

## Preclinical Evaluation of Antimicrobial Efficacy and Biocompatibility of a Novel Bacterial Barrier Dressing

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**Abstract:** Wounds that become infected can lead to devastating consequences for patients, resulting in substantially increased healthcare costs. Bacterial barrier dressings are a first line of protection against developing wound infections. Most bacterial barrier dressings contain microbicidal chemicals (eg, silver ions, iodine, chlorhexidine) that are released from the dressings, which can be toxic to wound cells. A need exists for cost effective bacterial barrier dressings that absorb wound exudate and do not release toxic materials into the wound or increase the risk for developing bacterial resistance to the microbicidal chemical. The present study reports the development and properties of a novel bacterial barrier dressing that meets these needs. *Methods.* A high molecular weight (~250 k Daltons) polymer containing a high density of quaternary amines (polydiallyldimethylammonium chloride [polyDADMAC]) was permanently bonded onto cellulose fibers (gauze). Microbicidal and mammalian cell cytotoxicity tests were conducted using standard methods. Development of bacterial resistance to the microbicidal fibers was assessed over 10 passages. *Results.* The polyquat-treated bacterial barrier gauze dressing (BIOGUARD [BBD]) had high microbicidal activity even in the presence of proteinaceous fluid against a wide range of Gram-positive and Gram-negative bacteria. The BBD dressing passed all mammalian cell toxicity tests due to the non-leaching of the bactericidal polymer. Furthermore, the BBD dressing did not demonstrate any ability to induce bacterial resistance in selection vector testing. *Conclusion.* This novel dressing featuring a bound microbicide offers another choice for wound caregivers to provide patients with an antimicrobial barrier dressing safe enough for prophylactic use to protect against wound infections.

**W**ound infections occur in both acute and chronic wounds, and lead to substantial patient morbidity, mortality, and increased expense. The ideal treatment for any wound has been well articulated in the principles of wound bed preparation and management, and involves creating the optimal environment for the wound to re-epithelialize.<sup>1</sup>

Factors to help ensure optimal wound healing include wound bed preparation (proper debridement of compromised tissue), proper moisture management, and the exclusion of exogenous influences that would retard healing, such as bacteria that can cause infection or agents that could impede wound healing.<sup>2</sup>

A common strategy to protect wounds from bacterial colonization is to use a bacterial barrier dressing, which can be physical barriers such as films (commonly polyurethanes) that are gas-permeable but have the ability to block liquids, or moisture-permeable dressings containing antiseptics that block bacterial transmission by killing bacteria. The latter class is largely dominated by treated gauze products, alginates, and other dressings utilizing antiseptic molecules (ie, silver ions, polyhexamethylene biguanide [PHMB], iodine, or chlorhexidine) that are released from the dressings into the wound bed. These released broad-spectrum agents are not selective for bacteria (like antibiotics that affect specific bacterial target sites), and each agent can show some level of toxicity toward mammalian cells in the wound bed.<sup>3</sup>

Silver products have the largest market share of antimicrobial products and comprise the bulk of bacterial barrier dressings. Concerns exist regarding the cytotoxic effects of silver on cultured cells. Delayed healing of clean, superficial wounds such as donor sites and superficial burns has been documented with silver dressings.<sup>4,5</sup> Further concerns regarding silver dressings include the potential to develop or enrich populations of silver resistant bacteria in chronic wounds as silver resistance genes have been known for decades.<sup>6</sup> The silver released from the antimicrobial dressings can be absorbed by the

body, as demonstrated by silver deposits in re-epithelialized burn scar tissue and amputation sites following application of silver-containing dressings,<sup>7-9</sup> argyria-like symptoms, and elevated liver enzymes reported in a burn patient treated with a silver coated dressing.<sup>10</sup>

BIOGUARD bacterial barrier gauze dressings (BBD) incorporate a high molecular weight polycationic biocidal polymer (polyDADMAC), which has a very high concentration of positively charged quaternary amines (polyquats) bound to the gauze fibers. PolyDADMAC is chemically in the same class of biocides as PHMB, which has an excellent history of safe usage in Covidien's AMD™ line of products. PolyDADMAC is differentiated by being permanently bound to the dressing instead of leaching into the wound bed and by being a much larger molecule—two factors that contribute to the safety of the product. BBD dressings have extremely high microbicidal activity against Gram-positive, Gram-negative and antibiotic resistant bacteria, even in the presence of high concentrations of plasma proteins and salts that can inactivate or impair most microbicides<sup>11,3</sup>

The mechanism by which polycationic dressings, such as BBD and AMD, function was previously detailed by Gilbert and Moore<sup>12</sup>: positively charged quaternary nitrogen(s) coordinate to the head groups of acidic phospholipids that comprise the bacteria's cellular membrane. Displacement of the phospholipids disrupts the cellular membrane as it dislodges them from their natural sites in the membrane. The initial effect is disruption of normal metabolic and transport properties, while the displacement of sufficient numbers of phospholipid groups compromises the structural integrity of the entire membrane,

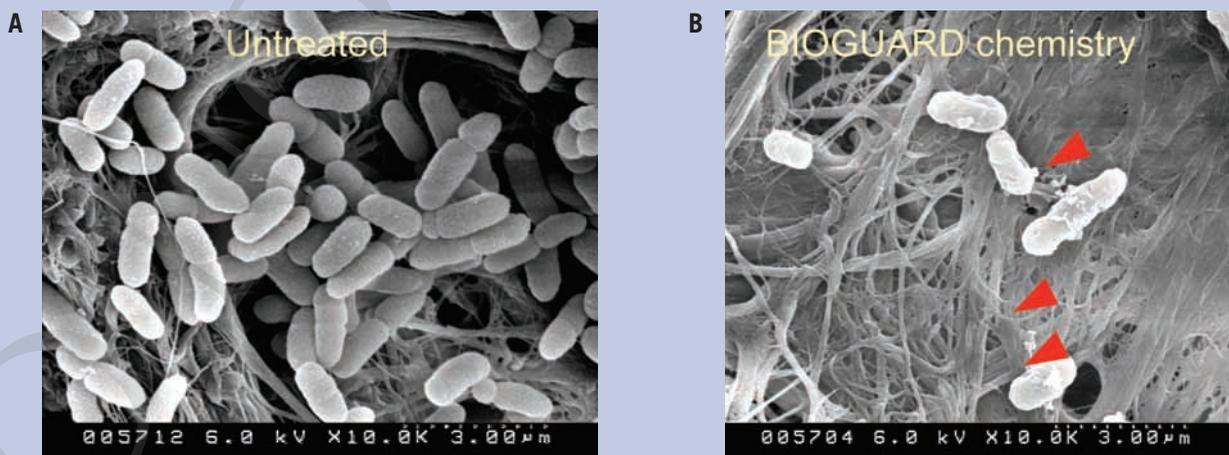


Figure 1. *E coli* A) on an untreated surface and B) with BBD-type surface treatment.

thus lysing the cell. This mechanism takes place on the outside of the cell, therefore making the development of resistant bacteria unlikely because known mechanisms of acquired resistance are dependent on the internalization of agents that target disruption of metabolic or reproductive bacterial processes.<sup>15</sup> Figure 1 shows this effect on *E coli* cells exposed to surfaces treated with BBD chemistry.<sup>14</sup>

This pre-clinical evaluation of BBD dressings is performed in contrast with commercial silver dressings. This comparison highlights the safety of BBD dressings for prophylactic usage, for which silver dressings are less well suited.

#### KEYPOINTS

- A need exists for cost effective bacterial barrier dressings that absorb wound exudate and do not release toxic materials into the wound or increase the risk for developing bacterial resistance to the microbicidal agent
- The present study reports the development and properties of a novel bacterial barrier dressing that meets these needs

## Materials and Methods

**Dressings.** Sterile samples of the BBD dressings were provided by the manufacturer (Derma Sciences, Princeton, NJ). The dressing is cotton gauze that has 0.3% (wt/wt) of a cationic polymeric biocide (polyDADMAC) bonded to the surface. In addition to BBD samples, conventional cotton gauze and a silver-containing dressing (Acticoat™ Antimicrobial Barrier Dressing, Smith & Nephew, Fort Lauderdale, FL) were selected as reference substrates.

**Bacterial strains.** Nine bacterial strains commonly associated with wounds were sourced from American Type Culture Collection (ATCC) for use in this study (Table 1). *Escherichia coli* ATCC 15597 was used both for antimicrobial efficacy testing and for testing of bacterial resistance. Reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless specified.

**Antimicrobial efficacy.** Antimicrobial efficacy was assessed by modified American Association of Textile Chemists and Colorists (AATCC) method 100-2004.<sup>15</sup> This method is designed to demonstrate the efficacy of antimicrobial woven and non-woven textile materials when swatches of antimicrobially-treated materials are directly exposed to bacterial inocula. Modifications included adding an organic burden (10% Fetal Bovine

Serum or FBS) to the bacterial inoculum to simulate wound exudate, increasing the number of replicates within sample sets to assess statistical variability, and calculating the bacterial reduction values using untreated control materials incubated at parallel exposure times (ie, overnight controls) for calculation of bacteriostatic effect, and initial inoculum or “ $t = 0$ ” controls for calculation of biocidal effect.

Zone of the inhibition (ZOI) assays evaluated the effectiveness of antimicrobial dressings with sustained-release pharmacological action.<sup>16</sup>

Bacterial barrier function testing was conducted to determine the efficacy of a BBD dressing, as described elsewhere, with minor modifications to accommodate a porous substrate.<sup>17</sup> The gauze samples, normalized for ratio of weight to inoculum absorbance, were directly inoculated on the topside of the material, allowing the inoculum to fully absorb into the dressing. *S aureus* and *P aeruginosa* were selected as challenge organisms. Inoculated control and test articles were placed onto an agar growth plate. Evaluation for microbial growth on and under the dressings took place after 24 and 48 hours incubation. After 48 hours of incubation, the dressings were removed and the agar plates were re-incubated and observed for evidence of microbial growth in each area from where the dressing material was removed.

**Change in bacterial susceptibility.** *In-vitro* assessment of changes in bacterial susceptibility to the BBD surface was carried out by repeated and continuous exposure (10 serial passages) of *E coli* to BBD samples by direct inoculation procedure similar to AATCC 100-2004.<sup>18</sup> When available, three or four isolated survivors colonies were selected and propagated into new inoculum, then re-exposed to the antimicrobial barrier dressing, and repeating the cycle for up to 10 passages of stepwise trained bacteria. Two independent experiments were performed at each time point to account for statistical variability. Minimum inhibitory concentration (MIC) was used to determine changes in susceptibility of *E coli* after prolonged exposure to BBD gauze by collecting MIC values of survivors in a confirmation experiment.

**Cytotoxicity testing.** Cytotoxicity testing of dressings was performed using the standard procedures described in the European standard EN ISO10993-5:2003, ASTM F895-84- 2001 “Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity,” and in ASTM F813-07 “Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices.”<sup>19</sup> Testing was performed using immortalized

**Table 1.** Activity of antimicrobial barrier wound dressing against microorganisms.

Wound pathogen	ATCC number of species	Average % kill vs. untreated control ( $t = 0$ ); (initial inoculum)	Average % kill vs. untreated control (overnight)
<i>S aureus</i> MRSA (Methicillin resistant <i>S aureus</i> )	ATCC 6538	99.9995%	99.999992%
<i>S epidermis</i>	ATCC BAA-44	99.9996%	99.999998%
<i>P aeruginosa</i>	ATCC 12228	99.9995%	99.999997%
<i>E faecium</i>	ATCC 15442	99.988%	99.99999%
<i>E coli</i>	ATCC 19434	99.9996%	99.999987%
<i>A baumannii</i>	ATCC 15597	99.9996%	99.999998%
VRE (Vancomycin resistant <i>E faecium</i> )	ATCC 19606	99.9989%	99.999999%
	ATCC 51299	99.9996%	99.999991%

cell line L929 derived from mouse connective tissue (American Type Culture Collection, Rockville, MD). Complementary *in-vitro* cytotoxicity data were generated by 1) placing test articles on a thin permeable agar layer to protect the cellular monolayer from physical damage, and 2) exposing L929 cells directly to the dressings.

**Biocompatibility testing.** *In-vivo* dermal irritation test was performed per Draize method (ASTMF 719–81). *In-vivo* repeated patch dermal sensitization test was performed according to the Buehler method (modified for medical devices).<sup>20</sup> Both tests were performed per GLP standards by an independent research laboratory (WuXi AppTec, St. Paul, MN).

#### KEYPOINTS

- BBD, conventional cotton gauze, and a silver-containing dressing (Acticoat™) were tested with nine bacterial strains commonly associated with wounds
- Bacterial susceptibility, cytotoxicity testing, and biocompatibility tests were performed

## Results

**Antimicrobial efficacy.** Test organisms selection was based on typical pathogens associated with acute and/or chronic wound infections. Gram-positive, Gram-negative, and the most prevalent resistant bacterial species associated with nosocomial infections were tested.<sup>21,22</sup> Results of the antimicrobial efficacy of the BBD gauze are presented as values of the average percent reduction compared to the initial inoculum, and as bacterial growth differences between the dressings and negative control materials (Table 1).

The results demonstrate that the BBD substrate effec-

tively suppresses high levels of bacterial burden ( $10^5$ – $10^6$  CFU/mL) compared to both overnight controls and initial inoculum levels.

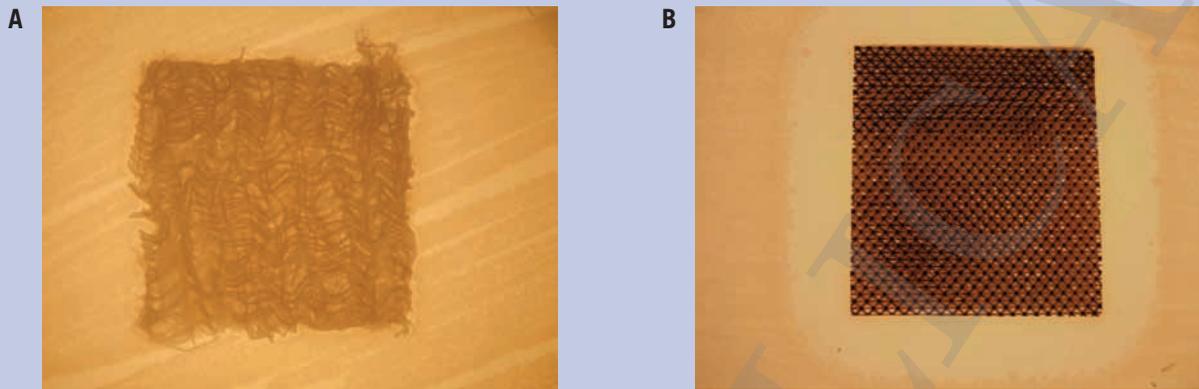
A study was performed to evaluate the barrier capability of the BBD gauze in resisting bacterial penetration. Due to the porous nature of the substrate, the barrier function test was performed on gauze samples normalized for weight per inoculum absorbance. Under the conditions of this study, 0.6 g of BBD gauze inoculated with 100  $\mu$ L of  $1 \times 10^6$  cfu/mL of bacterial species resisted microbial penetration of challenge organisms and showed no bacterial growth on the growth plate, confirming the bacterial barrier function of the treated substrate. In contrast, when untreated gauze was inoculated in the same manner, bacteria grew on the growth plate beneath the dressing.

Zone of inhibition (ZOI) assays are typically employed to evaluate the effectiveness of antimicrobial dressings with sustained-release pharmacological action.<sup>16</sup> The authors utilized this test to demonstrate the absence of biologically significant leachables. Plates with BBD dressings were compared to Acticoat barrier dressings (as a representative of silver-releasing dressings [Figure 2]).

The BBD dressing inhibited the growth of bacteria in the area underlying or in direct immediate contact with the dressing, but not in the distal areas (Figure 2A). This demonstrates the correct functioning of the BBD dressing in suppressing bacteria within and on the surface of the dressing, while not releasing the biocidal agent into

#### KEYPOINTS

- The BBD dressing inhibited the growth of bacteria in the area underlying or in direct immediate contact with the dressing, but not away from the dressing



**Figure 2.** Zones of inhibition of agar plates streaked with *E coli* culture as produced by A) BBD gauze and B) silver dressing.

the wound bed. The silver dressing demonstrated a significant zone of inhibition due to the leached microbicide (Figure 2B).

### Changes in Bacterial Susceptibility

Changes in bacterial susceptibility to BBD wound dressings were assessed through step-by-step adaptation training of *E coli* cultures to the active surface of the antimicrobial dressing. The sequential assessment of the minimum inhibitory concentration (MIC) of collected *E coli* survivors was used as a confirmation experiment.

Serial passages of bacteria were exposed to BBD dressings to create selection pressure. When available, three or more isolated survivor colonies were selected and propagated into new inoculums and exposed to the treated substrate. By combining several survivor colonies it was assured that selection was not accidentally limited to any one organism that avoided contact with the treated surface. The polyDADMAC treated BBD surface retained activity against the challenge organism even after repeated exposure, consistently demonstrating > 99.9% efficacy (Table 2).

In a complementary set of experiments, the MIC of *E coli* survivors at each passage fell within the same range of MIC as the reference *E coli* strain. These experiments demonstrated that *E coli* did not develop any measurable

tolerance against the BBD dressings after prolonged and repeated exposure under the test conditions, and that the efficacy of the dressings remained virtually unchanged.

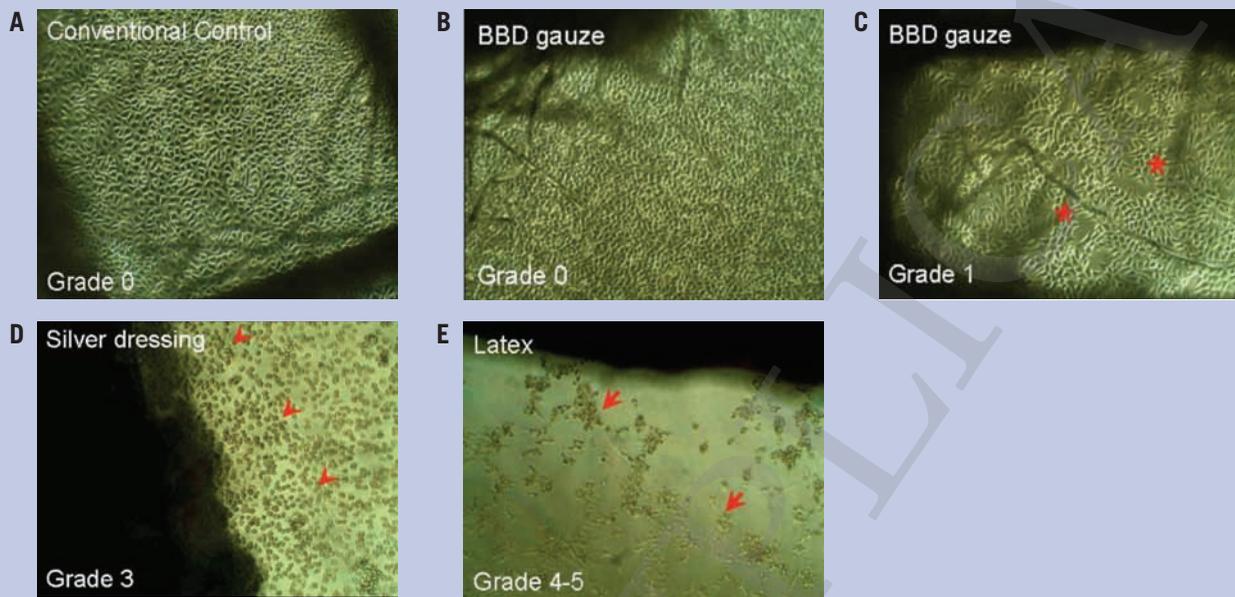
### Cytotoxicity

Evaluation of cytotoxicity by the “Agar Overlay” method demonstrated that BBD samples produced no detectable cytotoxicity zone under or around the samples, giving a score of “zero” on the cytotoxicity test scale. A more sensitive assay method was also employed that exposed a cellular monolayer directly to test samples by the protocol described in ISO 10993-5 and ASTM F813-07 “Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices.” Photographic images were collected to support the visual score assignments.<sup>23</sup> The microscopic findings (Figure 3) for the samples are shown with the assigned grades: BBD showed scores of 0 and 1 (3B and 3C), while the silver-releasing dressing showed grade 3 (3D), and the latex positive control confirmed the validity of the system with a score of 4–5 (3E).

**Silver dressing.** The L929 monolayer exposed to a silver dressing shows a significantly depleted (“sparse to none”) cell population (Figure 3D), demonstrating that silver eluted from the dressing compromised a significant

**Table 2.** Efficacy of BIOGUARD polyDADMAC-treated surface against step-wise trained *E coli* cultures.

Test iteration	1	2	3	4	5	6	7	8	9	10	
Inactivation (%)	99.988	99.994	99.999	99.999	99.999	99.999	99.999	99.999	99.990	99.991	99.999



**Figure 3.** Examples of L929 monolayer response to BBD and silver-based dressings, alongside positive and negative controls. (Asterisks [slide C] indicate areas of altered cellular morphology not associated with lysis; arrows [slides D and E] indicate areas with pronounced cellular lysis).

portion of the fibroblast population. The plates show round denucleated and detached cells indicating pronounced lysis. These observations affirm previous studies of the *in-vitro* cytotoxicity of silver-based dressings.<sup>4,24</sup>

**BIOGUARD dressing.** BBD gauze retained approximately 90% of the confluent monolayer, in a pattern typical for healthy cellular architecture. Isolated areas immediately underneath the substrate showed reduced cellular population with patches denuded of cells, or cells with distorted cellular appearance (Figure 3C). The remaining monolayer maintained a normal pattern of close apposition, with cells exhibiting a prominent polygonal shape with well-defined round or ovoid nuclei associated with normal cell morphology. No morphological changes representative of lysis, such as rounding, or detachment and loss of cytoplasmic inclusions were observed indicating no cytotoxic reaction to the tested antimicrobial barrier dressing. Based on the visual grading scale, the direct contact study of BBD substrates were evaluated as Grade 0 to 1 consequently demonstrating the high *in-vitro* biocompatibility of the BBD surface.

### Biocompatibility Testing

*In-vivo* skin irritation and sensitization study results confirmed the positive biocompatibility results of the *in-vitro* cytotoxicity assays. BBD did not evoke any signs of dermal irritation in the denuded skin area of rabbits, and

received the lowest (best) irritation index score possible according to Draize's table. Repeated patch dermal sensitization testing gave no indication that BBD gauze has any propensity to cause sensitization. These results are consistent with the BBD design to avoid leaching any substances from the dressing onto the dermal surface.

### Discussion

The high incidence of surgical site infections and the growing incidence of chronic wounds strongly suggest a need for an effective dressing that blocks penetration of bacteria to a wound. Most bacterial barrier dressings also release cytotoxic chemicals, which are toxic to wound cells as well as bacteria. To address the cytotoxicity issue, the authors developed a bacterial barrier gauze dressing with a microbicidal polymer that is bound to the gauze fibers and does not release cytotoxic chemicals, therefore avoiding any possible impairment of wound healing due to leached microbicidal chemicals.

This study evaluated the *in-vitro* antimicrobial efficacy of a novel bacterial barrier dressing against a broad range of bacterial strains. The cytotoxicity of this non-leaching wound dressing was assessed and compared to a commercial silver-releasing antimicrobial wound dressing. The ability of bacteria to develop tolerance to this dressing was also evaluated as part of the preclinical safety evaluation of the new antimicrobial dressing.

The antimicrobial efficacy of the BBD dressing is based on a bonded cationic polymer antimicrobial agent, polyDADMAC, which physically disrupts the prokaryotic cell wall. Polycationic microbicides, as a chemical class, demonstrate broad antimicrobial activity, while their large size and high-charge density precludes entry into the bacterial cell. This property makes the development of bacterial resistance development highly unlikely.<sup>25,26</sup> The excellent biocompatibility shown by the BBD dressing could be initially attributed to the high molecular weight of the polycationic biocide<sup>12</sup> and further enhanced by the absence of a leaching antimicrobial released into the wound bed.

The non-migrating nature of the bonded polymeric antimicrobial used in the BBD gauze is clearly demonstrated by the lack of a zone of inhibition (ZOI) on an agar plate populated with bacterial culture. Cytotoxicity studies confirm no influence by the BBD dressing outside the immediate contact area. A silver-based antimicrobial dressing showed a significant ZOI when tested under identical conditions, which correlated with cytotoxicity test results that showed a far-reaching cytotoxic zone in the mammalian cell monolayer immediately adjoining the dressing.

The biological significance of the presence of the zone of inhibition has mostly been represented as providing protection from bacterial colonization. The corollary to far-reaching prevention of bacterial growth is the effect on mammalian cells. The use of antimicrobial dressings with leachable agents represents a tradeoff in properties. The presence of a heavy bacterial burden in a wound would justify the use of a leachable agent if the condition was not addressed by debridement to a healthy margin or by some systemic pharmaceutical. Silver, for instance, has been demonstrated to have a significant cytotoxic potential, which can retard wound healing by impeding the activity of the regenerating fibroblasts and keratinocytes that are rebuilding and populating the matrix to heal the wound.<sup>27,28</sup> At the same time, in cases such as bacterially colonized chronic wounds, the use of silver dressings has proven highly effective and clinically beneficial, because the reduction of bacterial burden in the wound brings a great and necessary benefit, and cellular toxicity or chemical impediments to optimal wound healing are necessary side effects that do not outweigh the benefits of reducing the bacterial burden.<sup>29</sup> For clean surgical wounds or other uninfected sites where the goal is to protect the wound from bacterial colonization, the use of an aggressive

leaching product could conceivably impair wound healing and closure, possibly extending the time that the wound is more vulnerable to pathogens. When the primary goal is to protect a wound site from bacterial colonization, it makes sense to utilize a dressing that will provide the highest level of biocompatibility with the healing wound. For this type of prophylactic use, the best dressing design to utilize should have minimal interaction with the regenerating wound bed so as to minimize the chances of impeding wound healing, while maintaining a barrier to bacterial colonization and minimizing any opportunity for bacteria to develop resistance to the active agent.

#### KEYPOINTS:

- When the primary goal is to protect a wound site from bacterial colonization, it makes sense to utilize a dressing that will provide the highest level of biocompatibility with the healing wound
- The BBD antimicrobial barrier dressing fulfills its protective function without impeding cells relevant to wound healing and is safe enough to be used broadly as a prophylactic dressing

## Conclusion

The BIOGUARD dressing was tested extensively to demonstrate high efficacy against common wound pathogens, while maintaining a high level of biosafety. Biosafety was demonstrated through standard ISO models for cytotoxicity, dermal irritation, and sensitization, as well as through testing of mammalian cell culture models. This was consistent with the absence of a zone of inhibition in testing, demonstrating that the material was able to control pathogens in the dressing without exerting a physiological effect on the wound bed cells. Since the BBD antimicrobial barrier dressing fulfills its protective function without impeding cells relevant to wound healing and without causing concern over selecting for resistant bacterial strains, it is safe enough to be used broadly as a prophylactic device to protect vulnerable patients and wounds from infection without additional risks for the patient.

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