Efficacy of Polyhexamethylene Biguanide-containing Antimicrobial Foam Dressing Against MRSA Relative to Standard Foam Dressing

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Abstract: Many modern foam wound dressings possess a variety of attributes that are designed to create a supportive wound-healing environment. These attributes include absorbing exudate, providing optimum moisture balance at the wound surface, and preventing maceration of surrounding tissue. However, studies suggest that controlling wound bioburden should also be targeted when developing wound therapeutics. Thus, traditional foam dressings may absorb a copious amount of fluid, but may also provide an environment where microbes can grow unchallenged, leading to an increase in wound bioburden. However, antimicrobial foam dressings may prevent or reduce microbial growth, increasing the potential for wound healing. Studies reported herein evaluated the efficacy of 0.5% polyhexamethylene biguanide (PHMB) treated dressings to prevent the growth of methicillin-resistant *Staphylococcus aureus* (MRSA). An antimicrobial foam (Kendall™ AMD, Covidien, Mansfield, MA), which contains PHMB and a standard foam dressing (Copa™, Covidien, Mansfield, MA), which contains no PHMB (control), were directly inoculated with clinical isolate of MRSA and placed on a growth medium for selected time intervals. The presence or absence of microbial growth was quantified using plate counts and was visually assessed using scanning electron microscopy. At all time points, the antimicrobial foam dressing significantly reduced the MRSA growth compared to the control dressing. Similar results were also obtained in the microscopic evaluations.

Polyhexamethylene biguanide (PHMB), also known as polyaminopropyl biguanide (PAPB) or polyhexanide, is a broad-spectrum antimicrobial agent used in a variety of products including contact lens cleaning solutions, skin disinfectant solutions, and wound dressings. The antimicrobial activity of PHMB is attributed to its disruption of the bacterial cell wall. This polymeric biguanide reacts with acidic membrane lipids and induces aggregation, leading to increased membrane fluidity and permeability, and eventual organism death. Polyhexamethylene biguanide has also been reported to bind bacterial DNA, alter its transcription, and cause lethal DNA damage.[1] Wound care products containing PHMB include Kerlix™ AMD, Excilon™

AMD, Telfa™ AMD (all from Covidien, Mansfield, MA) and XCell® Cellulose Wound Dressing (Xylos, Corp., Langhorne, PA).

The efficacy of the AMD dressings against gram-positive and gram-negative bacteria as well as yeast and fungi has been demonstrated in several *in-vitro* and *in-vivo* studies.[2-5] Kerlix AMD (PHMB-impregnated gauze) was exposed to several bacterial pathogens isolated from veterinary patients. Using a disk diffusion antimicrobial susceptibility test, the PHMB-impregnated gauze significantly inhibited the growth of 4 out of 4 gram-positive organisms, and 6 of 6 gram-negative species compared to untreated gauze.[4] Furthermore, after direct inoculation of the Kerlix AMD dressings and 24 hours of incubation, there was no recovery of any colony forming units (CFU). However, CFU levels significantly higher than the initial inoculum (10^5 CFU) were recovered from the control gauze samples.[4] Using a logarithm reduction test, Kerlix AMD dressings displayed high activity against gram-negative bacteria, gram-positive bacteria, and yeast.[3] In addition, the log reduction values after 30 minutes of exposure were similar to the values obtained after 2 hours, indicating that the majority of the microbes were killed after a short exposure period.[3] *In-vivo* testing of Kerlix AMD dressings have demonstrated its ability to act as a barrier to bacterial invasion.[2]

The efficacy of Kendall AMD dressings was also demonstrated in clinical settings.[5-7] It was demonstrated that packing wounds with Kerlix AMD dressings may be beneficial toward reducing the bacterial bioburden in terms of both the total amount of microorganisms and the total number of species.[6] In another prospective, randomized, controlled, open-label clinical case series, it was determined that the Excilon AMD drain sponge could be an important instrument in the control of antibiotic-resistant bacterial infection, as well as other infections frequently found in patients with tracheotomies. The study results suggested that the antimicrobial drain sponges could help control organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* in an institutional setting.[5] Finally, sterile gauze dressings were replaced institution-wide with sterile antimicrobial gauze (Kerlix). Surgical site infections (SSI), and specifically MRSA-associated SSIs were tracked for the 11-month period both before and after the dressing switch. The switch to the AMD antimicrobial dressings resulted in a significant reduction in both SSIs and MRSA-associated SSIs. Accompanying the switch was a reduced morbidity rate, shortened patient hospital stays, and a reduction in postsurgical care costs.[7]

The standard foam dressing (Copa) is a soft foam dressing with high fluid absorption and retention. The antimicrobial foam dressing (Kendall AMD) is similar to the standard foam dressing; however, it has been developed to contain 0.5% PHMB—a broad-spectrum antimicrobial agent. The purpose of this study was to evaluate the efficacy of an antimicrobial foam dressing to prevent MRSA growth relative to a standard foam dressing. Test dressings were directly inoculated with a MRSA suspension and incubated for a period of time. The bacterial growth was quantified using plate counts and imaged using scanning electron microscopy (SEM).

Materials and Methods

Dressings. Sterile samples of the antimicrobial foam dressing and the standard, non-PHMB foam dressing were provided by the manufacturer (Covidien, Mansfield, MA). The dressing samples were pre-cut into 25-mm disks for testing.

Challenge organism. Dr. Mark Shirtliff (Department of Microbiology and Immunology, School of Medicine, University of Maryland, Baltimore) graciously provided a clinical isolate of MRSA. This isolate was cultured from a bone debridement specimen from a patient with osteomyelitis at the University of Texas Medical Branch.

Inoculation and bacterial growth. Dressing samples were inoculated on each side of the disk with 1 mL of 10^6 CFU/mL of MRSA in 10% tryptic soy broth (TSB). The disks were then placed on 50%-strength trypticase soy agar (TSA) plates and incubated at 37˚C for 0, 24, 72, or 168 hours. To ensure the dressings remained hydrated, each dressing was re-hydrated with 1-mL sterile phosphate buffered saline (PBS) after 24, 48, and 72 hours.

Quantification of bacterial growth. At selected time points (0, 24, 72, and 168 hours) the dressings were removed from the TSA plates and immersed in 25-mL of Dey/Engley (D/E) neutralizing broth and vortexed. The samples were then allowed to sit for 15 minutes, followed by an additional 1 minute of vortexing. Afterward, 1 mL of the solution was removed, serially 10-fold diluted, and plated on TSA. The plates were incubated at 37˚C overnight and the number of colony forming units (CFU) per dressing was calculated. Triplicate samples were used for each time point. Statistical analysis for significance was determined using two-tailed t-test assuming unequal variances with $\alpha = 0.5$ and $P \leq 0.05$ considered to be significant.

Scanning electron microscopy. At selected time

points (0, 24, 72, and 168 hours) the dressings were removed from the TSA plates and fixed with 4% paraformaldehyde for 3 hours. The samples were then dehydrated in a gradient of 50%, 70%, 95%, and 100% ethanol. Following dehydration, the samples were sputter coated with iridium and imaged on a Zeiss Supra 55VP field emission scanning electron microscope. Non-inoculated dressing samples were also evaluated to image the structure of the dressing.

Results

Quantification of bacterial growth. The efficacy of the antimicrobial foam dressings was evident as quickly as the T = 0 time point. There was approximately 7.17 ± 0.02 log CFU/dressing for the standard foam dressing and 4.99 ± 0.25 log CFU/dressing for the antimicrobial foam dressing (P = 0.004, Figure 1). This difference reflected a 2.18 ± 0.14 log reduction in MRSA levels with the antimicrobial foam dressing (Figure 2). By 24 hours, the antimicrobial foam dressing displayed an 8.87 ± 0.62 log reduction in MRSA growth. The log reductions at the 72 and 168 time points were 9.37 ± 0.03 and 8.88 ± 0.08, respectively.

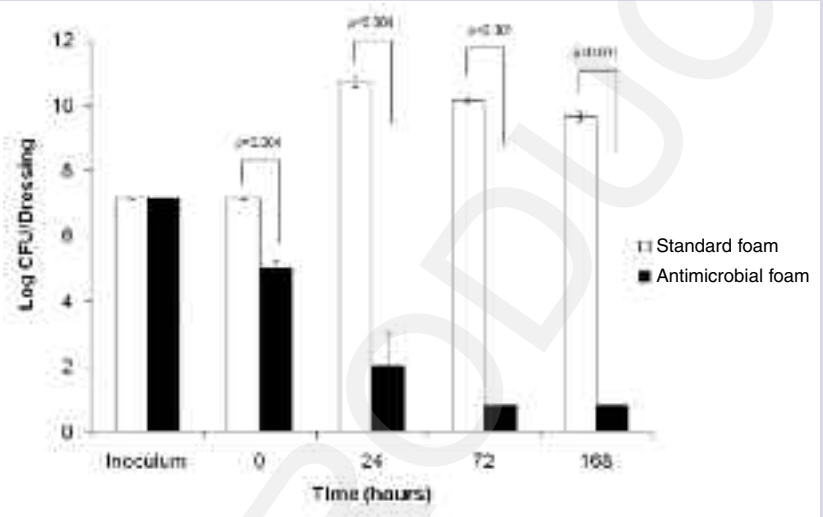

Figure 1. Log CFU/dressing for both standard foam dressing and antimicrobial foam dressings. Data represented as mean ± standard deviation.

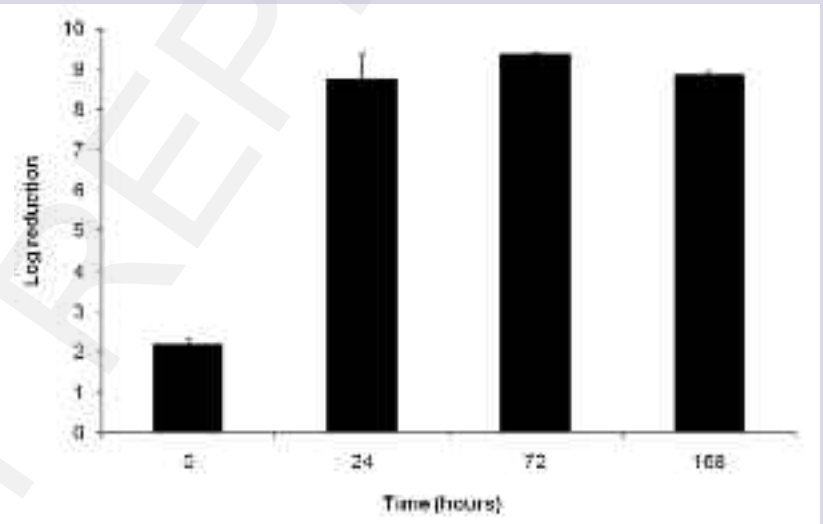

Figure 2. Log reduction of antimicrobial foam dressing with respect to the standard foam dressing. Data represented as mean ± standard error.

Scanning electron microscopy. The efficacy of the antimicrobial foam dressing against MRSA was also evident in the SEM evaluation (Figures 3). Images were collected after 0, 24, 72, and 168 hours of incubation. As the incubation period progressed, MRSA growth on the standard foam dressing was evident. There appeared to be more bacteria at the later time points, and the bacteria appeared to be imbedded in a matrix. However, few MRSA bacteria were present on the antimicrobial foam dressings at the 0- and 24-hour time points; bacteria were not found at the 72- and 168-hour time points. There were individual spherical-shaped objects found on the antimicrobial foam dressing, but these objects varied in diameter and were similar to those found on untreated dressings. Furthermore, the texture of the objects was similar to the texture of the dressing as opposed to the smooth texture of MRSA bacteria.

Discussion

Current wound research indicates that bacterial growth is a contributing factor in the chronicity of wounds.[8-11] Furthermore, research suggests that wound bioburden should be targeted when developing and testing wound therapeutics.[12-15] One principal component of wound care is dressing choice. Traditional foam dressings are designed to absorb a copious amount of fluid; therefore, they are particularly useful in the early

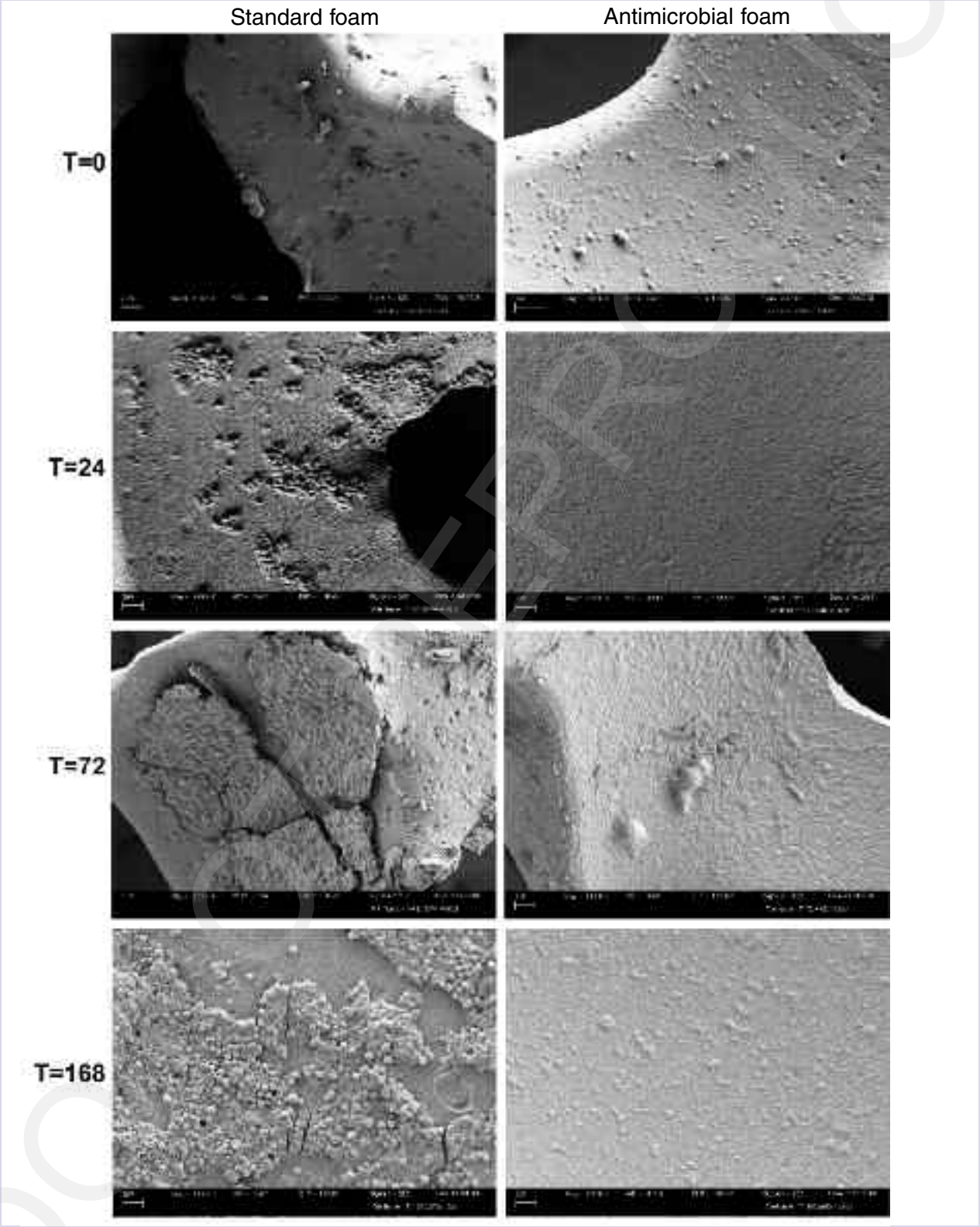

Figure 3. SEM images of both standard foam dressing and antimicrobial foam dressings at selected time points. All images at 3000x magnification.

stages of wound healing when drainage is the greatest, or in heavily exudating wounds. Traditional foam dressings may also provide an environment where microbes can grow unchallenged, leading to an increase in wound bioburden; however, antimicrobial foam dressings may prevent or reduce microbial growth, thereby reducing wound bioburden and increasing the potential for wound healing. The studies reported herein involved the direct inoculation of both traditional and antimicrobial foam dressings. Bacterial growth was recorded via plate counts and SEM.

Antimicrobial efficacy of the antimicrobial foam dressing became evident quickly. The antimicrobial foam dressing exhibited a 2.18 ± 0.14 log reduction in MRSA levels with respect to the standard foam dressing immediately after inoculation. By 168 hours, the difference had increased to 8.88 ± 0.082 log reduction by the antimicrobial foam dressing. These results were also reflected in the SEM analysis. The T = 0 and T = 24 samples had a few scattered cocci, but by 168 hours of incubation, large colonies of MRSA were present on the standard foam dressing, whereas MRSA was not found on the antimicrobial foam dressing.

Conclusion

PHMB-treated antimicrobial foam dressing exhibited antimicrobial activity against MRSA by resisting bacterial colonization within the dressing. At all time points, differences in the log counts were statistically significant, indicating that the antimicrobial foam dressing was dramatically more effective in reducing colony counts than the standard foam dressing. The use of an antimicrobial foam dressing may either prevent or reduce microbial growth in the wound environment, and reducing wound bioburden may improve wound-healing outcomes.

Acknowledgement

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