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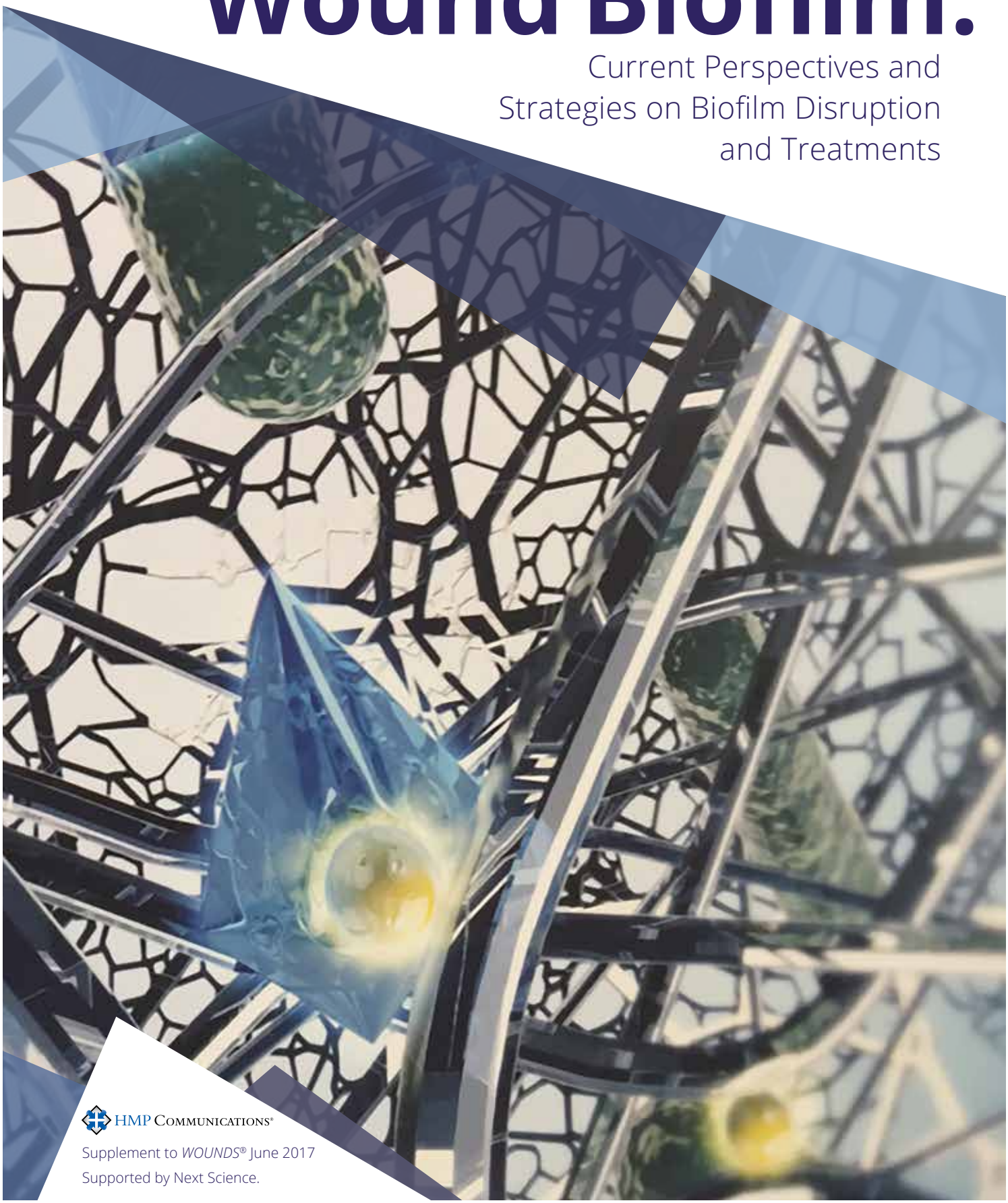
Wound Biofilm:

Current Perspectives and Strategies on Biofilm Disruption and Treatments



HMP Communications®

Supplement to *WOUNDS*® June 2017

Supported by Next Science.

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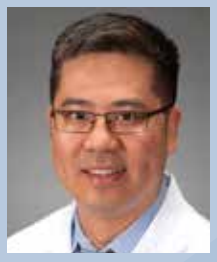

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Disclosure: Dr. Snyder received a consulting honorarium for facilitating the panel discussion. All participants received an honorarium for attending this panel meeting.

Wound Biofilm:

Current Perspectives and Strategies on Biofilm Disruption and Treatments

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Abstract: The presence of biofilm remains a challenging factor that contributes to the delayed healing of many chronic wounds. The major threat of chronic wound biofilms is their substantial protection from host immunities and extreme tolerance to antimicrobial agents. To help guide the development of wound treatment strategies, a panel of experts experienced in clinical and laboratory aspects of biofilm convened to discuss what is understood and not yet understood about biofilms and what is needed to better identify and treat chronic wounds in which biofilm is suspected. This article reviews evidence of the problem of biofilms in chronic wounds, summarizes literature-based and experience-based recommendations from the panel meeting, and identifies future and emerging technologies needed to address the current gaps in knowledge. While currently there is insufficient evidence to provide an accurate comparison of the effectiveness of current therapies/products in reducing or removing biofilm, research has shown that in addition to debridement, appropriate topical antimicrobial application can suppress biofilm reformation. Because the majority of the resistance of bacteria in a biofilm population is expressed by its own secreted matrix of extracellular polymeric substance (EPS), panel members stressed the need for a paradigm shift toward biofilm treatment strategies that disrupt this shield. High-osmolarity surfactant solution technology is emerging as a potential multimodal treatment that has shown promise in EPS disruption and prevention of biofilm formation when used immediately post debridement. Panel members advocated incorporating an EPS-disrupting technology into an antibiofilm treatment approach for all chronic wounds. The activity of this panel is a step toward identifying technology and research needed to improve biofilm management of chronic wounds.

Key words: biofilm, antibiofilm, bacteria, EPS-disrupting technology, chronic wounds, antimicrobial

Wounds 2017;29(6 suppl):S1–S17.

Biofilm is a structured community of microbial cells, enclosed in a polymeric matrix, and adherent to natural or artificial surfaces or to themselves.[1] These dynamic, heterogeneous communities maintain genetic diversity and variable gene expression (phenotype) that create behaviors and defenses that may be used to produce chronic infection.[2,3] They work together to advance their own survival as well as the chronic nature of the infection, surrounded by their own secreted matrix of extracellular polymeric substance (EPS) that provides structural integrity and protects them from external threats.[4] Biofilms are characterized by significant tolerance to antimicrobial agents,[5] disinfectants,[6] and the host's immune defenses.[2,7]

A biofilm can consist of 1 bacterial or fungal species, but more commonly exists as polymicrobial entities, containing diverse species of bacteria, fungi, viruses, and archaea.[8] Only 10% to 20% of a wound biofilm is composed of microorganisms; the other 80% to 90% is EPS.[9] The composition of EPS varies according to location of the biofilm and its microorganisms, but generally it is a heterogeneous mix of polymers

that include proteins, polysaccharides, metal ions, nucleic acids, glycoproteins, and phospholipids.[10,11]

In 1978, Costerton et al[12] found that bacteria have a natural tendency to exist in a biofilm phenotype by virtue of the "glycocalyx" of fibers that surround bacterial cells, encouraging adherence to surfaces and other cells. Since then, biofilm presence has been established in all natural ecosystems except in very harsh environments in the ocean and deep groundwater.[13] The association of biofilms with human health and disease is now universally accepted in tuberculosis,[14] periodontal disease and tooth decay,[15] cystic fibrosis,[16] and otitis media and other upper respiratory infections.[17] In fact, chronic biofilm infections affect every organ system in the human body, including skin.[18] There is growing evidence of biofilm infection in chronic wounds.[18-20] In addition, biofilms have long been known to form on surfaces of medical devices, such as urinary catheters, endotracheal and tympanostomy tubes, orthopaedic and breast implants, contact lenses, intrauterine devices, and sutures.[21,22]

The threat of biofilms is their substantial protection from host immunities and extreme tolerance to antimicrobial agents; the continuing rise in antimicrobial resistance has placed a greater emphasis on correctly diagnosing and managing biofilm-associated infections in nonhealing, chronic wounds. In a seminal 2008 in vitro study of 50 chronic human wound specimens obtained from 4 different wound types (diabetic foot ulcers [DFUs], pressure ulcers [PUs], venous leg ulcers [VLUs], and other chronic wounds) and 16 acute wound samples (blisters, skin tears, and other acute wounds),[18] biofilm was observed via microscopic analysis in 30 out of 50 (60%) of the chronic wounds and 1 out of 16 (6.2%) of the acute wounds. In another analysis of biopsy specimens obtained from nonhealing VLUs,[23] in vitro examination via transmission electron microscopy confirmed biofilm in all 45 (100%) specimens. From published studies, it has not been possible to determine whether biofilms are more prevalent in 1 particular chronic wound type due to small sample sizes.[24]

The fact that biofilms have been found to exist in the majority of nonhealing, chronic wounds sampled and rarely in acute wound specimens has led to the assumption that biofilms may contribute to wound healing delays and add to the complexity of wound treatment. However, the challenge in addressing biofilm in chronic wounds has been translating knowledge from the laboratory setting into clinical practice. The presence of biofilm is currently confirmed via methods of microscopic analysis, appearing as large aggregates of cells and/or a dense extracellular matrix closely associated with bacterial cells. Routine culturing techniques cannot identify the presence of biofilm. In addition, there are no specific clinical signs that clearly point to biofilm involvement in an infection. Neither is there a definitive quantity threshold or specific type of biofilm that definitively points to biofilm as the primary cause of stalled wound healing.

The most likely cause for injury and resulting inflammation in any chronic wound is repetition or resumption of the wound's original cause or patient comorbidities that delay healing. Good chronic wound care is patient- and wound-centered, holistic, multidisciplinary, and evidence-based.[25,26] When these principles are applied, chronic wounds can heal despite the presence of biofilm. Yet, there are many chronic wounds that persist despite good wound care. Thus, the extent to which biofilms impact wound healing is an area of controversy and ongoing research. Can an enhanced focus on antibiofilm strategies speed chronic wound healing, and to what extent should antibiofilm strategies be considered part of good wound care? Scientific research has shed light on the nature and ubiquity of biofilms in chronic wounds, yet many questions remain unanswered. A prospective, randomized controlled clinical trial is not yet available to support biofilm-guided care decisions; biofilm management care decisions are based on best available evidence and personal experience.

To help guide the development of wound treatment strategies, a panel meeting of wound healing specialists was organized to discuss what is understood and not yet understood about biofilms, and what is needed to better identify and treat chronic wounds in which biofilm is suspected. The purposes of this article are to review evidence of the problem of biofilms in chronic wounds, to summarize literature-based and experience-based recommendations from the panel meeting, and to identify future and emerging technologies needed to address the current gaps in knowledge.

Methods

A panel of experts experienced in clinical and/or laboratory aspects of biofilm convened on November 19, 2016 in Jacksonville, FL, to discuss the current state of practice in treating and identifying biofilm in chronic wounds. Panel members received an emailed selection of peer-reviewed studies[27-32] selected by the moderator (R.S.) to review prior to the meeting. Studies were selected via an online literature search to include recent, relevant studies on various aspects of biofilm identification and treatment. The meeting was moderated by 1 of the panel members, and notes from the meeting were recorded during the meeting by a medical writer and sponsor representative.

The meeting was divided into several topics determined by the moderator in advance. Discussion topics included the extent of the problem of biofilm, "good" versus "bad" biofilms, scientific evidence to support the existence of biofilm, culturing and diagnostic approaches, economic implications of biofilm in DFUs, challenges of general surgeons in treating biofilm, and current effective biofilm-disrupt-

ing treatment strategies/technologies. Each panelist was assigned to guide a roundtable discussion regarding each topic with respect to current evidence and clinical experience. Following the meeting, information presented and discussed was grouped into categories of "what we know," "what we don't know," and "what we need to be successful in treating biofilm." Follow-up email communication with panelists continued throughout the development of this manuscript. All subject matter contained in this publication was approved by all panel members.

Results

What We Know About Biofilm in Chronic Wounds:

Biofilms exist and are prevalent in chronic wounds. All panel members supported the concept that biofilms exist in chronic wounds and that most chronic wounds contain biofilm. The ability to identify the existence of biofilm in chronic wounds has been driven largely by advancements in molecular microbiology, microscopy technology, and techniques for the study of bacterial populations in situ.[32] While the majority of evidence regarding the ability of wound isolates to grow as biofilms is based on experimental in vitro models and in vivo animal data,[33,34] several human wound studies also demonstrate that chronic nonhealing wound samples harbor biofilm.[24,34,35] Biofilm has been found across all related etiologies, including VLUs,[36] PUs,[37,38] and DFUs.[18]

In a recent meta-analysis of 9 human studies (185 chronic wounds) detailing the presence of biofilm and bacteria in general through microscopy, Malone et al[24] determined the prevalence of biofilms in chronic wounds was 78.2% (confidence interval, 61.6–89, $P < .002$). Biofilm prevalence across studies, identified by the percentage of positive biofilm samples, was no lower than 60% in 3 studies, and equal to 100% in all remaining studies. The authors concluded that the results of the meta-analysis supported clinical assumptions that biofilms are ubiquitous in human, nonhealing, chronic wounds.[24] In contrast, biofilm has been found to be present in only 6% of acute wounds.[18]

Biofilms, in addition to other factors, are a barrier to wound healing. During the meeting, there was an indepth discussion on whether biofilms delay wound healing. Panel members concluded that biofilms delay wound healing at some level, but this is based largely on experience and mounting coincidental data versus controlled cause-and-effect research which is lacking. The bulk of evidence supporting the concept that biofilm complicates the healing process of chronic wounds is from the in vitro model and in vivo animal data.[24] For example, the first specific evidence on the effect of bacterial biofilms on cutaneous wound healing occurred in a murine cutaneous wound system that directly demonstrated delayed reepithelialization caused by the presence of staphylococcal biofilms.[39] In vitro and in vivo animal data do not necessarily translate to the clinical setting, and the extent to which biofilm stalls healing was a subject of debate among the panel members; this controversy is evident in practice as well as in ongoing research and will be covered in greater detail later in this article.

It is known that if biofilm formation is prevented, in every one of the medical conditions known to harbor biofilm (ie, chronic sinusitis, burn infection, catheter infection, pulmonary infection in cystic fibrosis patients, ventilator-associated pneumonia, and urinary stent infection), the condition disappears.[40] Panel members affirmed that in their experience, when all barriers to wound healing were addressed and the wound remained recalcitrant, applying antibiofilm therapies generally improved healing of the wound. However, in order to achieve good outcomes during use of and without use of antibiofilm wound treatment strategies, panel members emphasized that it is critical to simultaneously employ a multidisciplinary approach that involves established principles of holistic wound care and good wound bed preparation including offloading. Microorganisms rarely invade healthy tissue unless it is compromised by drying out,[41] for example, and this is true for acute and chronic wounds. Antibiofilm treatment cannot substitute for adequate patient and wound optimization, including adherence to the TIME framework (tissue, infection/inflammation, moisture balance, and edge of wound) in chronic wound care.[25,26] Identifying and addressing all cause(s) of tissue injury is a vital first step toward healing any chronic wound that displays signs of inflammation or unexplained healing delay.

Routine culture is not an effective means of identifying biofilm bacteria. There was unanimous agreement among panel members that a routine clinical wound culture is an ineffective method of analyzing biofilm populations in chronic wounds and is therefore not recommended. The recommendation is based on the experiences of the panel members as well as general knowledge that the success of conventional bacterial wound culture methods is based on assessing free-floating populations of a single species during its logarithmic growth phase.[32] Bacteria in their planktonic versus biofilm states differ significantly in their morphology, mode of communication, and metabolism.

Conventional culturing methods lack sensitivity for identifying bacteria within their complex polymicrobial communities of immobile organisms embedded in an EPS matrix, and studies have consistently demonstrated failure of culture methods in detecting the types of organisms present in wound biofilms.[42-45] Deoxyribonucleic acid (DNA)-based technologies, or molecular methods, are capable of identifying and quantifying a wide range of microorganisms and have been shown to be better suited than traditional cultures for evaluating the microbial biofilm community.[46] In a

comparative study[44] of culturing versus molecular identification of bacteria in 168 chronic wounds, 17 different bacterial groups were identified with culture, whereas 338 different bacterial groups were identified with molecular testing. While most bacteria identified with culture testing were also identified with molecular testing, the majority of bacteria identified with molecular testing were not identified with culture testing. A separate study[45] showed standard bacteriological cultures identified an average of 3 common bacterial species in wound cultures, in contrast to high-throughput pyrosequencing, which identified an average of 17 genera in each wound. Implementation of personalized topical therapeutics guided by molecular diagnosis may result in statistically and clinically significant improvements in outcome.[28,47]

Dowd et al[48] used deep sequencing molecular methods (pyrosequencing) in an in vitro model to identify major populations of bacteria present in the wound fluid samples of 3 different wound types — DFUs, VLUs, and PUs — and found that there are specific major populations of bacteria in all chronic wound types, including *Staphylococcus* and *Pseudomonas,* as well as markedly different bacteria populations in each of the 3 different wound types. In a larger study using 16S ribosomal DNA (rDNA) pyrosequencing in an in vitro model to analyze the makeup of the bacterial communities present in samples obtained from patients with chronic DFUs (N = 910), VLUs (N = 916), PUs (N = 767), and nonhealing surgical wounds (N = 370), Wolcott et al[32] reported wound samples contained a high proportion of *Staphylococcus* and *Pseudomonas* species in 63% and 25% of all wounds, respectively. However, a high prevalence of anaerobic bacteria and bacteria traditionally considered commensal was also observed. Results suggested neither patient demographics nor wound type influenced the bacterial composition of the chronic wound environment and empiric antibiotic selection need not be based on or altered for wound type.[32]

Surgical or conservative sharp wound debridement is effective in removing biofilm from an open wound surface. There was strong agreement among panel members that surgical or conservative sharp wound debridement and physical removal/disruption of biofilms are critical to promote healing in wounds in which biofilm is suspected. The importance of debridement is well established in national and international guidelines[27,49]; although the exact impact of debridement is unclear, definitive research has shown physical removal/debridement of wound biofilm reduces biofilm burden.[47] Panel members acknowledged that while debridement is one of the most important treatment strategies against biofilm, it does not remove all biofilm or prevent biofilm regrowth, partly because biofilm typically spreads perivascularly below the surface of the wound.[50] Sharp debridement has been shown to reduce microbial numbers by 1 to 2 logs,[51] highlighting the need for additional topical treatment to suppress regrowth.

In addition to the physical removal of biofilm, clinical, animal, and in vitro models have demonstrated that debridement opens a time-dependent window during which applied topical treatments can suppress biofilm reformation.[52] Serial debridement is recommended to continually remove mature biofilm, immediately followed by multimodal biofilm wound management strategies.[47,52] Immediately following debridement, while biofilm microbes are disorganized and insufficiently protected by the disrupted matrix, they are forced to become metabolically active to reconstitute the matrix and thus more susceptible to antiseptics, biocides, and antibiotics. In a study using 4 different in vitro and ex vivo models,[52] all models demonstrated that at least within the first 24 hours after sharp debridement, the biofilm community was more susceptible to selective topical antibiotics, and after maturing for up to 48 hours became increasingly tolerant. Original tolerance levels were reached by 72 hours.[52] Topical dressings and lavage or therapeutic irrigation are among the recommended strategies immediately post debridement to suppress regrowth of the biofilm or to further reduce microbials through killing microbial cells.[34,52,53]

Use of ultrasound debridement received mention during the panel meeting as an employed method of removing mature biofilms. In vitro data using semisolid agar or a relevant pigskin explant model has demonstrated the effectiveness of ultrasound debridement in reducing mature biofilms.[54,55] Simultaneous use of several modalities (eg, ultrasonic wound debridement together with conservative sharp wound debridement using a scalpel or loop curette) may improve success,[56] but data on combination debridement techniques is limited.

Biofilms have a natural ability to rebuild rapidly. There was consensus among panel members that a major challenge in treating biofilms is their natural ability and strength to rapidly rebuild after sharp debridement and biofilm removal. Biofilm may reform in a wound by the growth of fragments left behind following debridement or cleansing, the spread of planktonic bacteria released from the remaining biofilm, and the growth of biofilm by newly introduced microorganisms.[57] In vivo, the regrowth of mature biofilms can occur within 72 hours, but early presence of biofilms can be detected within 24 hours post debridement.[52]

Systemic antibiotics are of limited use in managing biofilm. Panel members maintained that the planktonic concept of a single antibiotic or single biocide to eradicate the microbial pathogen is not valid for chronic infections. There is no strong evidence to support the use of empiric or traditional, culture-guided systemic antimicrobial agents to prevent or treat biofilm infections in the treatment of wound-associated infec-

tions.[28,58,59] For more than a decade, systemic antibiotics have been known to have limited use in treating biofilms due to various protective mechanisms that include: 1) restricted penetration by the EPS; 2) nutrient limitation and the dormant state of bacteria in the biofilm, which creates little or no activity for antibiotics to disrupt; 3) adaptive responses (resistance); and 4) formation of persister cells.[5]

Evidence in at least one in vitro study[30] has shown oxygen limitation inside the biofilm likely plays a role in the tolerance of *Pseudomonas aeruginosa* biofilm to ciprofloxacin and tobramycin. While tobramycin and ciprofloxacin penetrated biofilms of *P aeruginosa*, they failed to kill the bacteria. Phillips et al[30] suggested this reduced antibiotic susceptibility is likely due to oxygen depletion within the biofilm, which restricts bacterial metabolic activity to a narrow zone adjacent to the air interface.[60] Further, facultative and obligate anaerobic bacteria and bacterial strains, such as *Staphylococcus aureus*, *Streptococcus pneumonia*, and enterococci strains, which live or grow without the presence of oxygen, have shown ever-increasing phenotypic resistance to a variety of antibacterial treatments.[61]

Improved results have been reported with systemic antibiotics that have been personalized based on molecular-guided diagnostics in identifying biofilm. A retrospective cohort study[28] was performed to compare the wound healing outcomes of 3 large cohorts that received different solutions to manage wound bioburden: 1) standard of care (SOC) patients who were prescribed systemic antibiotics on the basis of empiric and traditional culture-based methodologies, 2) group 2 patients who were prescribed an improved selection of systemic antibiotics based on the results of molecular diagnostics, and 3) group 3 patients who received personalized topical therapeutics (including antibiotics) based on molecular diagnostics identification. Patients in all cohorts were otherwise subject to the same biofilm-based wound care protocol. Results showed that in the SOC group, 48.5% of patients (244/503) healed completely during the 7-month study period. This increased to 62.4% (298/479 [sic[28]]) in treatment group 2 and 90.4% (358/396) in treatment group 3. Cox proportional hazards analysis revealed the time to complete closure decreased by 26% in treatment group 2 ($P < .001$) and 45.9% in treatment group 3 ($P < .001$) compared with SOC.[28]

Importantly, antibiotics should be used only after ensuring the diagnosis is correct and all of the patient's risk factors for tissue breakdown and delayed healing have been addressed. A wound's bacterial bioburden is typically not a sole cause of tissue breakdown, and prophylactic antibiotic use without confirmed infection has been associated with delayed healing of all etiologies of leg ulcers.[62] Rigorously applied, basic, good clinical practice is a powerful tool to use before exposing patients to the risk of developing antibiotic-resistant organisms. For example, heavy microbial burdens have been shown to decline as venous ulcers heal when managed solely with moist wound healing and sustained graduated compression.[63]

Appropriate topical antimicrobial application can suppress biofilm reformation. In addition to following established principles of patient and wound bed preparation, the addition of appropriate topical antimicrobials immediately following sharp debridement can positively affect wound healing in which biofilm is suspected. Because of the rapidity with which biofilm reforms, quickly identifying the type and susceptibility of bacteria involved using rapid polymerase chain reaction (PCR) allows directed strategies such as application of specific topical antibiotics and biocides to increase the effectiveness of the debridement.[64,65]

Application of antimicrobials is time-dependent (within 24–48 hours). Wolcott et al[47] determined that debriding the wound every 7 days assisted in wound healing for the first 3 days (43% of the week), while adding appropriate topical biocides and personalized systemic antibiotics (based on results of molecular diagnostics) had a lasting effect in wound healing for 6 days, or approximately 86% of the week.

What We Do Not Know About Biofilm in Chronic Wounds:

It is not possible to determine which biofilms protect and which are virulent. The concept of when a wound biofilm could have a helpful or neutral effect was discussed during the panel meeting. The lack of definitive published research on this concept has resulted in extrapolations and integration of data from multiple fields and is far from conclusive. There are numerous examples of biofilms that are "good" for health; these commensal (normal) bacteria are present in humans in vast numbers. Commensal bacteria produce biofilm communities that help the "good" bacteria compete more effectively with other bacteria that could produce an "opportunistic" infection. Examples include *Lactobacillus* in the vagina, *S epidermidis* on the intact epithelium of skin, and several species in the lower intestine and colon. These organisms protect people from pathogens and toxins, help boost immune defenses, digest cellulose and salvage energy, and synthesize vitamin K.[66,67] An imbalance of bacteria in the gut — especially from antibiotic use, stress, or lack of dietary fiber — increases the risk of disease.

However, these nonpathogenic commensal biofilms can revert to pathogenic or virulent biofilms under stress.[9] In fact, when these beneficial commensal bacteria penetrate the epithelial cell layer of their respective tissues, they always produce destructive infection.[68] None of these normal, beneficial commensal bacteria is actually inside the epithelial cell layer that serves as a barrier to bacterial penetration. What we do not know is how much biofilm can exist in a wound before it becomes a barrier to healing. Do we want to get rid of all the biofilm?

In the SIDESTEP study, Lipsky et al[69] found that many methicillin-resistant *S aureus* (MRSA)-positive patients displayed positive responses to antibiotic treatments that were insufficient for this organism. Patients' chronic wounds colonized by *Pseudomonas* also healed when treated with ertapenem similar to wounds treated with antipseudomonal therapy. From this and other studies, authors have concluded that certain bacteria can colonize wounds without impairing wound healing. However, these results are based primarily on studies that were performed using culture-based approaches, which are inadequate for assessing polymicrobial samples.[32] Investigators have suggested it is not the biofilm as such that represents the greatest obstacle in healing a chronic wound, but rather its virulence and pathogenicity.[9] Numerous factors, including the composition of the biofilm, its physiochemical properties, the native microbiota and their virulence/pathogenicity, microbial numbers, the host's pathophysiology, and immunological fitness, control the effect of a pathogenic biofilm in a wound and its resistance to interventions. Owing to these variables, there is still question as to why some biofilm-infected wounds heal whereas others do not.

The exact mechanisms by which biofilms can delay wound healing are unknown. Biofilms share a common pattern of development that includes attachment, microcolony formation, maturation, and dispersion. While the initial attachment is reversible, attachment becomes stronger as microbe cells begin to multiply and differentiate, changing their gene expression patterns in ways that promote survival.[10,22] This is usually the result of a bacterial communication process called *quorum sensing* that enables bacteria to control and react to changes in cell population density.[70] Once firmly attached, the microbes begin to secrete a surrounding EPS, resulting in the formation of microcolonies. Fully mature biofilms continuously shed these microcolonies as well as planktonic bacteria and biofilm fragments, which are then able to spread and attach to other parts of the wound bed or to other wounds, forming new biofilm colonies.[21,22]

However, the exact mechanisms by which biofilms can delay wound healing remain the subject of ongoing research. Panel members emphasized that virtually all evidence comes from in vitro or animal model data, which does not necessarily translate to the clinical setting. Several mechanisms have been proposed. At least some biofilms are thought to delay wound healing by producing sustained hyperinflammation, feeding on plasma exudate, and damaging host tissues.[36,71-74] Controlled animal model studies have suggested the presence of biofilm in wounds delays healing by interfering with granulation tissue formation, epithelialization,[39,75,76] and host defenses.[77,78]

Recent research[79] involving oxygen microsensors and transcriptomics has suggested that bacterial biofilm and responding leukocytes consume oxygen in chronic wounds, which may impede wound healing by depleting oxygen required for healing. Anaerobes that flourish in this oxygen-depleted state are increasingly pathogenic and resistant to antibacterial treatments.[61] In addition, because the biofilm matrix protects enclosed bacteria from systemically administered antimicrobials, antibodies, complement, and phagocytosis,[80,81] the typical host immune response (eg, neutrophils and macrophages and their products, matrix metalloproteinases, neutrophil elastase, and reactive oxygen species [ROS]) appears to be less effective against biofilms compared with planktonic bacteria.[82,83] Large clinical studies are needed to confirm the mechanisms by which biofilms delay wound healing to inform product development and treatment.

The relative effect of biofilm presence on stalled wound healing is unknown. Panel members emphasized the need for more high-level science to determine the functional biofilm effect on wounds, which is currently unknown. Wound healing is a complex, multifactorial process, and there are a myriad of reasons wounds stall, including biofilm. Elevated proteases, ROS, and exotoxins all cause chronic wounds. These inflammatory factors could be caused by the host immune system, planktonic bacteria, biofilm, repeated physical injury, nutrition, and ischemia (**Figure 1**).[84,85] In addition, composition and virulence of biofilms differ, as do pathophysiological conditions of the host — all of which vary the effect of the biofilm on wound healing.

Likewise, the complex etiology as well as lack of robust data to quantify the level of biofilm bacteria in DFUs makes it impossible to know the relative impact of biofilm on DFU healing. Currently available evidence is anecdotal.[18,86,87] Particularly because it involves a weight-bearing structure, each DFU is complex, with many factors at play. Inflammation in a DFU can have many causes other than biofilm, including weight-bearing, repetition of injury, a lower prealbumin level, and an impaired host who may not be mounting a physiological response. Perfusion and biomechanics need to be prioritized, with consideration of total contact casting in appropriate cases.

However, due to the risk of limb loss, pursuing an antibiofilm strategy in treating DFUs has particular relevance. A heightened awareness of biofilm presence in DFUs is needed because of the potential for amputation if untreated. Employing biofilm-based wound management strategies in treating DFUs may also save health care system costs. A retrospective analysis[87] reported a reduction in total charges of 68% for patients with DFUs that were treated with biofilm-based wound management guided by molecular diagnostics, personalized gels, and commercially available topical antibiotics versus conventional wound care. More research needs to be performed to determine true cost savings.

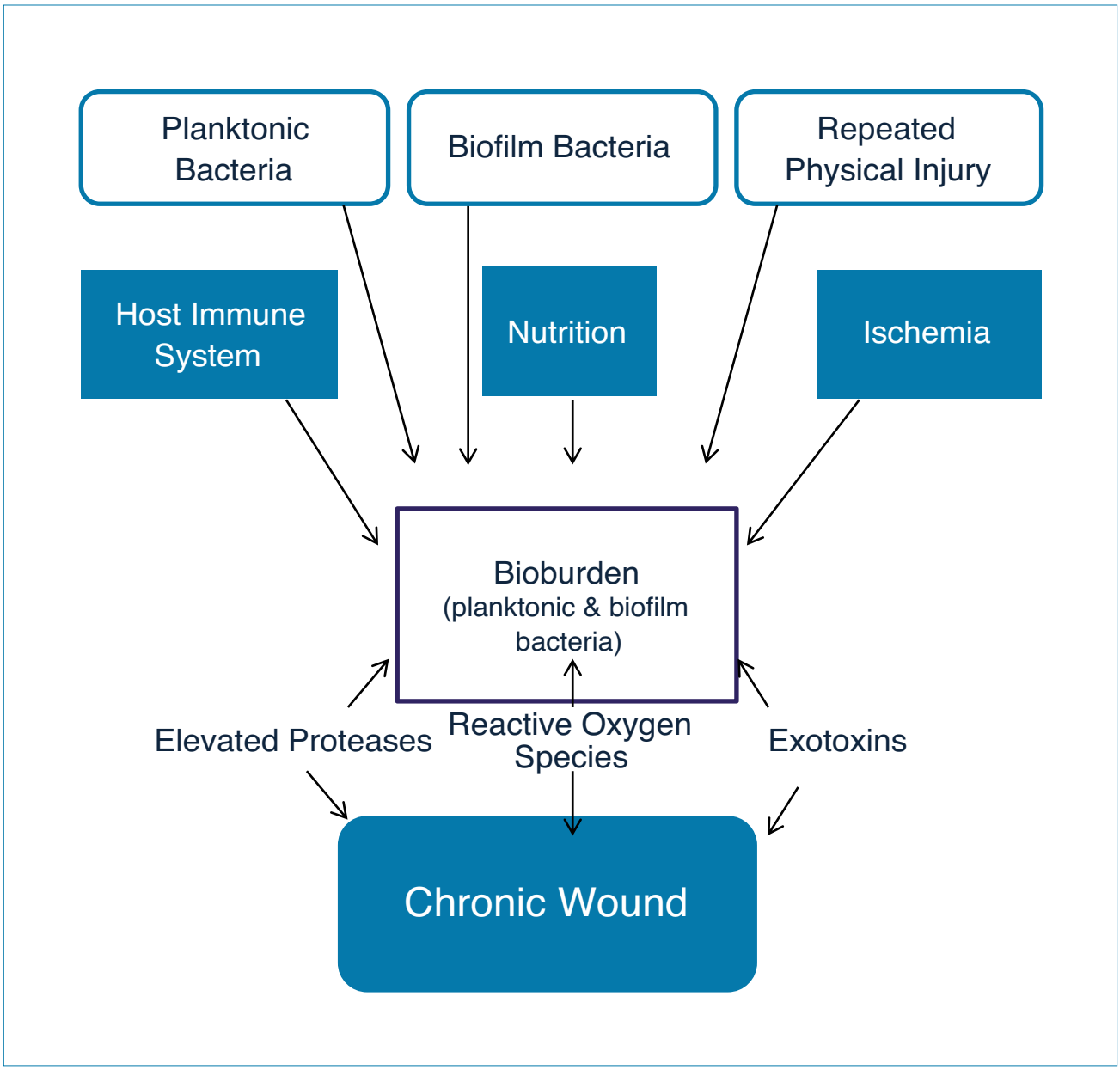

Figure 1. Factors that contribute to the existence of a chronic wound.

Similarly, antibiofilm strategies should be aggressively pursued in surgical wounds that contain sutures and implanted devices because of the high risk and cost of surgical wound complications caused by infection[88] such as dehiscence and removal of infected implanted devices. These risks highlight the importance of a multifaceted approach in treating wounds in which biofilm is suspected. Even after more than 30 years of biofilm research, there are still no definitive, classic, nondestructive, and noninvasive clinical indicators that can positively reveal the presence of a wound biofilm, particularly a virulent pathogenic biofilm.[9]

Panel members recommended that when a biofilm is suspected in a nonhealing wound, clinicians should initially focus on aggressive debridement and broad-spectrum antibiofilm management strategies to combat these multicellular organisms before biofilm is confirmed via molecular methods. A "step-down/step-up" approach has recently been proposed as the current best antibiofilm treatment strategy (**Figure 2**),[89] and it was advocated by panel members. The principle of this strategy is to aggressively initiate multiple broad-spectrum therapies first to rapidly and effectively reduce wound biofilm levels and reduce inflammation, ROS, and protease levels. Once the wound transitions out of the inflamma-

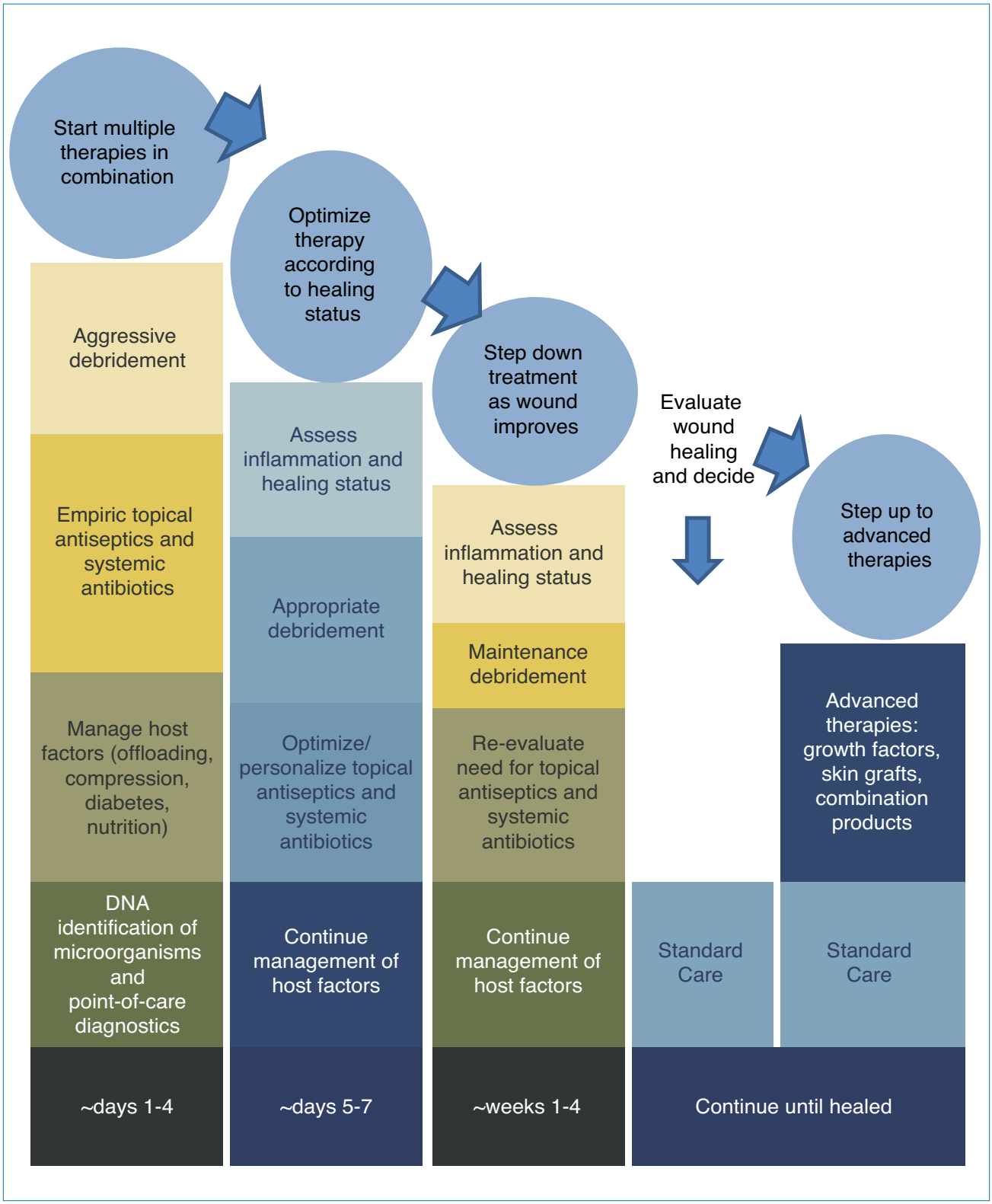

Figure 2. Outline of the "step-down/step-up" approach to biofilm-based wound care. (Adapted from Schultz et al[83]).

tory stage, therapy would be gradually stepped down to include personalized topical antiseptics, advanced wound care therapies, debridement, and continued management of host factors. Goals of the "step-down/step-up" approach are to speed wound healing, lower overall cost, and reduce the risk of amputation. Ultimately, to best serve clinical practice, it is vital to understand whether biofilms play a causal or an associative role in delaying healing and whether biofilm-guided decisions are as effective, reliable, valid, and accurate as those guided by well-established signs and symptoms of infection. Testing this hypothesis requires a well-designed, randomized controlled study.

Molecular analyses cannot yet differentiate between planktonic and biofilm bacteria. Panel members recommended molecular analysis over cultures in identifying and quantifying biofilm bacteria in wounds. Rapid PCR was the favored diagnostic approach, as supported by the literature.[44] Even so, it is not yet possible with this method to absolutely determine the type of bacteria within a wound bed. An array of different biofilms can exist throughout a wound environment, including on the wound surface, as collective cells dispersed within the wound exudate, in slough or on necrotic tissue, on the wound dressing, or on anything that falls into the wound. In a wound bed with both abiotic and biotic biofilms, as well as nonpathogenic and pathogenic biofilms, it is difficult to determine the presence of biofilm.

Nevertheless, studies have shown significantly better accuracy in detecting diverse polymicrobial communities and the presence of bacteria, including strictly anaerobic bacteria, with molecular analyses versus standard culture techniques.[18,44] Culture-free 16S rDNA sequencing, an advanced clinical molecular microbiological method increasingly employed to investigate the microbiota of chronic infections, can quickly quantify bacteria at a species level in a wound fluid sample.[44] However, a major limitation to the observations made by 16S rDNA and ribosomal ribonucleic acid (rRNA) analyses is that molecular techniques based on the amplification of DNA do not differentiate between live, dead, and dormant community members.[46] Current 16S rDNA and rRNA analyses provide a low-resolution snapshot of microbial life living on surfaces and cannot determine whether bacteria are in a planktonic or a biofilm state.

In fact, no current advanced method of molecular identification/detection can discriminate between planktonic and biofilm-growing bacteria or identify organisms responsible for delayed healing. Although panel members acknowledged most bacteria exist in a biofilm state and molecular tests can be used to identify the major types and quantities of bacteria present in chronic wound fluid, they considered it a "leap" to declare all molecular-identified bacteria as biofilm. Therefore, even clinical studies using modern sequencing technologies to identify bacteria lack robust evidence that the identified bacteria exist as a biofilm. Until it is possible to determine actual levels of biofilm bacteria in a wound, it is not possible to accurately compare effectiveness of different treatment strategies on biofilm reduction.

Results from in vitro and animal testing do not often translate to clinical practice. Practical considerations and host factors not yet understood affect the extent to which in vitro and animal tests of antibiofilm agents translate to clinical practice. The vast majority of biofilm testing has been in vitro or animal testing. In biologically diverse environments, factors such as chloride ions, proteins, phosphates, and lipids in particular are known to affect antimicrobial efficacy.[90] The in vivo wound environment contains sera, blood, and tissue fluid, which can all affect the bioavailability of any agent applied to the wound bed.

The delivery material or platform is also important to ensure sustainability and efficacy of the antimicrobial. As an example, the extent of silver activity will vary in different in vitro and in vivo environments. It has been reported that even at low concentrations (5 μg/mL^{-1}) ionic silver (Ag+) is highly efficacious on microorganisms in vitro.[91,92] In addition, silver has been shown to be effective in reducing biofilms in and on medical devices.[93] While silver has been shown to have antibiofilm efficacy in liquids,[94] as with all antimicrobials, at low levels it also has a reduced efficacy on biofilms.[95] Bjarnsholt et al[96] evaluated the efficacy of silver on *P aeruginosa* biofilms and found the concentration of silver in currently available wound dressings was much too low for treatment of chronic biofilm wounds. Panel members concluded that considerably more in vivo research is required to determine the extent in which in vitro testing translates to clinical practice.[96]

Evidence is insufficient to compare effectiveness of current therapies/products in reducing or removing biofilm. Antimicrobial agents, which include topical disinfectants, antiseptics, and antibiotics, are used extensively in antibiofilm treatment. However, very few in vitro or in vivo comparative studies have been performed with the scientific rigor required to determine efficacy of any 1 commercially available topical antimicrobial agent commonly used to treat biofilm, such as iodine, silver, silver sulfadiazine, polyhexamethylene biguanide (PHMB), sodium hypochlorite, methylene blue and gentian violet, or mupirocin. The methodologies of these few studies differ widely, making it impossible to perform a systematic review of therapy results across studies. Panel members acknowledged that because of this lack of comparative data, they rely on weak evidence and their own experience when choosing commercialized antimicrobial dressings as an antibiofilm strategy. Adding to the complexity is that all biofilms differ, as do host factors.

As an example of differing endpoints and comparators, a comparative in vitro test of biocompatibility (measurement of activity in relation to its cytotoxicity)

by Müller and Kramer[97] demonstrated the superiority of PHMB compared to chlorhexidine, povidone-iodine, triclosan, silver, and sulfadiazine. In a clinical study evaluating a PHMB-containing biocellulose dressing, Lenselink and Andriessen[98] showed a significantly reduced mean wound area and increased granulation tissue coverage over 24 weeks in wounds where biofilm was suspected, but biofilm presence and/or type of organisms was not identified or measured in any of these patients. In a different systematic in vitro comparison of antimicrobial wound dressings, 5 strains of *Acinetobacter baumannii*, *P aeruginosa*, and MRSA were tested against 4 antimicrobial wound dressings containing silver, honey, or PHMB using both a planktonic and immobilized cell model.[99] Across all species and models used, the nanocrystalline silver-coated dressing exhibited the best antimicrobial activity, being at least as good as all the other dressings.[99]

The most compelling evidence, although weak, appears to favor effectiveness of cadexomer iodine in treating biofilm. Phillips et al[30] recently reported that 100% cadexomer iodine has superior efficacy compared with diverse dressings including time-released silver, PHMB gel, calcium alginate with silver, and povidone iodine against *P aeruginosa* biofilms in an ex vivo model. In an in vivo mouse model and in vitro study using confocal laser scanning microscopy, Akiyama et al[100] suggested cadexomer iodine soaks up *S aureus* cells encircled by glycocalyx, directly destroys biofilm structures, collapses glycocalyx during dehydration, and can subsequently kill *S aureus* cells within biofilm.

Superior in vitro efficacy of cadexomer iodine versus silver-based dressings was further demonstrated against MRSA using multiple biofilm models with log reduction. In an additional mouse model, cadexomer iodine had a significantly greater impact on MRSA biofilm in mouse wounds than silver dressings or mupirocin-based dressings on gram-stained histology sections and quantitative microbiology from biopsy samples (> 4 log reduction in CFU/g versus 0.7–1.6, $P < .0001$).[101] Lastly, in a study designed to compare the antimicrobial effectiveness of silver- and iodine-containing wound dressings against preformed mature *P aeruginosa* and *S aureus* biofilms of pathogenic wound bacteria grown in vitro,[102] both test dressings displayed an antimicrobial effect against the target species biofilms, although the iodine dressing was more efficacious under the set experimental conditions.

Cadexomer iodine has been suggested to provide sufficient iodine for biofilm suppression without causing significant damage to the host,[100] but controversies remain regarding potential cytotoxicity and systemic absorption with prolonged use.[103,104] In an in vitro 3-dimensional fibroblast-populated collagen gel model, a matrix component native to the wound environment, all iodine products tested, including cadexomer iodine, were shown to be toxic to fibroblasts beyond 24 hours of application.[104] Conclusions from this study are in line with the US Food and Drug Administration guidelines, which advise short-term use of iodine-based antiseptics.

EPS-disrupting technology is emerging. High-osmolarity surfactant solution technology is emerging as a potential multimodal treatment that has shown promise in EPS disruption and prevention of biofilm formation when used immediately post debridement. This technology is composed of a surfactant (benzalkonium chloride) and a high osmolarity citrate buffer at 4 pH in a poly(ethylene) glycol hydrogel.[29] Osmolarity refers to the concentration of a solution expressed as the total number of solute particles per liter. Antimicrobial activity of the high-osmolarity solution (HOS) is focused primarily on degrading the biofilm matrix and then lysing the bacteria within it.[31]

With help of the surfactant, the highly concentrated acid component within the gel breaks down biofilm EPS by removing ionic metal bonds (x-links) between EPS polymers and allowing for penetration. This allows bacteria to be down-regulated. The solution has high osmolarity, which buffers the product to remain effective despite depletion. Its pH is favorable to biofilm disruption throughout multiple biofilm microenvironments. Persister cells are also exposed to treatment when biofilm is removed; the gel remains present and prevents EPS-bond formation so persisters cannot regrow biofilm. Adequate, continuous antimicrobial efficacy has been reported to be maintained for up to 5 days,[29] and an HOS gel has shown synergy with topical antibiotics.[31]

Preliminary research regarding HOS gel is scant but promising. In vitro quantitative analysis using strains isolated from wounds showed HOS gel reduced the viability of 5 different wound pathogens — *S aureus*, *S epidermidis*, *P aeruginosa*, *A baumannii*, and *Klebsiella pneumonia* — by 6 to 8 $\log^{10}$ CFU/disc.[29] In vivo, the gel prevented biofilm formation for 72 hours when applied at the time of wounding and infection, and eliminated biofilm infection when applied 24 hours post wounding and infection.[29] In a clinical study[31] that compared wound volume reduction over 4 weeks with SOC biofilm-based wound care treatment versus HOS gel versus SOC + HOS gel, the wound volume reduction was 47%, 62%, and 72% ($P < .05$) for SOC, HOS gel, and SOC + HOS gel, respectively, and the percentage of wounds healed during the 4 weeks was 53%, 80%, and 93% ($P < .05$). Wolcott[31] concluded that the study demonstrated the value of multiple, simultaneous strategies in managing chronic wounds. The **Table**[29-31,100,103-118] displays the comparative mechanisms of action of HOS gel and other commonly used topical antimicrobials in disrupting biofilm formation.

Discussion

Identification and Treatment Strategies Needed:

Develop a test model that identifies planktonic cells versus pathogenic biofilm.

Table. Comparative mechanisms of action of topical antimicrobials in disrupting biofilm formation

			Mechanisms Addressed			
Type/ Compound Base	**Trade Name Example[a]**	**Actives**	**Physical and Chemical Disruption of Biofilm Supportive Structures (Matrix)**	**Application of High-continuous Concentrations of Cidal Strategies to Individual Microbial Cells**	**Preservation of Host Healing Cells**	**Bacterial Resistance**
Quaternary ammonia (detergents)	MicroKlenz Antimicrobial Solution	Benzethonium chloride	No	Yes	Surfactant at low concentrations does not harm host tissue.	Development of microbial resistance is well-documented.[105]
Polyhexamethylene/betaine/biguanide	Prontosan	Undecylenamidopropyl betaine, polyaminopropyl biguanide	Polyhexamethylene biguanide (PHMB) solutions may block microbial attachment to surfaces; 0.02% PHMB solution has effectively removed an artificial *Pseudomonas aeruginosa* biofilm in vitro.[106]	Yes	Low concentrations of betaines and biguanides may not harm host tissue, depending upon chemical structure.[105,107] PHMB-containing dressings have shown high toxicity in vitro for various tested cell lines[107]; however, low toxicity with PHMB solutions has also been reported. Cytotoxicity influenced by additives and biomaterials.[106]	Poor chance of development of resistance to PHMB; resistance not reported.[105]
Collagen with PHMB	PuraPly Antimicrobial	PHMB	No published evidence showing effectiveness of collagen-PHMB dressings against biofilm.	Unknown	Unknown	Poor chance of development of resistance to PHMB; resistance not reported.[105]
Hypochlorite	Dakin's Solution	Bleach; chlorine	Yes at high concentration[108]	Yes	There is evidence that hypochloric acid may contribute to the tissue injury associated with inflammation; toxic to fibroblasts and keratinocytes.[109,110]	Reports of development of acquired resistance to certain pathogens to chlorine is very limited.[111]
Silver	SilvaSorb; Thermazene	Ionic silver; silver sulfadiazine	No	No	Silver nitrate and silver dressings have been found to be cytotoxic in vitro, but results have not translated to in vivo settings.[112]	Microbial resistance is rare.[110]
Topical antibiotics	Neosporin; Bactroban Cream	Bacitracin zinc salt, neomycin, polymyxin B; mupirocin	No	Yes — Mupirocin (gram-positive only)	Various concentrations of topical antibiotics have shown in vitro tissue toxicity.[113] No evidence of cytotoxicity found with mupirocen.[114]	Increased resistance rates have been associated with increased use of neomycin, bacitracin, and mupirocin.[115,116]
Iodine	IODOSORB	Cadexomer iodine	Limited ex vivo, in vivo, and in vitro evidence that cadexomer iodine reduces biofilm in wounds and may destroy biofilm structures[30,100]	Yes	May provide sufficient iodine for biofilm suppression without causing significant damage to the host,[100,117] but controversies remain regarding potential cytotoxicity and systemic absorption with prolonged use.[103,104]	No — bacteria are not able to develop a resistance to denaturing. Iodine-resistant microbial strains are exceptionally rare.[118]
Biofilm disruption technology	BlastX	Benzalkonium chloride, citrate	Yes[29,31]	Yes[29]	Yes — high osmolarity + surfactant does not harm host tissue.[31]	No — bacteria are not able to develop a resistance to cell lysis.[31]

[a] MicroKlenz Antimicrobial Solution: Medline Industries, Mundelein, IL; Prontosan: B. Braun Medical Inc, Bethlehem, PA; PuraPly Antimicrobial: Organogenesis, Canton, MA; Dakin's Solution: Century Pharmaceuticals Inc, Indianapolis, IN; SilvaSorb: Medline Industries, Mundelein, IL; Thermazene: Crown Laboratories, Inc, Johnson City, TN; Neosporin: Johnson & Johnson, New Brunswick, NJ; Bactroban Cream: GlaxoSmithKline, Research Triangle Park, NC; IODOSORB: Smith & Nephew, Andover, MA; BlastX: Next Science, Jacksonville, FL

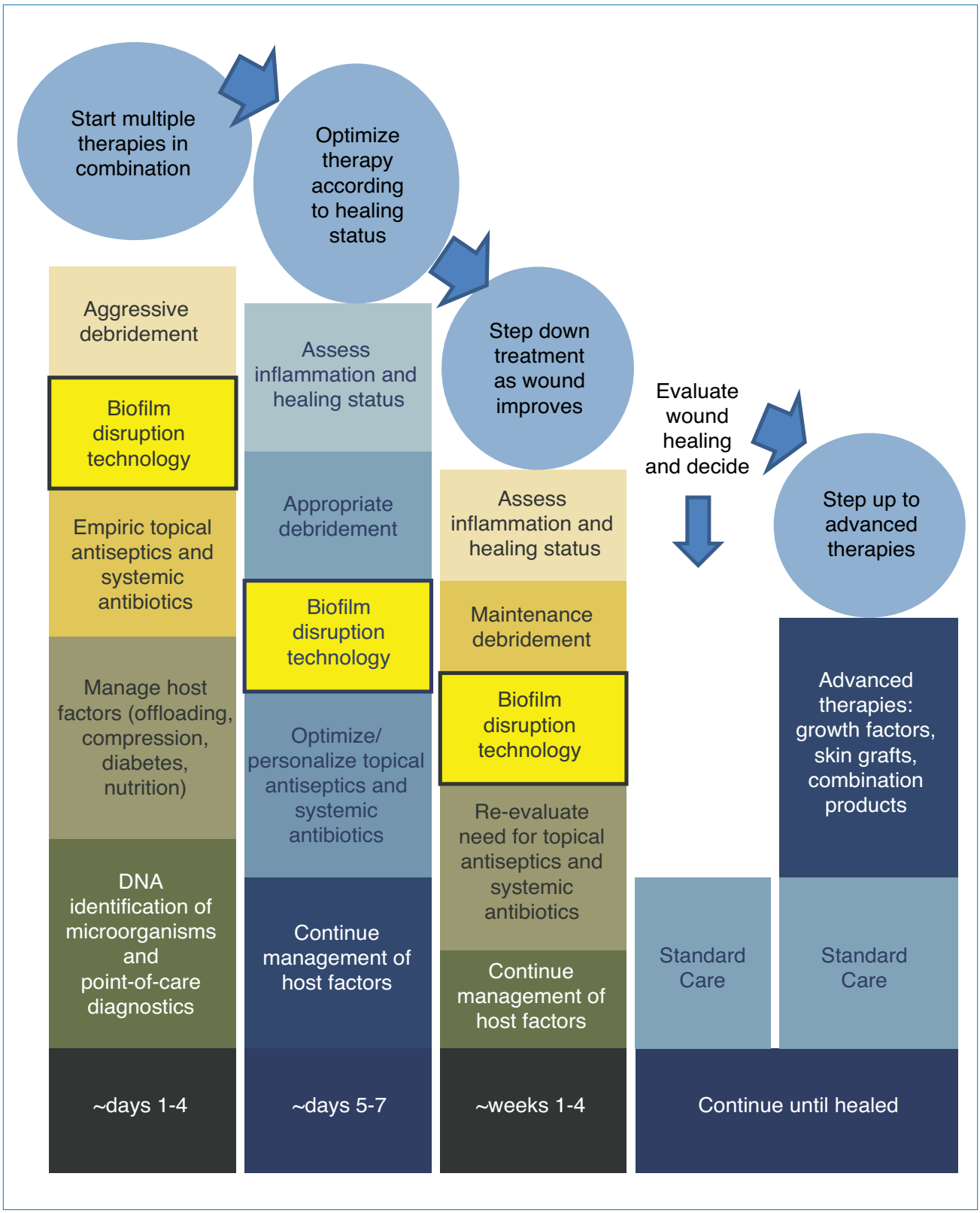

Figure 3. Recommended step-down/step-up approach with use of biofilm-disruption technology for antibiofilm treatment of wounds.

All panel members identified the urgent need for an accurate method of identifying planktonic cells versus biofilm in measuring biofilm levels. This is needed both for research and clinical practice to create a consistent process of comparing the efficacy of all antimicrobial strategies in biofilm reduction. Research performed using a novel biofilm model that would be established by first killing all planktonic cells was advocated by panel members. Planktonic cells would be eradicated from the model via a bleach solution. This method can be available now to serve as an interim solution for evaluating efficacy of antibiofilm treatments in laboratory testing. (Personal communication with Greg Schultz, PhD.)

Create a readily available, clinical point-of-care method to identify type of microbes in a biofilm. A quick, point-of-care clinical method of identifying microbes in a wound biofilm is needed. Current best practices of molecular identification are relegated to a few research labs, which is not practical for the vast majority of clinicians. A simple point-of-care RNA biofilm test made available to all clinicians would replace traditional culture techniques and guide biofilm-specific treatment of chronic wounds in an unprecedented way.

Incorporate EPS-disrupting materials/technology into antibiofilm treatment approach for all wounds. Much of the resistance of bacteria in a biofilm population is expressed by the EPS matrix. In addition to the physical barrier of an EPS matrix, RNA, proteins, and waste products excreted by the bacteria contained within the EPS matrix react with active treatment chemicals, preventing treatments from interacting with the bacteria.[31] The bacteria within biofilms have developed phenotypic-resistance mechanisms; they are not actively dividing, and may contain persister cells that are capable of recreating the biofilms after any treatment application that is not completely effective. Since the EPS matrix provides so much protection, panel members stressed the need for a paradigm shift toward biofilm treatment strategies that disrupt this shield.

Existing technologies classically used to treat biofilms are intended to either penetrate the EPS matrix or to use dispersing agents, which typically target a narrow range of bacterial biofilms.[31] These chemicals can be cytotoxic and/or damaging to the environment as well. Since wound biofilms generally consist of great diversity of microbial species including bacteria, yeast, and fungus, pursuing multiple concurrent strategies (multimodal approach) for treatment is critical. According to Wolcott et al,[31] a multimodal approach should include a physical means to disrupt the EPS matrix. One way to accomplish this is to break down biofilm EPS by removing bonds of bacterial ions between EPS polymers to allow rapid penetration.[31]

There should also be a chemical means to disrupt the wound biofilm matrix. This could involve a material that would allow penetration of 4 mm to 5 mm to break through the ions that cross-link the network together. Disrupting synergies between different microbial species within the biofilms is also necessary for effective treatment, as is disruption and prevention of attachment of microbial cells. Furthermore, persister cells need to be exposed to treatment when biofilm is removed. A material that remains present and prevents EPS-bond formation so that persister cells cannot regrow the biofilm is necessary. Lastly, the strategy should disrupt the communication language within the biofilm and provide application of high, continuous concentrations of cidal strategies to the individual microbial cells composing the biofilm. This biofilm disruption strategy would ideally be incorporated into the step-down/step-up approach as shown in **Figure 3**.

Conclusion

Although the understanding of biofilms has grown considerably during the past decade, much remains unknown. It is well established that biofilms are ubiquitous in nature and are prevalent in chronic wounds. The primary threat of biofilms is their substantial protection from host immunities and extreme tolerance to antimicrobial agents. While biofilms are known to be a barrier to wound healing, to what extent and by which mechanisms remains a subject of continued research. There are no established clinical signs of biofilms in wounds or readily available, accurate methods of bacteria identification. Thus, prospective, controlled clinical studies to evaluate treatment strategies have been difficult to perform, resulting in weak evidence. Results from in vitro and animal testing have not necessarily translated to clinical practice.

Surgical or conservative sharp wound debridement is a well-accepted means of effectively removing biofilm from an open wound surface. However, it does not remove all biofilm or prevent biofilm regrowth. There is a need for appropriate topical antimicrobial treatments in addition to debridement to suppress biofilm reformation. Rapid, molecular identification of the types and susceptibility of bacteria involved is recommended and available in certain laboratories; the procedure allows directed strategies such as the application of personalized topical antibiotics and biocides that may improve wound healing. However, current methods of molecular analyses cannot yet differentiate between planktonic versus biofilm bacteria in order to quantify efficacy of various topical treatments on biofilm reduction. Panel members identified an urgent need for diagnostics that can accurately identify planktonic cells versus biofilm as a means to evaluate efficacy of treatments in reducing biofilm. In addition, a point-of-care tool is needed in the clinical setting to quickly identify microbials in a biofilm to guide treatment.

A paradigm shift toward EPS-disrupting technology is needed to

improve healing rates of wounds in which biofilm is suspected. The EPS-disrupting technology would employ a multimodal approach to remove biofilm and prevent its reformation. The multimodal approach should cause physical and chemical disruption of the EPS matrix, disruption of synergies between different microbial species, disruption and prevention of microbial cell attachment, exposure of persister cells to treatment, and provide continuous cidal contact on the individual microbial cells making up the biofilm.[31] High-osmolarity surfactant solution technology is emerging as a potential multimodal treatment that when used in tandem with debridement shows promise in EPS disruption and prevention of biofilm formation with no cytotoxicity.

The activity of this panel is a step toward identifying technology and research needed to address current gaps in knowledge of biofilm management. Innovations in biofilm-disrupting technology and molecular diagnostics are required to move wound biofilm research and treatment forward. New technologies need to be inexpensive, not harmful to host cells or the environment, and easily accessible for wide clinical adoption. The hope is that large, controlled, prospective studies would follow and provide robust evidence needed to improve antibiofilm treatment of chronic wounds. ■

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