = 3. C, Effect of BM + 60%AT coating weight on the antimicrobial efficacy against *E. coli*, MRSA, and *P. aeruginosa*. The green dashed line indicates the initial inoculum concentration, n = 3. Antimicrobial performance against (D) *E. coli*, (E) MRSA, and (F) *P. aeruginosa* for uncoated gauzes, Silverlon® dressings, bacitracin, and coated gauzes, n = 3. All coated gauzes tested had a 15.5 mg/cm^2 coating weight. The green dashed line indicates the initial inoculum concentration. All error bars indicate 1 SD. BM + 60% AT, Baymedix® + 60 wt% alpha-terpineol.

species. Coated dressing **1** displayed a 5-log reduction against *E. coli* and a 4-log reduction against MRSA, while dressing **2** had a 5-log reduction against *E. coli* and MRSA. As expected, coatings **1** and **2**, which only contain AT, were not as effective against *P. aeruginosa*. To achieve antimicrobial efficacy against *P. aeruginosa*, we formulated two coatings with a mixture of AT and CMA. Both coatings maintained the overall 60 wt% of oil but varied the ratio of AT and CMA. The coated dressing that contained 50% AT and 50% CMA (**3**), remained just as effective as coating **1** against *E. coli* and MRSA demonstrating that the wt % of AT could be decreased without sacrificing efficacy. Even more impressive is that coating **4**, which contained only 5% AT and 95% CMA, was extremely effective in killing *E. coli* and MRSA, reaching the lowest limit of detection in our testing (5 CFU/mL). Additionally, coating **4** achieved a 5-log decrease in CFU/mL against *P. aeruginosa*.

Skin irritation

As wound dressings come in direct contact with open skin wounds, it is important to investigate whether any of the coating components cause skin irritation. Therefore, ISO 10993-10 test for *in vivo* skin irritation was performed in a rabbit model. Results indicated a primary skin irritation index of 0.0 for the BM control and the BM + 60%AT. However, the BM + 60%CMA had a skin irritation index of 1.8 indicative of slight erythema on the skin. Therefore, the BM + 60%AT coating was selected for the *in vivo* porcine study.

In vivo full-thickness burn infection efficacy

To evaluate the *in vivo* effectiveness at decreasing the bacterial load in the wound, and within the wound dressing, we chose to use a porcine full-thickness burn infection model. Pig skin is anatomically and physiologically similar to human skin and is considered the gold standard for use as a model for human wound healing (48). As seen in Figure 3, 1 day after the full-thickness burn wound creation, the wounds were inoculated with *S. aureus*. We selected *S. aureus* for this study because it is the most common bacteria found in burn wounds (49,50). Four different dressing conditions were tested in the full-thickness burn infection model: **1**) control (petroleum ointment), **2**) bacitracin, **3**)

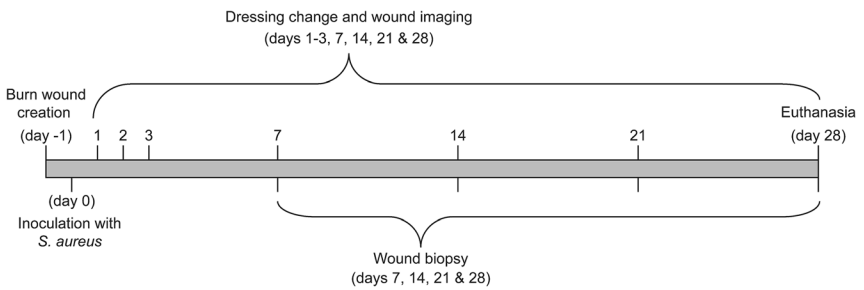

Fig. 3. **Timeline of the *in vitro* porcine infected burn wound model**. Burn wounds were created on day −1 and inoculated with *S. aureus* the day after (day 0). On days 1, 2, 3, 7, 14, 21, and 28, the wound dressings were changed, and the wounds were imaged for wound size quantification. On days 7, 14, 21, and 28, punch biopsies (3 mm diameter) of the wound were taken for histopathologic analysis. On day 28, the pigs were euthanized followed by final sample acquisition (6 cm × 6 cm).

BM + 60%AT, and **4**) BM + 60%AT & petroleum. The BM + 60% AT & petroleum condition was examined to determine if wound healing was due to the antimicrobial gauze, or the increased moisture retention associated with the use of thick occlusive ointments such as petroleum. The recommended frequency of dressing changes for burn wounds varies from twice daily to once a week depending on the amount of exudate, cost, and stage of healing. Therefore, to compare the dressings' performance at different change frequencies, the dressing was changed daily during the first 3 days, and weekly starting on day 7.

Wound size

We found that the dressing type and changing frequency had no significant effect on the wound size over time (Fig. 4, C and D). Most importantly, the BM + 60%AT and BM + 60%AT & petroleum treatments did not delay healing compared to the wounds treated with bacitracin ointment and were at par with this clinically utilized dressing.

Dressing bacterial load over time

On days 1, 2, 3, 7, 14, 21, and 28, the dressings were removed, and the number of live bacteria adhered to the dressings was quantified. Figure 4, E and F, show the CFU/mL recovered from the wound dressings after removal. For the control dressings, which contained no antimicrobial agent, all the dressings collected had an increased bacterial load compared to day 1. On days 2–3, the dressings with bacitracin ointment lowered the bacterial load by 1.7-log (Fig. S2, http://links.lww.com/SHK/C33). Comparatively, the BM + 60%AT and BM + 60%AT & petroleum dressings had a > 5-log reduction on days 2 and 3 (Fig. S2, http://links.lww.com/SHK/C33). This was a significant improvement over the bacitracin ointment during the early time period of dressing changes. When the dressing changes occurred weekly,

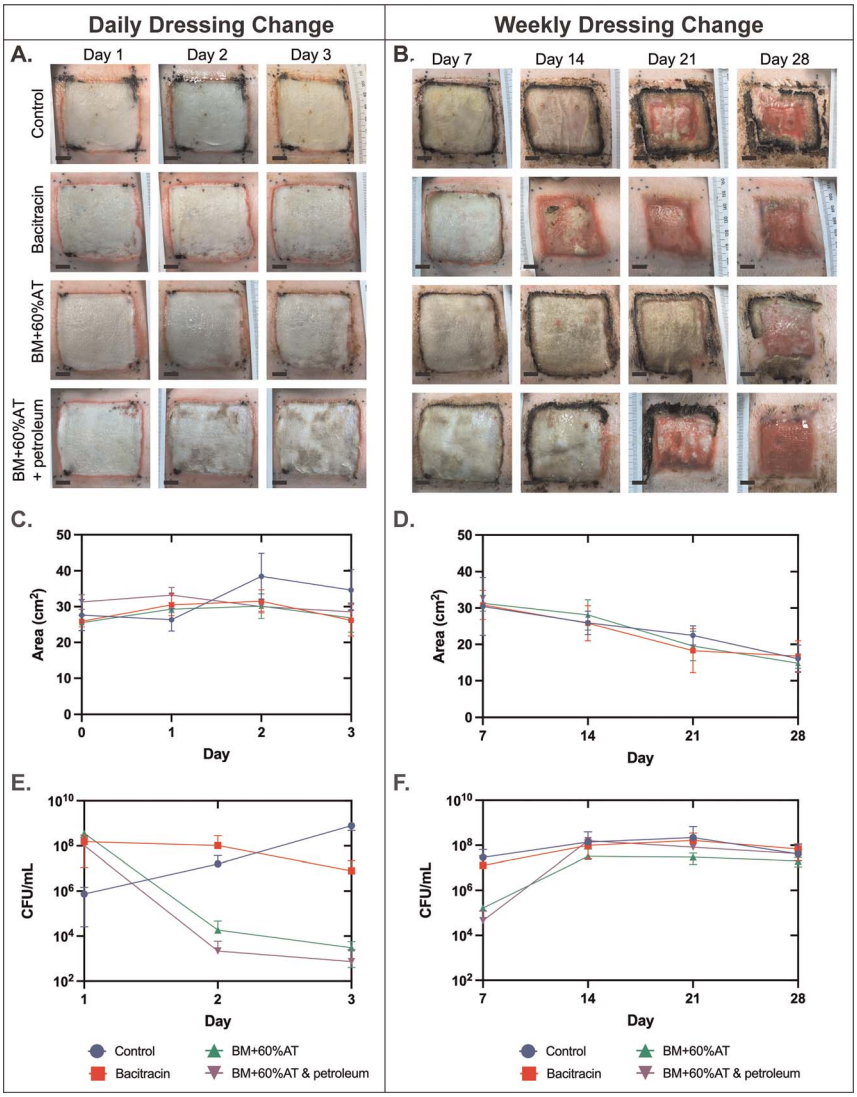

Fig. 4. ***In vivo* efficacy of antimicrobial wound dressings**. Representative images of wounds for the control, bacitracin, BM + 60%AT, and BM + 60%AT & petroleum on (A) days 1, 2, & 3 (daily changes) and (B) days 7, 14, 21, & 28 (weekly changes). Black scale bars are 1 cm. Wound area on (C) days 1, 2, & 3 and (D) days 7, 14, 21, & 28. The size of wounds treated with BM + 60%AT and BM + 60%AT & petroleum did not significantly differ from the control wounds and bacitracin treated-wounds over the duration of the experiment. CFU/mL of *S. aureus* recovered from the wound dressings on (E) days 1, 2, & 3 and (F) days 7, 14, 21, & 28. On days 2, 3, & 7, the bacterial load on the BM + 60%AT and BM + 60%AT & petroleum wound dressings was significantly lower than the control and bacitracin dressings. Error bars indicated 1 standard deviation. BM + 60%AT, Baymedix® + 60 wt% alpha-terpineol.

the log difference in bacteria load for the bacitracin, BM + 60% AT and BM + 60%AT & petroleum dressings were minimal (~0.5-log reduction).

Ex vivo skin adhesion test

Another consideration in wound dressing is the adhesion to the wound and surrounding skin (51). It has been demonstrated that there is a significant correlation between adhesion and pain intensity. Additionally, wound dressings that adhere too strongly to the wound bed can cause further injury upon removal. In a 180-degree peel test on an excised wound tissue (Movie S1, http://links.lww.com/SHK/C104), the uncoated gauze dressing had a peak adhesion force of 55 gf, while the gauze coated in BM + 60%AT had a peak adhesion force of 0 gf. The coated gauze's lower peak adhesion force indicates that it would be less painful to remove from the wound making it a better choice than uncoated gauze.

Histopathology

In order to measure the differences in the wound healing responses as a function of the coating composition, we performed blinded histopathology quantification. Blinded analysis of biopsied tissue removed on days 7, 14, 21, and 28 revealed similar trends in epidermal damage, dermal necrosis, depth of necrosis, granulation tissue, fibrosis, superficial inflammation, deep inflammation, chronic dermal inflammation, and bacterial load over time between the bacitracin, BM + 60%AT, and the BM + 60% AT& petroleum wound dressing treatments (Fig. S3, http://links.lww.com/SHK/C34). The BM + 60%AT and BM + 60% AT & petroleum treatments were noninferior to the bacitracin treatment and did not increase inflammation or delay the healing process (such wounds typically close fully in 6–8 weeks). Therefore, the polyurethane coated wound dressings with BM + 60% AT present a viable alternative, with added capabilities of reduced microbial load with daily dressing changes, and lower adhesion to the tissue for relieving pain associated with dressing changes.

LIMITATIONS

In this study, we did not measure the systemic absorption of AT. Future work should include more detailed studies on the percutaneous absorption and retention of the compounds.

CONCLUSION

In this work, we fabricated antimicrobial wound dressings in a simple two-step process, by utilizing medical grade polyurethane coated gauze. The *in vitro* broth culture tests revealed that the wound dressings are effective against the most common Gram-positive and Gram-negative bacteria responsible for burn wound infection. Furthermore, the coatings can be customized using a variety of EO components, thereby targeting specific bacteria and absorptive capacity. In a porcine full-thickness burn infection model, the antimicrobial dressings significantly decreased the bacterial burden during the first week of healing, when dressing changes were more frequent, compared to uncoated gauze and bacitracin ointment treatment. Based on our porcine burn wound model results, our recommendation for future wound dressings includes more frequent changes, such as every day or every other day, throughout the duration of wound healing. Because the coated gauze dressings have a lower adhesive force to skin, the frequent dressing changes should be less painful and have higher patient compliance. Future studies should evaluate the wound dressings effectiveness against other silver-based wounds dressing, bacteria, and wound types.

Supporting information

Table S1, http://links.lww.com/SHK/C35. A table containing the scoring criteria for the histopathological analysis. Figure S1, http://links.lww.com/SHK/C32. TGA of the antimicrobial coatings. The furnace was first heated to 100°C at a rate of 50°C/min and then held at 100°C for 100 min. The BM sample remained at 99.1%. The BM + 60%AT samples leveled out at 44.7%, 43.1%, and 43.2%, respectively. The BM + 60% (0.5AT + 0.5CMA) sample leveled off at 45.8% and the BM + 60% (0.05AT + 0.95CMA) at 47.5%. Figure S2, http://links.lww.com/SHK/C33. Log difference in CFU/mL of *S. aureus* recovered from the wound dressings Figure S3, http://links.lww.com/SHK/C34. Plots comparing the epidermal damage, dermal necrosis, depth of necrosis, granulation tissue, fibrosis, superficial inflammation, deep inflammation, chronic dermal inflammation, and bacteria load of the control, bacitracin, BM + 60%AT, and BM + 60%AT & petroleum wound dressings. Movie S1. The 180-degree peel test on an excised wound tissue, with the uncoated gauze and gauze coated in BM + 60%AT.

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