Expert Recommendations for Optimizing Outcomes in the Management of Biofilm to Promote Healing of Chronic Wounds

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Biofilm is a common form of wound contamination that is now recognized to be a major factor in delaying the healing of wounds. However, awareness of the biology of biofilm, its prevalence, its clinical significance, and optimal treatment approaches needs to be improved.

Ten experienced wound care specialists reviewed the current status of evidence-based management of biofilm with a focus on the optimal use of PuraPly™ Antimicrobial, a purified collagen-based wound care matrix containing the antimicrobial polyhexamethylene biguanide (PHMB).

The overarching goal of this meeting is to empower clinicians caring for patients with chronic wounds to optimize clinical outcomes by providing an educational resource about the management of biofilm that is concise and useful in their clinical practices.

The meeting participants represented a variety of perspectives including academic settings, teaching hospitals, and stand-alone wound care clinics. Gregory Schultz, PhD, from the University of Florida and Director of the Institute for Wound Research, and Professor Stephen C. Davis, from the University of Miami Department of Dermatology and Cutaneous Surgery, led the discussions. Following the conference, a summary of the presentations and group discussions was written and finalized with input from all meeting participants.

**Meeting Participants**

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Dr. Schultz is Professor of Obstetrics and Gynecology, and Director of the Institute for Wound Research at the University of Florida. Dr. Schultz completed a PhD in biochemistry from Oklahoma State University and postdoctoral training in cell biology at Yale University. Dr. Schultz’s research focuses on the molecular regulation of wound healing with an emphasis on anti-scarring therapies and the roles of elevated proteases and bacterial biofilms in chronic wounds. Dr. Schultz has authored over 320 scientific publications that have been cited more than 11,700 times, is principal investigator or co-investigator on grants totaling over $35 million, is an inventor on 26 patents and a co-founder of two biotech companies. He was recognized by *Time* magazine as an Innovation Leader in 2006. He served as a member of the National Pressure Ulcer Advisory Panel from 2007 to 2010, and served as President of the Wound Healing Society from 1999-2001.

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Professor Davis is a professor in the Department of Dermatology and Cutaneous Surgery at the University of Miami. He has over 32 years experience using various wound healing and infection models in swine. He has worked with a large number of companies in research and development of various products that are on the market today (e.g. Duoderm®, Iodosorb®, Procellera®, and Kerlix A.M.D.®). He has authored several book chapters, and over 100 published articles and abstracts on wound healing and infection. He is currently on the editorial advisory board for the journal *WOUNDS*, and is a reviewer for several journals including the *Journal of Clinical and Experimental Dermatology*, the *British Journal of Dermatology*, and *Wound Repair & Regeneration*. Some of his interests include: occlusive therapy, electrical stimulation, low energy light therapy, and bacterial biofilm formation. For the past several years, he has been funded by the Defense Advanced Research Projects Agency (DARPA), Canadian Defense, United States Army, Office of Naval Research, National Institutes of Health and the National Science Foundation (NSF).

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Dr. Carpenter is a Certified Wound Specialist Physician, specializing in wound care and hyperbaric medicine since 2004. He graduated from the University of California at Berkeley, majoring in molecular and cell biology. He received his medical doctorate from Tulane Medical School, and subsequently became board-certified in emergency medicine after completing his residency at LSU Charity Hospital in New Orleans. Over the past 10 years, Dr. Carpenter has developed an aggressive wound management program for hospitals, nursing homes, and clinics. He is the inventor of TeleWound™, a process by which homebound patients can receive aggressive wound care via telemedicine in the home. He also has several patents pending for wound care products and devices, including the Carpenter Curette and the True-See Digital Image Calibration system. He serves as Director of Wound Care for Northshore Specialty Hospital, Baton Rouge General Medical Center, and Promise Hospital of Baton Rouge. Dr. Carpenter is the founder and CEO of MedCentris, a comprehensive wound management company.
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Dr. Fitzgerald is a podiatric surgeon with extensive training in elective and reconstructive surgery of the foot and ankle. Dr. Fitzgerald’s expertise includes minimal incision arthroscopic surgery, diabetic foot care, lower extremity wound care, plastic surgery techniques, external and internal fixation, and reconstructive limb salvage. As a founding member of the Greenville Health System (GHS) Center for Amputation Prevention, a collaborative clinical and research alliance at GHS, Dr. Fitzgerald is dedicated to advancing the care of lower extremity ulcerations and preventing amputations in high-risk patient populations. Additionally, Dr. Fitzgerald serves as Assistant Professor of Surgery at the University of South Carolina School of Medicine-Greenville, where his responsibilities include lower extremity reconstruction and limb salvage techniques, wound care, research and medical education.

Dr. Fitzgerald has authored numerous peer-reviewed journal articles and trade publications, and he has been featured as a speaker at both national and international conferences. Dr. Fitzgerald is board-certified in reconstructive rearfoot and ankle surgery as well as foot surgery by the American Board of Foot and Ankle Surgery (ACFAS), and a member of the American Podiatric Medical Association (APMA).

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Ms. Gehling is a board-certified family nurse practitioner with over 20 years of experience in wound care. She has been credentialed as a CWOCN since 1992 and practices with Dr. Samies in wound care and infectious disease medicine at the Center for Advanced Wound Care and Hyperbaric Medicine at the Regional Medical Center in Orangeburg, SC. She was elected Southeast Region WOCN Nurse of the Year for 2012, and has presented numerous educational lectures, research posters and publications on advanced wound care topics.

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Dr. Samies is a board-certified specialist in infectious diseases and internal medicine with more than 25 years of experience in the treatment of acute and chronic wounds. He is the hospital epidemiologist and Director of the Center for Advanced Wound Care and Hyperbaric Medicine at the Regional Medical Center in Orangeburg, SC. He is a Fellow of the Society for Healthcare Epidemiology of America, and a Certified Wound Specialist. He has an active clinical practice in infectious disease medicine and wound care. His clinical and research interests are in infection control in wound care. He has published numerous articles and lectures on infection control, wound care and infectious disease topics.

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Dr. Teichman is a board-certified foot and ankle surgeon, and is a Fellow of the American College of Foot and Ankle Surgeons. A graduate of the California College of Podiatric Medicine, he completed a 3-year surgical foot and ankle reconstruction residency at St. Mary’s Hospital in Hoboken, NJ. Dr. Teichman is Chief of Podiatric Surgery at Sacred Heart Hospital in Allentown, PA. He currently serves on the limb salvage team at Sacred Heart Hospital, where he is also a panel physician specializing in lower extremity limb preservation and management. Dr. Teichman has been in private practice in Allentown, PA since 2005, and is co-founder of PA Foot and Ankle Associates. His current practice is focused on conservative and surgical management of the diabetic foot. He lectures nationally on wound management and limb preservation.
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Meeting Participants (Continued)

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Ms. Weir has been a registered nurse for 40 years; 36 of those dedicated to the practice of wound and ostomy care. She has practiced in acute care, home care and long term care, spent seven years in industry, and has practiced in outpatient care since 2001. She has been board-certified by the Wound, Ostomy and Continence Nursing Certification Board since 1985 and the American Board of Wound Management since 2004. She practices outpatient wound management at Osceola Regional Medical Center in Kissimmee, FL, and at Health Central Hospital in Ocoee, FL. Ms. Weir is the Co-Chair of the Symposium on Advanced Wound Care, was on the founding Board of the Association for the Advancement of Wound Care and held the positions of the first Treasurer and the third President. She has been on the faculty of the Wound Certification Prep Course since 2004. She has been a member of the Wound, Ostomy and Continence Nurses Society since 1980, the Florida Association for Enterostomal Therapists since 1979, and has held regional board positions with both. She has been a member of the Wound Healing Society since 2008, and was one of the Founding Editors of the journal *Today’s Wound Clinic*. Ms. Weir is a frequent lecturer on all aspects of wound management, has authored and co-authored many journal articles and eight book chapters. She is on the Speakers Bureau and medical advisory boards with several manufacturers.

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Biofilm Impedes Healing of Chronic Wounds

Chronic non-healing wounds remain a major challenge for all wound care practitioners. These include diabetic ulcers, venous ulcers, pressure ulcers, vascular ulcers, trauma wounds and surgical wounds. It is axiomatic among experienced wound care practitioners that wound infection can delay (or completely prevent) wound healing. However, it is now known that an important factor that delays wound healing in many patients is biofilm, a typically polymicrobial infection in a protective proteinaceous matrix.¹

There have been significant advances in our understanding of why biofilms are so prevalent, how they resist interventions to eliminate them, and how they slow wound healing. This knowledge has resulted in the development of novel wound therapies that promise to improve management of chronic wounds.

Biofilm is the most common type of wound bioburden and represents an intermediate stage in a continuum of bioburden that ranges from simple contamination to localized infection to sepsis. In simple contaminations, microorganisms within the wound are free-floating (planktonic), but as they multiply, these microbes firmly attach to the wound’s surfaces, differentiate, and change (“switch”) their gene expression patterns to a biofilm phenotype that promotes their survival.² Virtually all

Figure 1. Events and approximate times in the normal wound healing process. Acute wounds begin the repair phase early, and normally achieve healing in two to three weeks. Most chronic wounds that fail to heal are “stuck” in the inflammatory phase.

wounds contain a combination of both free-floating microbes and biofilm; however, biofilm is much harder to eradicate (see section #3 “Why Biofilm is Hard to Kill” on page 9) and delays wound healing to a greater degree.3

Most chronic wounds that fail to heal are “stuck” in the inflammatory phase of the normal healing process (see Figure 1 on page 5). In this inflammatory phase—which follows the normal clotting and vascular response phases—neutrophils and macrophages are attracted to the wound site where they secrete large quantities of a variety of enzymes, including matrix metalloproteinases (MMPs) and elastases, that break down damaged matrix. As invading microbes and damaged tissues are cleared, inflammatory cells present in the wound die off, the influx of additional inflammatory cells stops, and inflammation subsides.4 This is followed by the formation of new collagen, re-epithelialization, contraction, and ultimately scar formation.

The presence of biofilm impedes the normal healing process by continuously stimulating immune-mediated inflammation within the wound (see Figure 2 below). In fact, it has been clinically observed that patients with chronic wounds had wound inflammatory protease levels as much as 100 times higher than patients with normally healing acute wounds.5 The effect of inflammatory factors in the wound is greater when the biofilm composition is polymicrobial compared to monospecies infections, and least in

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**Figure 2.** Repeated injury, ischemia, and infection can cause a wound to remain in a chronic inflammatory phase and not heal.

wounds that do not contain biofilm. In addition to stimulating wound inflammation, biofilm slows healing by serving as a microbial reservoir for infection of healthy tissue, and by competing with normal cells for oxygen and nutrients.

Importantly, studies at Northwestern University have demonstrated that biofilm is a direct cause of delayed wound healing, and that the association between biofilm and delayed healing is not secondary or coincidental. Polymicrobial biofilms, which are associated with most wounds, have also been shown to reduce healing more as compared to single bacterial strains.

**EXPERT PANEL DISCUSSION AND RECOMMENDATIONS**

The panel members agreed that although the term biofilm is often recognized by wound care practitioners, its biology and clinical significance are often not well appreciated. A key point is that even when biofilm is not visually apparent and the wound “looks good,” biofilm is frequently present and delays wound healing significantly.

In addition, it was also discussed that high proteolytic activity within wounds is often a critical factor in non-healing wounds, but this fact is underappreciated by many practitioners. The panel concluded that there is an urgent need for education in this regard because

![Figure 3. Schematic representation of the continuum from contamination and colonization, which involves free-floating (planktonic) bacteria to biofilm to systemic infection. Biofilm and more advanced infection require therapeutic intervention. Source: Adapted from Phillips PL, Wolcott RG, Fletcher J, Schultz GS. Biofilms made easy. Wounds International. 2010;1(3):1-6.](image-url)
there are important implications for therapy: proteolytic enzymes generated by biofilm can degrade collagen dressings when bioburden is not adequately controlled.

2 Biofilm Is Common But Difficult to Diagnose

Many practitioners correctly believe that essentially all chronic wounds are contaminated or colonized with bacteria (i.e., they are not sterile), but these low levels of planktonic bacteria typically do not stop wounds from healing. However, when planktonic bacteria convert into communities of biofilm, as discussed above, the biofilm becomes clinically significant and bioburden control becomes critical to wound management (see Figure 3 on page 7). But how common is biofilm in real world settings? The answer is that a large majority of chronic wounds (60% to 90%) contain biofilm, while only 6% of acute wounds do.\(^2,3,9\) In fact, it is likely that almost all chronic wounds have biofilm on at least part of the wound bed.\(^2\)

Importantly, the presence of biofilm should not be excluded based on the absence of gross signs of infection. Unfortunately, biofilm can be underdiagnosed precisely because it is not associated with visual evidence of infection.

Practitioners can not necessarily rely on standard wound cultures performed by clinical microbiology labs to determine if biofilm is present. Typically, wound samples are processed by additional techniques to first kill all planktonic bacteria by brief exposure to a microbicidal antiseptic such as bleach, followed by neutralization of the antiseptic and dispersal of biofilms into single cells, followed by plating on culture dishes. From a practical perspective, the definitive determination of biofilm is probably unnecessary, if all chronic wounds are treated on the presumption that biofilm is already present or there is high risk of biofilm formation. Culturing the wound may not provide useful results in this regard, as standard cultures detect free-floating microbes on the wound surface and not biofilm microbes that lie deeper within the wound site. In fact, wound cultures may point to antimicrobial treatment that will not be effective against the biofilm.\(^2\)

![Figure 4: Mechanisms by which biofilm bacteria resist killing by antibodies and antibiotics, and contribute to the spread and persistence of infection in neighboring healthy tissue.](image)

Therefore, when faced with wounds that are slow to heal, it is reasonable for practitioners to assume that all non-healing wounds contain biofilm, and to treat the patient accordingly.

EXPERT PANEL DISCUSSION AND RECOMMENDATIONS

There was a strong consensus among the panel members that practitioners need to be more aware that wound biofilm is extremely common (i.e., affecting up to 80% of wounds). Panelists agreed that commonly there is failure to recognize that essentially all open wounds will likely develop biofilm within three days. In addition, it is also important to understand that while wound cultures are helpful in guiding antibiotic therapy of single free-floating (planktonic) bacteria within wounds, such cultures do not provide reliable information on biofilm bacteria, which “hide” more deeply within the wound bed. A key teaching point here is that wiping the surface of a wound with a swab samples only free-floating bacteria, but does not sample biofilm-associated bacteria. Given these considerations, the panel recommends that essentially all wounds should be treated on the assumption that biofilm is already present, or is highly likely to develop if adequate bioburden management is not achieved.

Why Biofilm Is Hard to Kill

Studies have shown that biofilm can be very difficult to eradicate. While free-floating bacteria are usually eliminated by the immune system and/or antibiotics, biofilm poses a much greater challenge for several reasons (see Figure 4 on page 8).

1. Extracellular Polymeric Substance – Biofilm-associated microbes produce a protective substance known as extracellular polymeric substance (EPS) that accelerates biofilm formation. EPS is a very dense collection of proteins, sugars, and other factors that impair diffusion of antimicrobial molecules, including antibodies and antibiotics. In addition, due to its highly negative ionic structure, EPS acts as an “anionic screen” that blocks many cationic agents, such as silver and many antibiotics. EPS may also act as a diffusion barrier against small molecule antimicrobial substances released by

![Figure 5: Laboratory gentamicin susceptibility of cultured biofilm bacteria after initial debridement. Three wounds were debrided with standard surgical technique and dressed with no antimicrobial treatment. Three days later (day 0/baseline), each wound was debrided again, with three different zones within each wound debrided again on the subsequent three days, each zone on a different day. Samples collected at days 0-3 were divided in half with one half serving as control and the other half being placed in a solution containing 200 g/mL gentamicin; all samples were cultured and colony-forming units (CFUs) were counted. The chart shows gentamicin reduction of CFUs among treatment samples on day 2, but recovery of bacteria to near baseline resistance on days 2 and 3 post-debridement.](source: Adapted from Wolcott RD, Rumbaugh KP, James G, et al. Biofilm maturity studies indicate sharp debridement opens a time-dependent therapeutic window. J Wound Care. 2010;19(8):320-328.)
inflammatory cells.\(^2\)

2. **Persistor Bacteria** – Biofilm contains “persistor bacteria” that have low metabolic activity. Since antibiotics only kill metabolically active microbes by inhibiting metabolic enzyme systems, inactive bacteria not using these systems may not be sensitive to antibiotics.\(^14\)

3. **Anaerobic Bacteria** – Oxygen diffusion to the center of the biofilm is reduced, creating a hypoxic environment in which more anaerobic bacteria can thrive.\(^15\) Anaerobic bacteria are generally more resistant to antimicrobial therapies. One study found that 60% of biofilm bacterial species in pressure wounds were strict anaerobes.\(^16\)

4. **Synergism Between Bacteria** – Examples of such synergism include MRSA that secrete resistance proteins within the biofilm that help other wound bacteria survive,\(^17\) and *Pseudomonas* that secrete catalase, which destroys cytotoxic hydrogen peroxide.\(^18\)

**EXPERT PANEL DISCUSSION AND RECOMMENDATIONS**

The panel members discussed that wound care practitioners need to appreciate that biofilm-associated bacteria can be extremely difficult to kill for the reasons described above. A key teaching point here is that antibiotics are effective only against *metabolically active* bacteria, which explains why they are not effective against *biofilm-associated* bacteria, many of which are metabolically inactive. This is in contrast to the antimicrobial agent PHMB, which is capable of killing quiescent biofilm-associated bacteria by “punching holes” through their membranes (see section #6 “PHMB: An Excellent Topical Antimicrobial” on page 13).

An important distinction that was discussed was that resistance to therapy and tolerance to therapy are not synonymous. Resistance means there has been a permanent genetic alteration within the bacteria’s DNA that allows it to resist killing. In tolerance, there is no genetic change but the bacteria are less susceptible to killing. Both resistance and tolerance may cause antimicrobials, both topical and systemic, to be ineffective against biofilm.

**Effect of Tobramycin on Free-floating and Biofilm-associated *P. aeruginosa***

![Figure 6: Biofilms are highly tolerant to antibiotics. For example, in this study, tobramycin rapidly killed free-floating (planktonic) *Pseudomonas aeruginosa* (blue) very effectively, but was not effective against biofilm (red). Source: Adapted from Walters MC 3rd, Roe F, Bugnicourt A, Franklin MJ, Stewart PS. Antimicrob Agents Chemother. 2003;47(9):317-23.](image)

**4. Aggressive Debridement is Essential but Biofilm Reforms Rapidly**

Successful management of biofilm requires two therapeutic objectives to be achieved: (1) remove biofilm that has already formed, and (2) prevent biofilm reformation.

Chronic wounds usually have both free-floating, single, planktonic bacteria and biofilm-associated microbes on their surface. These can be wiped or washed off, but beneath the surface of the wound, there are potentially many additional biofilm colonies. Aggressive debridement is necessary to remove the deeper biofilm. Fortunately, effective debridement can reduce bacterial counts to less than 1/100th of baseline.

However, even with aggressive debride-
ment, biofilm can start to reform within 24 hours and can fully mature within 3 days (see Figure 5 on page 9). In a porcine biofilm model, it has been shown that various debridement methods can initially reduce biofilm-associated bacteria by a few log CFU/g. However, high levels of these bacteria can remain in the wound for several days.

Frequent aggressive debridement is a necessary part of wound care and biofilm management. Practitioners commonly perform frequent debridements to care for wounds, however, due to the rapid reformation of biofilm, debridement alone is not sufficient to control bioburden.

EXPERT PANEL DISCUSSION AND RECOMMENDATIONS

A key point of consensus is that successful wound management requires successful biofilm management. Treating and preventing biofilm formation allows the wound to utilize the normal healing mechanisms. While some utilize a “step-up” approach in which increasingly potent therapies are utilized starting with the least potent, the alternative may be a better option, namely, the “step down” approach in which aggressive treatment (surgical debridement followed by antimicrobial therapy) is used initially. There was agreement that in many wound care centers, debridement is performed on a once-weekly basis. However, this was characterized as a “disconnect” because, as discussed above, biofilm starts to reform within 24 hours of debridement. In other words, there is a “window of opportunity” after debridement in which it is critical to prevent biofilm formation. Practitioners must address this by ensuring that topical antimicrobial wound therapy is applied that will prevent biofilm formation between debridement visits (i.e., as a “bridge” to the next debridement). If not, biofilm is likely to recur and interfere with wound healing. In addition, there was consensus that in wounds that are not healing well, it is critical to control bioburden before advancing to bioengineered living cell-based therapies (Dermagraft® and Apligraf®). The panelists agreed that complete wound bed preparation with adequate control of biofilm must be achieved before cell-based therapies are utilized. If adequate biofilm control is not achieved, the graft can be “consumed” by high levels of microbial enzymes and host inflammatory cell enzymes (MMPs and neutrophil elastase) and reactive oxygen species, which may explain why these therapies fail in some patients.

5 Limitations of Antimicrobial Therapy of Biofilm

Given that biofilm is formed by the activity of microbes, antimicrobial treatment of wounds is appropriate. Antimicrobial products are commonly used, both topically and systemically, but the ability of certain topical and systemic products to effectively manage biofilm is limited in several ways.

Topical antibiotics. Several studies demonstrate that treatment of biofilm

Figure 7: The mechanism of action of PHMB on the microbial cell membrane. There is a progressive interaction of positively-charged PHMB with the negatively-charged microbial cell membrane, leading to membrane dissolution and microbe death.

with topical antibiotics can be ineffective. For example, an *in vivo* study of two topical antibiotics in pig wounds inoculated with *S. aureus* showed that these preparations were effective at suppressing free-floating, single, planktonic bacteria but neither product was able to completely eradicate the biofilm. An *in vivo* study of topical tobramycin and ciprofloxacin against *P. aeruginosa* showed similar results (see Figure 6 on page 10). Antibiotics penetrated the biofilm but only eradicated the organisms in the outer layers where there was exposure to oxygen and the bacteria were metabolically active.

Systemic antibiotics. Clearly, systemic antibiotics do have a role when infection has progressed beyond biofilm to a gross infection, but such treatment is not targeting the biofilm per se. As discussed earlier, extracellular polymeric substance (EPS) secreted by biofilm in the wound protects bacteria from antibiotic exposure. It also harbors large numbers of inactive microbes, and such antibiotics act primarily against metabolically active organisms. Furthermore, as is always the case with antibiotic use, the presence and emergence of genetically resistant organisms in a polymicrobial infection is a common occurrence. For example, methicillin-resistant *S. aureus* (MRSA) has been isolated from chronic wounds.

Topical antimicrobials. A number of topical antimicrobials are available to reduce wound bioburden and these can be highly effective. However, some topical antimicrobial agents can have deleterious effects on healthy cells in or adjacent to the wound (cytotoxicity), which may contribute to poor wound healing. Silver, for example, has been shown to be non-specific in its mode of action, killing both bacteria and host keratinocytes and fibroblasts, leading to delayed healing. In addition, not all topical agents...
have a broad antimicrobial spectrum, which may limit their clinical utility, and bacterial resistance to certain antimicrobial agents has been documented.

EXPERT PANEL DISCUSSION AND RECOMMENDATIONS

The panel discussion focused on the limitations of topical antimicrobials, and that this needs to be more deeply appreciated by wound care practitioners. A key point that the panel wished to emphasize is that while some topical antimicrobials are very effective at killing bacteria, they may also have cytotoxic effects on normal cells. For example, bleach-containing agents and some silver dressings have been shown to have cytotoxic effects.

PHMB: An Excellent Topical Antimicrobial

In light of the foregoing discussion of the challenges that biofilm poses, the ideal topical antimicrobial for combating biofilm should have the following properties:

• Broad antimicrobial spectrum – Biofilm is often polymicrobial, including both bacteria, fungi, and unidentified pathogens
• No microbial resistance – Ideally, the mechanism of action would target a universal trait of all biofilm microbes that cannot be genetically modified
• Low microbial tolerance – It should have a proven ability to thwart the strategies microbes use to tolerate antimicrobials
• High tissue compatibility – Microbicidal effects should not damage healthy tissue
• Deliver antimicrobial therapy over a sufficiently prolonged period of time and remain in the wound to prevent biofilm reformation
• Well tolerated by host wound cells

Polyhexamethylene biguanide (PHMB), a positively-charged polymer that has been extensively studied for more than 25 years, has all of the above characteristics. Its mechanism of action has been well characterized. PHMB kills microbes through direct physical contact rather than chemical reactions (as is the case for silver and most antibiotics). The positively-charged polymer interacts with negatively-charged phospholipids in microbial membranes, forming “punched holes” within the membrane. This results in loss of cellular integrity and is quickly followed by bacterial cell death, while maintaining low cytotoxicity to surrounding mammalian cells. (See Figure 7 on page 11.)

This mechanism has additional advantages. Since it does not rely on cellular activity (unlike antibiotics), PHMB is effective against quiescent cells within biofilm. Since the mechanism is a direct effect on microbial membranes, there is no known microbial resistance. Given that virtually all microbes have negatively-charged phospholipids in their cell membranes, PHMB has a broad antimicrobial spectrum that includes gram-positive bacteria (including MRSA), gram-negative bacteria (including P. aeruginosa and E. coli), anaerobic bacteria, spore-forming bacteria, intracellular bacteria (including Chlamydiae and Mycoplasma), and fungi (including C. albicans and Aspergillus niger).

EXPERT PANEL DISCUSSION AND RECOMMENDATIONS

There was consensus among the panel members that many wound care practitioners are not sufficiently aware of the advantages of PHMB as a biofilm barrier, despite the fact that it has been extensively studied for many years. In addition, it is important to understand that the mechanism of action of PHMB is very different from other antimicrobials, and that this results in significant clinical advantages, including broad antimicrobial spectrum, no resistance, and high tissue compatibility. Unlike antibiotics, which only kill metabolically active microbes, PHMB kills metabolically inactive microbes as well. In addition, PHMB kills both free-floating and biofilm-associated microbes.

The Importance of Natural Collagen Matrix in Wound Healing

Although in the past collagen was thought to act only as a passive support structure, it is now known that intact collagen plays an active role in all phases of wound healing—hemostasis, inflammation, proliferation and remodeling. As a wound heals, a network of collagen fibers is formed, serving as a framework for fibroblasts to migrate along and close the wound. Collagen controls many cellular functions, including cell shape and differentiation, migration, and protein synthesis.

As noted earlier, in chronic wounds, the repair phase can be thwarted by excessive chronic inflammation caused by biofilm. Proteinases (such as MMPs and neutrophil elastase) released by inflammatory cells, which target denatured and damaged proteins, can also damage intact proteins. An imbalance in proteinases plays a critical role in the disordered remodeling of extracellular matrix during delayed wound healing. Studies have shown that collagen with native
structural and functional characteristics can effectively address the protease imbalance seen in chronic wounds.25

**EXPERT PANEL DISCUSSION AND RECOMMENDATIONS**

Several points were made during the panel discussion regarding the use of collagen products in wound management. Collagen can be used to sequester proteolytic enzymes (such as MMPs), however this may not be effective if bioburden is not controlled because bacteria will produce additional MMPs that will consume the collagen. It was discussed that there is some evidence that collagen with native structure inhibit a wider range of MMPs, and are therefore more effective at addressing the proteolytic environment than collagens that are reconstituted. There was consensus that if adequate bioburden control is not achieved, collagen products may be less effective in supporting wound healing.

**PuraPly AM: Sustained PHMB Delivery Via Intact Collagen Matrix**

**CHARACTERISTICS AND BENEFITS**

PuraPly™ Antimicrobial Wound Matrix (PuraPly AM) is composed of two layers of intact Type I collagen sheets that are saturated with the antimicrobial PHMB.

Intact Type I collagen is purified from a porcine source. The collagen material is treated chemically to remove cells and non-collagen materials that could cause an inflammatory or immunologic response, and the process also inactivates viruses and bacteria. This proprietary purification process preserves the natural structure of the collagen matrix, which is important for strength, function, and bio-compatibility during wound healing. The two sheets of intact collagen are cross-linked with ethyl dimethylamine carboxylic acid (EDC) and then laminated together. This combination of cross-linking and lamination increases resistance to enzymatic degradation and breakdown within the wound.

Prior to lamination, the cross-linked collagen sheets are soaked in PHMB. The subsequent double layering provides a greater surface area for PHMB availability in the product. The collagen matrix is fenestrated to allow for wound drainage through the dressing.

The collagen matrix in PuraPly AM supports healing while the PHMB is an effective barrier against microbial colonization, and has been shown to reduce biofilm formation in preclinical studies.26

**INDICATIONS AND CONTRAINDICATIONS**

PuraPly Antimicrobial is approved for the management of wounds as an effective barrier to resist microbial colonization within the dressing, and reduce microbes penetrating through the dressing. It is indicated for multiple types of wounds:

- Partial and full-thickness wounds
- Pressure ulcers
- Venous ulcers
- Diabetic ulcers
- Chronic vascular ulcers
- Tunnelerd or undermined wounds
- Surgical wounds (e.g., donor sites/grafts, post-Mohs surgery, post-laser surgery, podiatric, wound dehiscence)
- Trauma wounds (e.g., abrasions, lacerations, second-degree burns, skin tears)
- Draining wounds

Contraindications are a known sensitivity to porcine material or PHMB, and use of the product for the treatment of third-degree burns.

**PRECLINICAL STUDIES OF PURAPLY AM**

In a preclinical wound infection model, 36 partial thickness wounds in an animal model were infected with MRSA and covered for 24 hours to promote biofilm formation. Various treatments were then applied to the wounds: PuraPly Antimicrobial, 2-layer nanosilver, 1-layer nanosilver, Acticoat® and Bioclusive. At 72 hours, PuraPly Antimicrobial reduced bacterial counts to a greater extent compared to the silver dressings and Bioclusive film.26 (See Figure 8 on page 12.)

**EXPERT PANEL DISCUSSION AND RECOMMENDATIONS**

There was panel agreement that PuraPly AM offers the advantage of having both a collagen matrix and a highly effective antimicrobial (PHMB) in one product. Panel members discussed that this has the potential for providing a sustained release of microbicide rather than the burst release that may be less effective; however, there is no data on this issue at present. PuraPly AM can therefore be useful in preventing biofilm reformation which, as discussed above, can begin within 24 hours after wound debridement. For patients who are receiving frequent debridements, PuraPly AM can be used as a “bridge” between debridement visits.

There was also discussion regarding the use of PuraPly AM in diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs). The panel recommended PuraPly AM as a first-line therapy to address bioburden and high proteolytic activity in such wounds. If the wound responds adequately, then PuraPly AM should be continued. However, if the DFU or VLU does not adequately shrink in size as expected, despite adequate bioburden control, clinicians should consider advancing therapy to bioengineered living cell-based products (Dermagraft and Apligraf).
PuraPly AM: Case Studies

CASE 1
Pressure ulcer (heel) closed in 10 weeks with PuraPly Antimicrobial.
Before

After

Photographs courtesy of Ryan Fitzgerald, DPM, FACFAS

CASE 2
Before

With autologous skin graft applied

After

Photographs courtesy of Daniel L. Kapp, MD
CASE 3
Failed surgical flap wound treated with PuraPly Antimicrobial.

Before

After

Photographs courtesy of Adam Teichman, DPM, FACFAS

CASE 4
Sacral pressure ulcer closed with PuraPly Antimicrobial.

Before

After (6/15/15)

After (11/12/15)

Photographs courtesy of Marie Gehling, APRN, MSN, NP-C, CWOCN, and John H. Samies, MD, CWS
CASE 5
Diabetic foot ulcer treated with PuraPly Antimicrobial.

Before

After

Photographs courtesy of Shaun Carpenter, MD, FAPWCA, CWSP

CASE 6
Diabetic foot ulcer treated with PuraPly Antimicrobial.

Before

After

Photographs courtesy of Jeffrey Karr, DPM
SUMMARY OF EXPERT PANEL KEY RECOMMENDATIONS

✓ Be highly aware that biofilm affects virtually all wounds and delays wound healing.
✓ Treat all wounds assuming that biofilm is present, even when wounds “look good.”
✓ Focus on controlling bioburden as the first step in successful wound management.
✓ Don’t rely on wound cultures since they are not accurate in diagnosing biofilm.
✓ Debride wounds aggressively but realize debridement alone is insufficient.
✓ Remember that biofilm starts reforming with 24 hours of debridement.
✓ Use topical antimicrobials to prevent biofilm reformation between debridement visits.
✓ PHMB is an excellent topical antimicrobial agent with several advantages—a broad spectrum of activity, no resistance, and high tissue compatibility—that can act as a barrier against biofilm.
✓ PuraPly Antimicrobial provides both PHMB and a natural collagen matrix in one wound care product.
✓ In DFUs and VLUs that fail to adequately progress despite bioburden control, transition to bioengineered living cell therapies.
✓ Do not use collagen products in presence of uncontrolled wound infection.
✓ Do not advance to bioengineered living cell-based products until bioburden is adequately controlled.
✓ Keys to success: Control bioburden and support healing.

10 | Summary

Biofilm is the most common bioburden in wounds and lies at an intermediate stage in the continuum of infection severity from light contamination to sepsis. Biofilm is clinically significant because it has been shown to delay wound healing by causing wounds to become “stuck” in the inflammatory phase of healing.

Virtually all chronic wounds have or are at risk of developing biofilm, which is a thick, sticky mass of pathogens, often polymicrobial, residing in a protective extracellular polymeric matrix (EPM) that protects the microbes from both endogenous (e.g., antibodies) as well as exogenous (e.g., antibiotics) antimicrobial attack. This makes biofilm difficult to eradicate.

Aggressive debridement is critical in biofilm management, but studies show that biofilm starts reforming within 24 hours and mature biofilm can form by three days post-debridement. Therefore, antimicrobial therapy is essential. However, both topical antimicrobial and systemic antibiotics have significant limitations in effectively managing biofilm. Antibiotics may not penetrate the biofilm, and have limited effect as biofilm microbes tend to be inactive and antibiotics require metabolically active targets. Resistance, tolerance, and cytotoxicity to normal tissues also pose challenges with some topical antimicrobial products.

PHMB is a topical antimicrobial with many advantages. It has a wide antimicrobial spectrum, no mechanism by which resistance can emerge, very low cytotoxicity to normal tissues, and excellent tolerability.

Collagen-based dressings have been shown to promote wound healing, but collagen products are not interchangeable. Research has shown that products that retain the native collagen architecture are able to inhibit a wider range of proteolytic enzymes compared to reconstituted collagen. PuraPly Antimicrobial combines PHMB within an intact type I collagen matrix. The collagen matrix supports healing while the PHMB acts as an effective barrier against microbes, and has been shown to reduce biofilm.

PuraPly Antimicrobial has been approved for use in a wide variety of wound types. It is also an appropriate choice for the initial phases of chronic DFU and VLU care to control biofilm in those wounds that may require a bioengineered cell-based therapy to promote wound healing and closure.

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