A Guideline for the Assessment and Treatment of Bacterial Biofilms in Chronic Wounds

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Executive Summary

Bacterial biofilms are a contributing factor to the development and perpetuation of chronic wounds. Thus they add a significant burden to the healthcare system and adversely affect quality of life for many patients. A multi-faceted approach is necessary in order to effectively manage biofilms so that wound healing can progress; this includes the use of accurate diagnostic tests, wound bed preparation with regular debridement and the application of appropriate topical therapies. To date, while there are many studies, most of them in vitro, concerning the diagnosis and treatment of bacterial biofilms in chronic wounds, there is no single guideline or systematic review that provides clinical recommendations for the management of bacterial biofilms in chronic wounds. An evidence-based guideline would help clinicians to select appropriate treatments efficiently, leading to improved patient outcomes and a better quality of wound care.

The objectives of this project were to develop an evidence-based guideline for the management of bacterial biofilms in chronic wounds and to disseminate this guideline to local wound care clinicians. A systematic process was followed to review the current literature and grade the available evidence in order to create well-supported recommendations. An algorithm was also generated to facilitate use of the guideline and provide a reference tool for clinicians. A guideline addressing diagnosis, wound bed preparation, and topical management was developed based on the review of the evidence. The guideline was evaluated for quality and clarity by content experts and wound care clinicians using the AGREE II instrument. Because of a paucity of strong clinical research evidence regarding biofilm management, the final product was a systematic review with recommendations rather than a guideline. The finished systematic review with recommendations was then submitted to a local wound care clinic for evaluation and possible implementation. Additionally, a poster was prepared and presented to a regional audience at the Eastern Idaho Regional Medical Center wound care conference.

I was assisted in the completion of this project by the guidance of my committee chairperson, Dr. Linda Edelman, and my content experts, Dr. Harriet Hopf of the University of Utah, and Drs. Mary Cloud Ammons and Elinor Pulcini of the Center for Biofilm Engineering of the University of Montana.
A guideline for the assessment and treatment of bacterial biofilms in chronic wounds

Bacterial biofilms are a widespread and common contributing factor in the development and perpetuation of chronic wounds, with estimates of up 80% of all chronic infections being caused by biofilms (Krivit & Heuertz, 2011). Chronic wounds present a growing burden to the healthcare system in the form of long-term therapy, patient suffering, treatment costs, hospital admissions and care provider time (Stremitzer, Wild & Hoelzenbein, 2007). Practitioners in the acute care setting often see patients with chronic wounds involving bacterial biofilms that vary in type, severity and level of prior care received. It is a challenge to manage wound care for these patients as the treatment plan involves management of many underlying etiological factors and comorbidities. There are a wide variety of treatment options available for the management of biofilms but there is no clear set of guidelines for the prevention and treatment of biofilms in chronic wounds.

The purpose of this project was to facilitate improved patient care and more effective management of patients with chronic wounds with biofilm involvement. This was achieved first by gathering information on the treatment strategies that are currently available and under investigation for the management of biofilms in chronic wounds, followed by an evaluation of the supporting evidence for these strategies. From this foundation I developed recommendations and an algorithm for use at a local acute care facility and associated wound clinic for the management of biofilms in chronic wounds. The use of evidence-based clinical recommendations designed to direct treatment of biofilms in chronic wounds will improve patient outcomes, reduce cost and promote healing.

Significance
Chronic wounds are a significant problem worldwide, with estimates indicating that 1 to 2% of the population among developed countries will develop a chronic wound at some point in their lifetime (Percival, Hill, Malic, Thomas & Williams, 2011; Siddiqui & Bernstein, 2010). The definition of a chronic wound varies, with descriptions including non-healing after 4 to 6 weeks to 3 months, or lack of reduction in surface area by 20-40% following 2 to 4 weeks of appropriate treatment (Siddiqui & Bernstein, 2010). The cost of treatment for chronic wounds is significant, with estimates ranging from worldwide expenditures of $13 to $15 billion annually (Siddiqui & Bernstein, 2010) to an excess of $25 billion each year in the United States alone (Percival et al., 2011). Chronic wounds are predominantly seen in the elderly population (age 60 and over), with the most common underlying pathologies being pressure ulceration, diabetes mellitus, peripheral vascular disease and venous stasis (Siddiqui & Bernstein, 2010). The treatment process for chronic wounds is complex and the impact of chronic wounds on quality of life, morbidity and mortality is also significant.

Biofilms play a key role in the development and perpetuation of chronic wounds. The U.S. Department of Health and Human Services, National Institute of Health (2006) reports that biofilms are accountable for more than 80% of infections, including those found in the lungs, oral tissues, and on implanted devices. Biofilms are composed of communities of microbial cells that have attached to a surface and secreted a matrix of extracellular polymeric substances (EPS) that is organized with channels to direct the flow of nutrients and metabolic wastes (Edwards & Harding, 2004; Percival et al., 2011; Saye, 2007; Siddiqui & Bernstein, 2010). The biofilm community provides bacteria with a protective environment that facilitates their ability to express a much greater (up to 1000 times) resistance to the action of antimicrobial compounds as well as increased virulence when compared to the free-floating phenotype (NIH, 2006; Percival et al.,
Because of these factors, bacteria within biofilms are much more difficult to eradicate, resulting in recurrent infections despite antibiotic therapy, thus contributing to the delayed and poor healing seen in chronic wounds (Saye, 2007).

While there are a wide variety of treatment options available for the management of chronic wounds in general and biofilms in particular, clear guidelines for the topical/local treatment of biofilms are lacking. Given the variety of options, it can be difficult for the clinician to quickly and accurately select an appropriate treatment plan. Clear recommendations describing evidence-based options for biofilm management in chronic wounds would facilitate care for patients with chronic wounds complicated by biofilm formation and lead to improved outcomes, more cost-effective care and better quality of life.

**Objectives**

My primary objective was to organize and follow a systematic approach to develop a guideline for the local/topical management of bacterial biofilms in chronic wounds for use in the acute care setting. The guideline answered the clinical question: “based on current evidence, what are appropriate local/topical treatment strategies for the management of bacterial biofilms in chronic wounds?” The resulting recommendations address the areas of wound bed preparation, debridement, cleansing, and topical treatment therapies.

My secondary objective was to disseminate the finished guideline to an audience of healthcare professionals. This objective was achieved in a two-fold manner. First, I planned to submit an abstract describing my clinical guideline to a wound care or nursing professional meeting for presentation to an audience of healthcare professionals. Secondly, I sought to have the guideline implemented at a local wound clinic by presenting the completed guideline to
appropriate leadership at the wound care clinic at Ogden Regional Medical Center for possible implementation.

I planned to follow a systematic process and use an organized timeline to direct the completion of this project in a timely manner (See Appendix A for a description of the implementation and evaluation plan).

**Literature Review**

A literature search was conducted to identify relevant articles in PubMed and CINAHL using the search terms “Biofilm”, “chronic wound”, “diagnosis” and “treatment.” Inclusion criteria included articles in English, peer reviewed journals, reviews, clinical trials, systematic reviews and guidelines. Exclusion criteria included articles that discussed biofilms not associated with wounds, such as biofilms on implantable devices. The initial search focused on articles from 2007-2012 to ensure that information was current; the search was later expanded to include older seminal articles referenced in the original search results in order to obtain a well-rounded view of previous knowledge. Additional searches were also conducted using less specific search terms (“Biofilm, wound”) in order to access more general information such as biofilm physiology, and using the terms “colonized, infected wounds,” “wound assessment,” and “chronic wound clinical guidelines” in order to see what treatment guidelines and assessment tools were available in wound care. Of note, a separate systematic literature review was conducted as part of the guideline development process (to be discussed later). The findings of that review are presented in the discussion portion of the guideline (see Appendix B) and are included and expanded upon below.

**Biofilm physiology**
**Continuum of infection.** Bacterial bioburden is the burden that is placed upon a wound and the body’s healing processes due to the presence of bacteria and bacterial toxins. Over 90% of all wounds face some form of bioburden (Tomaselli, 2006) as the defensive barrier provided by intact skin is broken when a wound occurs, opening the doorway to bacterial entry (Santy, 2008). The level of bacterial bioburden involvement in wounds varies along the continuum of infection from contamination to colonization and infection. Contamination indicates that non-proliferating bacteria are present in the wound, while colonization occurs when bacteria are proliferating but do not cause tissue damage or interfere with wound healing (Edwards & Harding, 2004; Santy, 2008). Infection results when invading organisms overwhelm the body’s protective mechanisms, causing tissue damage and immune responses (Santy, 2008). Critical colonization, or local infection, occurs where host defenses are insufficient to prevent bacterial growth and wound healing is compromised (Santy, 2008; Tomaselli, 2006). It is during the period of colonization that a bacterial biofilm can develop and impair wound healing, leading to critical colonization and contributing to the development of infection (Tomaselli, 2006).

**Biofilm development.** A biofilm is a slimy polysaccharide matrix composed of extracellular polymeric substances (EPS) secreted by bacteria to link them together in eradication-resistant colonies (Clutterbuck, Cochrane, Dolman & Percival, 2007; Davis et al., 2008, Merckoll, Jonassen, Vad, Jeansson & Melby, 2009). Biofilm formation begins when planktonic (individual free-floating) bacteria adhere to a surface, become irreversibly attached and then develop into micro-colonies with complex three-dimensional structures involving multiple species of organism (Dallo & Weitao, 2010; Mohamed & Huang, 2007). Early biofilm organization with EPS production occurs within the first 24 hours after attachment, and progresses to a mature, sophisticated structure by day 6 (Charles, Ricotti, Davis, Mertz and
Kirsner, 2009; Dallo & Weitao, 2010). After about 9 to 12 days, individual cells can then break off from the biofilm and disperse to new areas, leading to the spread of infection (Dallo & Weitao, 2010; Davis et al., 2008).

**Biofilm resistance mechanisms.** The biofilm environment provides many benefits to the bacteria residing within it. Bacteria within the densely packed biofilm structure are able to work synergistically to promote antibiotic resistance using intercellular signaling, a process called quorum sensing (Kirketerp-Moller et al., 2008; Mohamed & Huang, 2007). Through quorum sensing, bacteria are able to alter gene regulation and signal for increased production of virulence factors (Holby et al., 2011). Bacteria within biofilms also demonstrate phenotypic changes; they transition to a sessile growth pattern with decreased metabolic activity and a slower growth rate (Edwards & Harding, 2004). This enables them to avoid the action of certain antibiotics such as beta-lactams, which interfere with the bacterial growth cycle (Percival et al., 2011).

The EPS offers additional physical protection by making the bacteria less vulnerable to environmental dangers such as drying out or being washed away (Merckoll et al., 2009). Aggregate grouping within the biofilm environment also provides protection to the bacteria by inhibiting antibiotic diffusion and interfering with the host’s immune system response (Davis et al., 2007; Kirketerp-Moller et al., 2008; Percival et al., 2011). Within the biofilm there are also micro-environmental niches that vary in pH and include aerobic and anaerobic environments. Percival et al. (2011) suggest that these microenvironments allow a variety of bacteria to grow under different conditions and further inhibit antibiotic effectiveness. All of these benefits mean that bacteria are able to survive longer and lead to delayed wound healing and infection.

**Impact on wound healing.** Chronic wounds differ from acute wounds in that they experience alterations in the regulation of various cytokines, increased levels of matrix
metalloproteases and a prolonged inflammatory phase (Bjarnsholt et al., 2008; Black & Costerton, 2010). Bacteria promote wound chronicity by altering the function of leukocytes and impairing the formation of granulation tissue, an effect which is exacerbated by biofilms (Black & Costerton, 2010). Biofilms interfere with the host’s immune response by interfering with opsonization, preventing phagocytosis, and in some cases even releasing extracellular signals that have been theorized to lead to neutrophil cytotoxicity and lysis (Bjarnsholt et al., 2008; Thomson, 2010). The biofilm structure also interferes with the healing process by providing a mechanical barrier to the migration of keratinocytes, resulting in impaired epithelialization (Black & Costerton, 2010). Kirker et al (2009) found that *S. aureus* biofilms significantly reduced the viability of human keratinocytes and increased apoptosis in an *in vitro* setting.

**Diagnosis of Wound Infection and Biofilms**

**Clinical assessment.** Appropriate wound treatment is dependent on accurate wound assessment, including causative factors and wound characteristics; however wound assessments can often be quite subjective and inadequately documented, resulting in frequent treatment plan changes and failure to heal (Stremitzer et al., 2007). The use of an objective system for wound assessment improves consistency in assessment, enables monitoring of wound progress and can indicate the need for an adjustment in therapy (Stremitzer et al., 2007). Accurate assessment also facilitates early detection of problems such as wound infection, which can drive earlier and more effective treatment (Moore & Cowman, 2007).

Identification of bacterial biofilms in wounds is difficult because the signs and symptoms of wound colonization are not always consistent among all patients and many of the commonly recognized signs of infection, such as localized heat, redness, pain and swelling, do not occur until bacteria have caused a clinical infection (Tomaselli, 2006). Poor quality granulation tissue
that is friable, pale, dull red or edematous and changes in the amount and character of pain or exudate can all be indicators of critical colonization (Tomaselli, 2006). The following criterion, developed by Parsek and Singh, has been proposed to determine if a biofilm infection is present: (a) the pathogenic bacteria have adhered to a surface, (b) a matrix formed of bacterial or host components is visible by direct examination, (c) the infection is localized and (d) it demonstrates resistance to appropriate antibiotic treatment (Chen & Wen, 2011; Wolcott et al., 2010a). Based on these factors, it is clear that a directed and organized approach to wound assessment is necessary if biofilms are to be recognized and treated appropriately.

**Diagnostic tools.** Once a bacterial biofilm is suspected there are difficulties that arise in accurately diagnosing the causative organism. Semi-quantitative swab cultures are a common technique for diagnosis of bacterial infection, but there are multiple problems associated with this approach. Samples from swabbing only reflect a small portion of the wound, not the wound in its entirety, and swabbing is often done improperly, which gives results that only reflect the organisms present on the surface rather than those in the tissues (Stotts, 2007).

Current culturing techniques have also been criticized as being inaccurate even when properly performed, especially in the case of biofilms. Wolcott et al. (2010a) point out that clinical cultures are limited in their effectiveness because they are selectively biased towards bacteria such as aerobic Gram-positive organisms that are easily grown in laboratory conditions, which results in the vast majority of bacterial species going unidentified and overlooked. Standard cultures are not able to show if a biofilm is present, and in comparing the results between standard wound cultures and direct bacterial detection techniques Kirketerp-Moller et al. (2008) found a lack of correlation between the culture results and what was actually present in the wounds. Additionally, the standard antibiotic susceptibility testing used to guide therapy is
based on cultures using agar plates which are not adequately representative of the natural growth state of biofilms and can thus present an unrealistic view of bacterial sensitivities (Clutterbuck, Cochrane, Dolman & Percival, 2007).

In view of these findings, Kirketerp-Moller et al. (2008) made the recommendation that newer diagnostic strategies be developed and used in the assessment of wounds so that they can be appropriately treated. Molecular diagnostic techniques such as 16S ribosomal DNA sequencing, quantitative polymerase chain reaction (qPCR), peptide nucleic acid fluorescent in situ hybridization (PNA FISH) and confocal laser scanning microscopy (CLSM) are newer options for identifying bacterial biofilm populations in wound tissues (Leake et al., 2009, Malic et al., 2009, Rhoads et al., 2012). Molecular methods are beneficial as they can identify organisms that are not able to be cultured and they describe the bacteria quantitatively based on relative abundance (Rhoads et al., 2012).

**Biofilm Prevention and Treatment**

**Debridement.** Once the presence of a biofilm is identified, the clinician faces the question of what treatment options to implement. Debridement of the wound bed is a key component of biofilm eradication, and can be accomplished either mechanically or chemically. With mechanical debridement, devitalized tissue and biofilm are removed to leave a clean wound bed; this can be accomplished through sharp surgical debridement, hydrosurgery, wet-to-dry dressings and larvae application (Black & Costerton, 2010). Chemical debridement can be accomplished through the use of a variety of topical agents, but many of these agents are ineffective or require much stronger concentrations when used against biofilms (Black & Costerton, 2010).
Debridement opens a time-sensitive window wherein bacteria are more susceptible to treatment. Wolcott et al. (2010b) demonstrated that biofilm resistance to antibiotics was decreased for the first 24 hours following sharp debridement; resistance increased over time until reaching original levels at 72 hours. Wolcott et al. (2010b) hypothesized that the increased susceptibility is because debridement forces bacteria into a more “immature,” metabolically active state wherein they respond more readily to antibiotics; they also no longer have the physical protection provided by the EPS barrier. The importance of debridement was supported by Nusbaum et al. (2012) who found that debridement via either plasma-mediated bipolar radiofrequency ablation, hydrosurgery or sharp debridement resulted in a significant reduction of bacterial counts compared to no debridement in a porcine model.

**Chemical and biological agents.** A wide variety of agents are currently in use and under investigation for the treatment of bacterial biofilms (Ammons, 2010). These treatments include chemical and biological agents that are directed at different aspects of bacterial and biofilm structure. Antimicrobial chemicals such as silver and honey, as well as antibiotics have been used topically to damage and disrupt bacterial cell walls and function (Tomaselli, 2006; Stotts, 2007). Other agents are also in use and development, such as anti-biofilm agents and quorum sensing inhibitors, to prevent biofilm growth and reduce virulence (Ammons, 2010). While these approaches are promising, the evidence in support of many of these agents is often limited to *in vitro* studies, a situation which highlights the need for additional well-organized research into biofilm treatment.

Antibiotics have been used both topically and systemically in the treatment of biofilms although this practice is controversial due to the often limited efficacy (Black & Costerton, 2010). Systemic antibiotic use has been shown to be effective only 30% or less of the time when
used against biofilms. Likewise, topical antibiotics are of little effect as bacteria protected by biofilms have 1000 times the resistance compared to planktonic bacteria (Black & Costerton, 2010). Topical antimicrobials can be of further detriment to the wound by possibly encouraging the overgrowth of resistant organisms such as *Pseudomonas* (Park, Copeland, Henry & Barbul, 2010). However, the recent development of personalized topical antibiotic selection based on molecular diagnostic assay has demonstrated significant improvements in biofilm treatment effectiveness, and is a promising area of investigation (Dowd, Wolcott, Kennedy, Jones, & Cox, 2011). Thus, while the use of antibiotics may be indicated for systemic and localized infection or in the treatment of planktonic bacteria, care should be used in the selection of this therapy for chronic wounds and biofilms.

Two chemical antimicrobial agents used extensively in wound care are silver and iodine. Silver in ionic form causes damage to bacterial cells through the release of ionic energy when in the presence of neutral liquids, leading to non-selective action against a broad range of bacteria, yeast and viruses (Tomaselli, 2006). Unfortunately, some bacteria are developing resistance to silver (Dallo & Weitao, 2010) and silver has limited tissue penetration, thus it is not indicated for infections of the deeper tissues such as cellulitis (Tomaselli, 2006). Higher concentrations of silver are needed to treat biofilms versus planktonic bacteria (Black & Costerton, 2010), although this should be employed cautiously as excessive concentrations can be damaging to host cells (Tomaselli, 2006). Cadexomer iodine has bactericidal properties and comes in a microsphere form that is capable of trapping bacteria while also releasing iodine, thus decreasing wound bioburden while remaining non-toxic to fibroblasts and human cells (Stotts, 2007).
Biological agents that have been explored in the management of biofilms include honey, lactoferrin and xylitol. Honey has multiple antibacterial properties including a high osmolarity that inhibits bacterial growth, an acidic pH that may help to inhibit the development of biofilm, enzymatic production of hydrogen peroxide in low concentrations and the presence of methylglyoxal, a substance that reacts with the glycolysis process in bacteria (Merckoll et al., 2009; Pieper, 2009). Honey provides further benefit to the wound environment by reducing edema and inflammation, promoting energy production within macrophages, and modulating the activity of immune cells (Pieper, 2009). Honey’s antimicrobial effects are slower than those achieved traditional antiseptics, but it has not yet been found to foster bacterial resistance and is effective against a wide spectrum of microbes, including antibiotic resistant strains of \textit{S. aureus} (Pieper, 2009). Lactoferrin is a protein that binds iron and has been shown to destabilize bacterial cell walls, leading to cell death; it also prevents bacterial surface adhesion which prevents biofilm formation (Ammons, 2010). Xylitol is a sugar alcohol that prevents bacterial surface adhesion through inhibition of bacterial metabolizing enzymes (Ammons, 2010).

Additional methods used in the management of bioburden and biofilms include dressings impregnated with various compounds such as bleach and polyhexamide. Bleach can be used in wound dressings or cleansing solutions, but does not penetrate to the base of biofilms (Black & Costerton, 2010) and can impair wound healing by causing tissue inflammation and fibroblast damage (Park et al., 2010). Polyhexamide is an antiseptic that acts on the phospholipids contained in bacterial membranes, leading to membrane instability and impairment of bacterial growth; it is well-tolerated, minimally toxic to host cells and active against a broad spectrum of organisms (Kaehn, 2010).
The topical application of negative pressure therapy (NPT) and low-frequency ultrasound are also potential treatments for bacterial biofilms. Ngo, Vickery and Deva (2012) studied the effects of topical NPT in an in vitro biofilm model, and found that there was a small but statistically significant reduction in biofilm after 2 weeks of therapy; biofilm thickness was reduced and diffusion distance was decreased. When silver impregnated foam was used, the bacterial biofilm reduction was observable within 24 hours and was much more effective (Ngo, Vickery & Diva, 2012). Seth et al. (2013) found reduced bacterial counts with the use of noncontact low-frequency ultrasound in a rabbit ear model. Further research into both of these modalities in the clinical setting would be helpful to determine the benefit of these treatments on chronic human wounds.

Researchers are investigating newer topical agents that focus on the EPS structure of the biofilm itself, and their use independently or in conjunction with other topical preparations such as antibiotics could prove to be effective treatments (Ammons, 2010). These include detachment-promoting agents and anti-biofilm agents. Detachment-promoting agents provide an enzymatic function to break down the carbohydrate structure of the EPS, leading to disassembly of the biofilm and prevention of biofilm formation (Black & Costerton, 2010). Antibiofilm agents reduce the bacteria’s ability to attach to a surface and produce EPS, thus inhibiting their ability to produce a mature biofilm (Black & Costerton, 2010). Without the protection afforded by the biofilms, bacteria are more susceptible to therapies such as the topical antibiotics that are so effective in the management of planktonic bacteria.

The combination of multiple concurrent therapies may also prove to be an effective approach to combat biofilms in chronic wounds. Ammons et al. (2009) found that combined therapy with silver, xylitol and lactoferrin in an in vitro model was effective at reducing P.
*Pseudomonas aeruginosa* viability. In another study, Ammons, Ward and James (2011) found a statistically significant reduction in an in vitro biofilm model using a gel formulation of lactoferrin/xylitol in combination with a silver dressing. To this end, Wolcott and Rhoads (2008) have proposed a “biofilm-based wound care” treatment approach in chronic wounds using multiple concurrent modalities including lactoferrin, xylitol and antibiotics such as silver and iodine. In a retrospective observational study, they found that the use of biofilm-based wound care significantly improved wound healing within the setting of a single wound clinic (Wolcott & Rhoads, 2008).

**Theoretical Framework**

In 2002, the Association for the Advancement of Wound Care (AAWC) organized a task force with the primary goal of improving the quality of wound care services across the continuum of care and across service sites (Paine et al., 2006). The task force spent four years conducting an extensive literature search and developing a conceptual framework, including a diagrammatic representation to describe the factors affecting quality patient care and the provision of a successful wound care program (Paine et al., 2006). The resulting diagram displays the factors affecting wound care quality as a building, with a foundation, supporting columns and a roof, each representing different concepts in the wound care quality system model (Paine et al., 2006). The foundation represents the patient and their relationship with caregivers; the pillars signify specific components of quality wound care and the roof is representative of the providers, regulatory agencies and organizations their overarching responsibility to achieve effective outcomes in health care delivery and benefit the patient’s quality of life (Paine et al., 2006). The pillars represent the following quality indicators: (a) safety, (b) wound care effectiveness, (c) patient-centered wound care, (d) timeliness in wound care, (e) efficiency in
wound care, and (f) equity in wound care (Paine et al., 2006). The AAWC intends for providers to use this framework as a tool to understanding the ingredients necessary for the delivery of quality wound care and as a paradigm for the implementation of quality wound care services (Paine et al., 2006).

**Methods**

**Implementation**

The purpose of my project was to develop an evidence-based guideline for the local/topical management of bacterial biofilms in chronic wounds. Guidelines are specific recommendations for practice that are derived from current evidence and synthesized into tools to inform practice (Harrison, Legare, Graham & Fevers, 2009). Harrison, Legare, Graham and Fevers (2009) discussed the process of adapting and applying guidelines for local use; their suggestions are useful in the development of evidence-based guidelines. They recommend the following principles: “use of reliable and consistent methods to ensure the quality of the… guideline,” “consideration of context,” and “transparent reporting to promote confidence in the recommendations” (p.3). In order to ensure that the guideline produced was of high quality, the above ideas were incorporated in the process of guideline development and I sought the feedback of content experts to guide and confirm my actions. Additionally, my goal was to disperse the guideline to a broader audience through the presentation of the completed guideline at a regional wound care conference and at a local healthcare facility for implementation at the outpatient wound care clinic. I sought to achieve these goals through use of the following implementation plan (please see Appendix A for further description of the implementation and evaluation plan).

**Guideline development.** The criteria outlined in the AGREE II instrument were used initially to direct the process of guideline development, and later in the project to evaluate the
quality of the final guideline. This instrument is designed to rate the quality of healthcare guidelines, and addresses the domains of: (a) scope and purpose, (b) stakeholder involvement, (c) rigor of development, (d) clarity of presentation, (e) applicability, and (f) editorial independence (AGREE Next Steps Consortium, 2009).

**Abstract presentation.** The Eastern Idaho Regional Medical Center (EIRMC) annual wound care conference was chosen as an appropriate venue to present the final guideline. The abstract, guideline and a copy of the poster presentation were submitted to the EIRMC wound care conference director in late March. The guideline was approved to be shared as a poster presentation.

**Facility implementation.** As part of the dissemination of this guideline, I planned to present the guideline to the medical director of the Wound Care Clinic at Ogden Regional Medical Center, Dr. Balcombe, to determine if the guideline could be adapted for use at the wound care clinic.

**Evaluation Plan**

In order to determine if the guideline was developed effectively and that the project was completed appropriately, the results of the project were evaluated for quality using a validated scoring tool, namely the AGREE II instrument. The final guideline was rated by an audience of professionals, including a doctor specializing in wound care, a registered nurse with experience in infection control and three content experts. Each evaluator used the same tool to evaluate the guideline for overall quality and comment on its clinical applicability based on their area of clinical experience and expertise.

**Results**

**Objectives and Evaluation**
Each of the originally planned objectives was achieved, although the process for completing them did not exactly follow the original plan in all cases. The primary objective, to create a guideline for the management of bacterial biofilms in chronic wounds, was adapted to a systematic review with recommendations. This decision was made based on the advice of my content experts due to the limited clinical evidence available concerning biofilm management in chronic wounds. The guideline development process was followed according to plan, but due to the limited nature of the evidence, my content experts and I felt that it was a little early to consider the final product a “guideline,” and that a more accurate description would be a “systematic review with recommendations.”

Guideline development. One of the highest priority considerations in completing this project was to create the recommendations in an organized and consistent manner. This was achieved first by reviewing and selecting an appropriate system for grading the level of evidence of articles, namely the GRADE system (GRADE working group, 2012). Next I conducted a thorough literature search, using appropriate search, inclusion and exclusion criteria. I then reviewed the article abstracts from the search results and applied inclusion and exclusion criteria to develop final list of articles. I reviewed the final articles, rated them according to level of evidence, documented the main findings of the articles and displayed findings from the articles using a table. Based on the review findings, I generated recommendations with supporting levels of evidence. I then wrote a paper describing the literature review, evidence grading, recommendations and discussion (Appendix B).

I planned to have 6 professionals to evaluate the recommendations using the AGREE II instrument by April 1, 2013. Currently, this objective is partially completed; the final paper with recommendations and the scoring tool have been submitted to an audience of 6 professionals.
Three evaluations have been returned, while the others are still in the process of completing and returning their evaluation. To this point, the results have been positive in that the reviewers felt that the overall quality of the recommendations was fairly high, and that a systematic process for development was followed. The reviewers felt that the weaknesses of the recommendations were that the development process did not adequately involve professionals from all relevant groups, nor did it include the views of the target population. As including these aspects is outside the scope of the current project, these suggestions will be considered for future directions in the implementation of the guideline.

Two barriers to the completion of this project were a) the lack of high quality clinical evidence from which to draw conclusions and develop a guideline, and b) the time it took to coordinate communication with multiple content experts who all had busy schedules and were occasionally out of the office. This experience will be beneficial in the future as it illustrated the time constraints that experts and consultants face, and the need to plan enough time in advance to work around their schedules more effectively.

**Abstract presentation.** I presented the systematic review with recommendations in a poster presentation at the EIRMC annual wound care conference on April 26, 2013. I had the opportunity to discuss my findings with wound care nurses and professionals during the pre-conference registration, as well as during the breaks between afternoon workshop sessions. Several of the conference participants who stopped to review the poster expressed interest in the recommendations and took copies of the recommendations to use in their own practice.

The one barrier to achieving this objective was actually getting in contact with the appropriate person at EIRMC in order to request an opportunity to present the poster. It took
over a month and repeated phone calls before I received a return contact; after that, the submission process progressed without difficulty.

**Facility implementation.** The final recommendations and algorithm were presented to the medical director at the Wound Care Clinic at ORMC in early April. She is currently in the process of reviewing the recommendations and will determine how to implement them.

The biggest barrier with the implementation of the recommendations will be adapting the recommendations to the current practices in place at the wound care clinic at ORMC. However, as the recommendations are directed to this particular audience and have multiple choices that are easily available to most wound care clinics, this should be a very feasible process.

**Unintended Consequences**

There are two consequences resulting from this project that I had not anticipated or intended. First, I have been encouraged by my project chair and my content expert, Dr. Hopf, to attempt to have my paper with the results of my systematic review and recommendations published in a peer reviewed journal. Secondly, I have found that association with my content experts has opened my understanding of the opportunities that come from collaborating with other experts in my field.

**Limitations**

As mentioned earlier, the biggest limitation was the lack of high quality clinical evidence from which to develop a guideline for the management of biofilms in chronic wounds. When I originally conducted my literature review, it appeared that there was ample evidence available. However, after assessing the evidence using the GRADE tool and really digging deeper into the available studies, I found that the majority of information was either very poor quality observational studies or based on *in vitro* studies (some of which were very high quality, but did
not provide in vivo clinical evidence). It was still possible to complete the systematic review process, and generate recommendations based on research findings and on the feedback of content experts, but the resulting recommendations were weak, with low levels of supporting evidence. Thus it was necessary to change the main objective from that of developing a clinical guideline to developing evidence-based recommendations.

**Recommendations**

Future directions for this project should address adapting and implementing the recommendations to the needs of specific wound clinic or acute care settings. After implementation it would then be possible to evaluate the effectiveness of the recommendations in the clinical environment and possibly use that information to generate more effective guidelines and contribute to the current body of evidence.

Additionally, this project would benefit from input from various sources and stakeholders such as wound care clinic nurses and patients so that the recommendations could take their preferences into account. This project could also be applied in other settings such as long-term acute care and extended care facilities; in order to do that however, the recommendations would need to be adapted to the resources and needs of the facility in question.

**Conclusion**

In conclusion, chronic wounds are a widespread problem throughout the world, causing a significant economic, medical and psychosocial burden. Bacterial biofilms have been shown to play a role in wound chronicity, and are the subject of much current research. It is clear that effective treatment strategies will require multiple approaches including effective assessment, diagnostic tools, physical debridement and topical antimicrobial strategies. A guideline that addresses the current research on these treatment modalities will assist clinicians in creating
appropriate treatment plans to achieve faster healing, improved outcomes and a decreased burden on society.
References


Appendices

Appendix A: Guideline objectives, implementation and evaluation

Appendix B: The Management of Bacterial Biofilms in Chronic Wounds: A Systematic Review with Recommendations
Appendix A

Guideline objectives, implementation and evaluation

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Implementation</th>
<th>Evaluation</th>
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</table>
| **Guideline development.**  
Follow a systematic approach to develop a guideline for the local/topical management of bacterial biofilms in chronic wounds. | 1. Review and select an appropriate system for grading the level of evidence of articles.  
2. Conduct a thorough literature search, using appropriate search, inclusion and exclusion criteria  
3. Review the articles, rate them according to level of evidence, document main findings of articles and display findings from articles using a table.  
4. Generate a guideline based on the results of the literature review; include levels of evidence with recommendations. | Guideline completed by March 13, 2013.  
Guideline rated by an audience of at least 6 wound care professionals using the AGREE II instrument by April 13, 2013. |
| **Abstract presentation.**  
Submit an abstract describing my clinical guideline to a wound care or nursing professional meeting for possible presentation to an audience of healthcare professionals | 1. Investigate possible venues for presentation of the abstract.  
2. Contact conference organizer and obtain submission requirements.  
3. Prepare the abstract submission according to requirements. | The abstract is prepared to be submitted to a wound care/nursing professional venue by April 1, 2013. |
| **Facility implementation.**  
Present guideline for implementation at a local wound care clinic. | 1. Determine whom to contact and set up an appointment to present the guideline.  
2. Prepare a Power-Point presentation and outline of the guideline for presentation. | Appointment set to present the guideline to the appropriate leadership at the wound care clinic at Ogden Regional Medical Center by April 1, 2013. |
Appendix B

The management of bacterial biofilms in chronic wounds: a systematic review with recommendations

Bacterial biofilms are a widespread and common contributing factor in the development and perpetuation of chronic wounds, with estimates of up 80% of all chronic infections being caused by biofilms (Krivit & Heuertz, 2011). Chronic wounds present a growing burden to the healthcare system in the form of long-term therapy, patient suffering, treatment costs, hospital admissions and care provider time (Stremitzer, Wild & Hoelzenbein, 2007). Practitioners in the acute care setting often see patients with chronic wounds involving bacterial biofilms that vary in type, severity and level of prior care received. It is a challenge to manage wound care for these patients as the treatment plan involves management of many underlying etiological factors and comorbidities. There are a wide variety of treatment options available for the management of biofilms but there is no clear set of guidelines for the prevention and treatment of biofilms in chronic wounds.

The purpose of this project is to facilitate improved patient care and more effective management of patients with chronic wounds with biofilm involvement. The specific objectives are to provide clear guidance on the most effective treatments for biofilm in chronic wounds in terms of diagnostic testing, wound bed preparation and topical therapies. The recommendations will answer the clinical question, “Based on current evidence, what are the diagnostic tests and topical treatments that are appropriate for the management of bacterial biofilms in chronic wounds?” The recommendations will be directed towards patients with chronic wounds (generally adults or elderly persons, male and female) resulting from common etiologies such as pressure ulcers, venous stasis and diabetic foot ulcers. The author will achieve these objectives
first by gathering information via systematic review on the treatment strategies that are currently available and under investigation for the management of biofilms in chronic wounds. The author will then conduct an evaluation of the supporting evidence for these strategies. The author will then provide evidence-based clinical recommendations for the management of biofilm in chronic wounds. The intended audience for these recommendations will be physicians and nurses in hospital and wound care clinic settings who manage chronic wounds with biofilm. The evidence-based clinical recommendations will be designed to direct treatment of biofilms in chronic wounds with the goal of improving patient outcomes, reducing cost and promoting healing.

Methods

Literature Review

A systematic review of the literature was conducted by the author. The author is a registered nurse certified in wound, ostomy and continence care with experience in the acute care setting in managing chronic wounds. The author declares no conflicts of interest or sources of funding in the completion of this review with recommendations. This review with recommendations was conducted by the author as a Senior Project in the completion of her Doctorate of Nursing Practice degree.

The author conducted a review of the current and past literature regarding biofilm management in chronic wounds using the search terms “Biofilm, Wound” in PubMed. Additional searches were also conducted to ensure that all applicable evidence was reviewed, using the narrowed search terms “Chronic wound bacterial biofilm treatment silver” and “Chronic wound bacterial biofilm treatment honey.” These searches yielded results that were all encompassed by the original search terms, thus it was determined to simply use the original search terms as these would likely yield all pertinent information. The search results were limited
to the English language. This resulted in 446 article references from the years 1996 to 2013. All types of study design from peer-reviewed journals were included (i.e. randomized controlled trial, observational and systematic review).

The author then assessed the abstracts of these articles to determine which articles would be appropriate for further review. Of the initial search results, 204 articles were excluded because they did not address chronic wounds, but were focused on other forms of biofilm growth, such as those found on medical devices, in the oral cavity and on water filtration systems. Sixty seven articles were excluded as they focused on biofilm physiology, not treatment. Eleven articles were excluded because they were about biofilm laboratory model development, not treatment. Thirty six articles were excluded as they focused on research into possible future treatments, but did not contain information on currently available treatments. Twelve articles discussed wound care dressings, but did not address the management bacterial biofilm growth in chronic wounds. Fifteen articles were editorials or letters to the editor and were also excluded. Lastly, there were five articles that were unavailable in either abstract or full text form and were also excluded from further review. This resulted in a final list of 96 articles that were determined to be appropriate for further review (Figure A).

**Grading of Evidence**

The GRADE system was chosen to use as a review tool for classifying and grading the quality of the articles reviewed (GRADE Working Group, 2012). This system evaluates the benefits versus the risks and cost of treatment, then makes recommendations that are classified as either “Strong” or “Weak” (UpToDate, 2013). The quality of evidence is further rated based on the consistency of results, risk of bias and the precision of the evidence; the quality of the evidence is then classified as either “High” (Grade A), “Moderate” (Grade B) or “Low” (Grade
In this paper, the GRADE system was adapted to indicate quality within the moderate and low categories; i.e. “Low (-)” indicates expert opinion, while “Low (+)” indicates evidence such as well-organized *in vitro* studies that were of high quality in terms of methods used, but still did not achieve a “High” classification in terms of clinical quality (see Table A). The author chose this approach because much of the evidence concerning biofilm in chronic wounds comes from *in vitro* studies which by GRADE methodology would be classified as “Low” in terms of clinical quality. However, as there was a wide range of quality within the “Low” category, and it was felt that some indication of the quality of these studies was needed. The GRADE approach has been utilized to develop evidence-based recommendations by organizations such as the Centers for Disease Control (2012).

**Guideline development**

After conducting a systematic review of the literature and grading of the evidence, the author used the resulting evidence to generate recommendations for the management of biofilms in chronic wounds. These recommendations were shared with three content experts who provided independent review of the recommendations. The content experts have all been previously published in peer-reviewed journals, and have experience in wound care and/or biofilm physiology and management (see Appendix A). The author used feedback from the reviewers to revise and clarify the discussion and recommendations.

**Results and Recommendations**

The purpose of this systematic review with recommendations is to address the clinical question, “Based on current evidence, what are the diagnostic tests and topical treatments that are appropriate for the management of bacterial biofilms in chronic wounds?” The author will first present the results of the systematic review with recommendations based on that review then
BACTERIAL BIOFILMS IN CHRONIC WOUNDS

discuss the findings. These recommendations are based on currently available evidence in the literature concerning biofilms in chronic wounds. Unfortunately, the literature is lacking in comprehensive, high-quality clinical evidence concerning the treatment of biofilms in chronic wounds. The majority of the evidence is in vitro (some of which is very high-quality) or based on expert opinion. Generally, there were 2-3 studies available concerning each treatment. If there happened to be more than five studies providing evidence for or against a specific treatment, then the author used the five studies that were of highest quality and level of evidence to generate the guideline. The selected studies are indicated in parentheses. Summaries of the main findings in the studies and grading of quality can be found in Tables C, D and E. A summary of the recommendations and algorithm can be found in Table B and Figure B, respectively.

**Diagnostic testing**

**Standard swab culture.** The use of standard swab culturing methods is not suggested for purposes of diagnosis of bacterial biofilms in chronic wounds. If swab cultures are used, clinicians should be aware that standard cultures are unable to show the biofilm phenotype of bacterial growth, may not correlate well with the results of direct detection methods and can be selectively biased towards organisms that are easily grown in lab settings.

Strength of recommendation = Weak

Level of evidence = Low (+)

(Kirketerp-Moller et al., 2008; Rhoads et al., 2012; Siddiqui & Bernstein, 2010; Wolcott et al., 2010a)

**Molecular Diagnostics.** Molecular diagnostic techniques are suggested for the diagnosis of bacterial biofilms in chronic wounds and/or for the identification of the organisms present in the wound. The choice of diagnostic test is left up to the clinician; available methods include:
a) qPCR (results are limited to the specific pathogens the assay is designed to detect, Leake et al., 2009)

b) Pyrosequencing (can detect, identify and quantify any bacteria or yeast, Leake et al., 2009).

Strength of recommendation = Weak
Level of evidence = Low (+)
(Leake et al., 2009; Rhoads et al., 2012; Wolcott & Dowd, 2008; Wolcott, Cox & Dowd, 2010)

**Wound bed preparation**

**Cleansing.** As there is little evidence addressing wound cleansing specific to biofilms, this guideline makes no strong recommendation other than to follow facility protocol in wound cleansing. Clinicians may choose to use cleansing solutions; 0.01% hypochlorous acid preparations are available in buffered saline solutions for cleansing and may help to decrease bacterial load and biofilm; topical polihexanide may also reduce biofilm. These products are suggested as options for wound cleansing.

Strength of recommendation = Weak
Level of evidence = Low
(Crew et al., 2012; Hubner et al., 2010; Kaehn, 2010)

**Debridement.** Regular debridement is, by general consensus, a mainstay for the treatment of bacterial biofilms in chronic wounds. Debridement is therefore recommended as part of the treatment plan for biofilms. Sharp debridement and hydrosurgery are suggested methods for debridement of biofilms. At this time, the author is unaware of any study that compares the various methods of debridement in the clinical setting, thus the clinician is advised to select debridement method and frequency according to the characteristics of the wound,
patient needs and what is available per their facility. Caution is advised in wounds with compromised circulation. The use of methylene blue dye has been recommended by some experts as a method to ensure that the entire wound is adequately debrided and may be useful in debriding wounds with biofilm (Endara & Attinger, 2012).

Strength of recommendation = Strong
Level of evidence = Low (+)

(Black & Costerton, 2010; Kim & Steinberg, 2012; Nusbaum et al., 2012; Wolcott & Rhoads, 2008; Wolcott et al., 2010b)

**Ultrasound.** The application of low-frequency ultrasound is suggested as an optional method to reduce biofilm in chronic wounds. Clinicians are advised to follow manufacturer guidelines concerning treatment time and the ultrasound frequency used; no evidence currently exists to specify best practice for these settings.

Strength of recommendation = Weak
Level of evidence = Low

(Karau et al., 2010; Kim & Steinberg, 2012)

**Maggot therapy.** Topical application of maggots or larvae is a suggested option to debride necrotic tissue and degrade biofilms in chronic wounds in patients where this is determined to be appropriate. Clinicians should be aware that wounds containing *Pseudomonas aeruginosa* may require more maggots to achieve biofilm disruption as virulence factors from *P. aeruginosa* can be toxic to maggots.

Strength of recommendation = Weak
Level of evidence = Low

(Blueman & Bousfield, 2012; Brown et al., 2012; van der Plas et al., 2008)
**Topical Management**

**Silver.** Topical silver is recommended as an appropriate therapy for biofilms in chronic wounds. The effectiveness of the silver can be related to the form; hydrophobic dressings provide sustained silver release and are less easily contaminated than hydrophilic dressings which provide higher silver concentrations short term (Kostenko et al., 2010).

Strength of recommendation = Weak

Level of evidence = Moderate

(Beele, Meuleneire, Nahuys & Percival, 2010; Bjarnsholt et al., 2007; Hill et al., 2010; Kostenko et al., 2010; Percival, Bowler & Woods, 2008)

**Iodine.** Iodine-containing dressings can reduce biofilm and are suggested for use in chronic wounds with biofilm present. Limited evidence shows that in experimental conditions iodine may be more effective than silver. Iodine has a larger antimicrobial effect initially, but its effects can wane over time and allow biofilm recovery (Thorn & Greenman, 2009). Thus it is not recommended for prolonged treatment.

Strength of recommendation = Weak

Level of evidence = Low

(Hill et al., 2010; Thorn et al., 2009; Thorn & Greenman, 2009)

**Antibiotics.** Topical antibiotic use should be undertaken with caution as biofilms have been shown to be resistant to many topical antibiotics, requiring a much higher dose in order to achieve inhibition. The use of specific antibiotic mixtures based on the results of molecular diagnostic identification of wound bacteria is suggested for management of biofilm in chronic wounds (Wolcott, Cox & Dowd, 2010).

Strength of recommendation = Weak
Level of evidence = Low

(Bjarnsholt et al., 2007; Davis et al., 2008; Hammond et al., 2011; Wolcott, Cox & Dowd, 2010)

**Polyhexamethylene biguanide.** Evidence is mixed concerning the effectiveness of polyhexamethylene biguanide (PHMB) in the management of biofilms. The use of PHMB dressings is therefore only cautiously suggested for use to prevent biofilms; clinicians should monitor closely for lack of effect.

Strength of recommendation = Weak
Level of evidence = Low

(Lenselink & Andriessen, 2011; Lipp et al, 2010; Woods et al., 2012)

**Honey.** Honey may help to reduce biofilm and is suggested as an option in topical agents for biofilm management in chronic wounds.

Strength of recommendation = Weak
Level of evidence = Low

(Merckoll et al., 2009; Percival & Cutting, 2009)

**Lactoferrin.** Lactoferrin has been shown to interfere with iron binding mechanisms and bacterial cell membrane stability. It is suggested for use as part of a comprehensive treatment plan for biofilm in chronic wounds, and may be combined with other therapies such as silver and xylitol.

Strength of recommendation = Weak
Level of evidence = Low

(Ammons et al., 2009; Ammons, Ward & James, 2011; Wolcott & Rhoads, 2008)

**Xylitol.** The mechanism of action for xylitol is still not completely clear, but it likely interferes with biofilm metabolism. It is suggested as an option for biofilm in chronic wounds
and may be combined with other therapies such as silver and lactoferrin as part of the comprehensive treatment plan.

Strength of recommendation = Weak

Level of evidence = Low

(Ammons et al., 2009; Ammons, Ward & James, 2011; Dowd et al., 2009; Wolcott & Rhoads, 2008)

**Negative Pressure Wound Therapy.** Negative Pressure Wound Therapy (NPWT) may help to reduce biofilm through mechanical debridement. It is suggested as an option for management of biofilms in chronic wounds. It may be combined with other treatments such as silver and instillation of antimicrobial solutions to achieve greater efficacy.

Strength of recommendation = Weak

Level of evidence = Low

(Bradley & Cunningham, 2013; Ngo, Vickery & Deva, 2011)

**Combination Therapy.** The combination of multiple concurrent therapies may also prove to be an effective approach to combat biofilms and is recommended in the management of biofilms in chronic wounds. Combinations that have been used include lactoferrin and xylitol, with and without silver, and alternating regimens of silver and iodine.

Strength of recommendation = Weak

Level of evidence = Low

(Ammons et al., 2009; Ammons, Ward & James, 2011; Wolcott & Rhoads, 2008)

**Discussion**

Bacterial biofilm establishment is a major barrier to wound healing and contributes to the development of chronic wounds (Ammons, 2010; Rhoads, Wolcott & Percival, 2008; Wolcott &
Rhoads, 2008). The author understands that chronic wounds also result from a wide variety of etiological sources such as pressure, diabetes and venous stasis, and does not attempt to address these issues in this review with recommendations. There are a multitude of guidelines available that address the management of these concerns. These recommendations focus solely and specifically on bacterial biofilm management. Appropriate management of the underlying cause of the chronic wound is still necessary and an essential component of standard wound care; clinicians are encouraged to refer to established guidelines from other sources for guidance in these areas.

**Assessment and Diagnosis**

Appropriate wound treatment is dependent on accurate wound assessment, including causative factors and wound characteristics; however wound assessments can often be quite subjective and inadequately documented, resulting in frequent treatment plan changes and failure to heal (Stremitzer et al., 2007). The use of an objective system for wound assessment improves consistency in assessment, enables monitoring of wound progress and can indicate the need for an adjustment in therapy (Stremitzer et al., 2007). Accurate assessment also facilitates early detection of problems such as wound infection, which can drive earlier and more effective treatment (Moore & Cowman, 2007). This guideline does not make any recommendations as to specific assessment systems for use with bacterial biofilms as there are no studies available that validate the use of any specific assessment tool for wounds with biofilm. However, as with all general wound care, clinicians are encouraged to use whatever system is currently employed by their facility in a consistent, objective manner.

Identification of bacterial biofilms in wounds is difficult because the signs and symptoms of wound colonization are not always consistent among all patients and many of the commonly
recognized signs of infection, such as localized heat, redness, pain and swelling, do not occur until bacteria have caused a clinical infection (Tomaselli, 2006). Current culturing techniques have been criticized as being inaccurate even when properly performed and limited in their effectiveness because they are selectively biased towards bacteria such as aerobic Gram-positive organisms that are easily grown in laboratory conditions, which results in the vast majority of bacterial species going unidentified and overlooked (Wolcott et al., 2010a). Standard cultures are not able to show if a biofilm is present, and in comparing the results between standard wound cultures and direct bacterial detection techniques Kirketerp-Moller et al. (2008) found a lack of correlation between the culture results and what was actually present in the wounds. Additionally, the standard antibiotic susceptibility testing used to guide therapy is based on cultures using agar plates which are not adequately representative of the natural growth state of biofilms and can thus present an unrealistic view of bacterial sensitivities (Clutterbuck, Cochrane, Dolman & Percival, 2007).

In view of these findings, Kirketerp-Moller et al. (2008) made the recommendation that newer diagnostic strategies be developed and used in the assessment of wounds so that they can be appropriately treated. Molecular diagnostic techniques such as 16S ribosomal DNA sequencing, quantitative polymerase chain reaction (qPCR), tag-encoded FLX amplicon pyrosequencing (TEFAP or pyrosequencing), peptide nucleic acid fluorescent in situ hybridization (PNA FISH) and confocal laser scanning microscopy (CLSM) are newer options for identifying bacterial biofilm populations in wound tissues (Leake et al., 2009; Malic et al., 2009; Rhoads et al., 2012). Malic et al. (2009) found that the combination of PNA FISH with CLSM was effective in examining the spatial organization of biofilms and identifying specific bacteria within the biofilm sample. Molecular methods are beneficial as they can identify
organisms that are not able to be cultured and they describe the bacteria quantitatively based on relative abundance (Rhoads et al., 2012). Of additional benefit is the reported speed at which results can be obtained using molecular diagnostics (between 4 and 24 hours depending on the test used; Leake et al., 2009). However, since most wound care centers do not have direct access to such methods, the time to obtain results would likely be similar to that of traditional culturing methods.

**Wound bed preparation**

Appropriate preparation of the wound bed is a key component of wound care in general, as well as specifically for biofilm eradication. Preparation includes cleansing and debridement, and can be accomplished either mechanically or chemically. With mechanical debridement, devitalized tissue and biofilm are removed to leave a clean wound bed; this can be accomplished through sharp surgical debridement, hydrosurgery, wet-to-dry dressings and larvae application (Black & Costerton, 2010). Chemical debridement can be accomplished through the use of a variety of topical agents, but many of these agents are ineffective or require much stronger concentrations when used against biofilms (Black & Costerton, 2010).

Debridement opens a time-sensitive window wherein bacteria are more susceptible to treatment. Wolcott et al. (2010b) demonstrated that biofilm resistance to antibiotics was decreased for the first 24 hours following sharp debridement; resistance increased over time until reaching original levels at 72 hours. Wolcott et al. (2010b) hypothesized that the increased susceptibility is because debridement forces bacteria into a more “immature,” metabolically active state wherein they respond more readily to antibiotics; they also no longer have the physical protection provided by the EPS barrier. The importance of debridement was supported by Nusbaum et al. (2012) who found that debridement via either plasma-mediated bipolar
radiofrequency ablation, hydrosurgery or sharp debridement resulted in a significant reduction of bacterial counts compared to no debridement in a porcine model.

**Topical Management**

A wide variety of agents are currently in use and under investigation for the treatment of bacterial biofilms (Ammons, 2010). These treatments include chemical and biological agents that are directed at different aspects of bacterial and biofilm structure. Antimicrobial chemicals such as silver and honey, as well as topical antibiotics have been used topically to damage and disrupt bacterial cell walls and function (Tomaselli, 2006). Other agents are also in use and development, such as anti-biofilm agents and quorum sensing inhibitors, to prevent biofilm growth and reduce virulence (Ammons, 2010). While these approaches are promising, the evidence in support of many of these agents is often limited to *in vitro* studies, a situation which highlights the need for additional well-organized clinical research into biofilm treatment.

The combination of multiple concurrent therapies may also prove to be an effective approach to combat biofilms in chronic wounds. Ammons et al. (2009) found that combined therapy with xylitol and lactoferrin in an in vitro model was effective at reducing *P. aeruginosa* viability. In another study, Ammons, Ward and James (2011) found a statistically significant reduction in an in vitro biofilm model using a gel formulation of lactoferrin/xylitol in combination with a silver dressing. To this end, Wolcott and Rhoads (2008) have proposed a “biofilm-based wound care” treatment approach in chronic wounds using multiple concurrent modalities including lactoferrin, xylitol and antibiotics such as silver and iodine. In a retrospective observational study, they found that the use of biofilm-based wound care significantly improved wound healing within the setting of a single wound clinic (Wolcott & Rhoads, 2008).
The author anticipates that implementation of these recommendations in the acute care or outpatient wound care clinic setting should be manageable. Barriers to implementation would be cost of supplies and access to certain products. This author has found that the majority of sites have access to at least some of the recommended topical therapies, such as silver and iodine. The recommendations in this review focus on treatment options that are currently available to clinicians across the majority of treatment settings. There are many additional therapies that have been or are currently under investigation, such as detachment-promoting agents and nanoparticle applications, that are not addressed in these recommendations as they are either unavailable to the vast majority of practitioners or are still in experimental stages. The use of molecular diagnostic studies might prove to be difficult in many centers due to the fact that these tests are not commonly performed in many laboratories; however it is possible to send samples to distant sites for assessment when an accurate diagnosis and measurement of wound biofilm is determined to be necessary. The author suggests using available therapies empirically, such as topical silver in conjunction with regular debridement, until test results are available to direct more targeted therapy.

**Limitations**

Limitations of this study include the lack of high quality clinical evidence from which to draw conclusions regarding the management of biofilms in chronic wounds. As mentioned before, the majority of evidence was based on *in vitro* laboratory studies and expert opinion. There was very little evidence available concerning patient outcomes such as reduced pain or increased quality of life to direct the recommendation process surrounding biofilm management in chronic wounds, and no information comparing these outcomes between therapies.
Additionally, these recommendations constitute a preliminary guideline that has not been used in clinical practice and therefore is not proven as to effectiveness or validity.

In the future, these recommendations would be strengthened by devoting attention to patient opinions and outcomes such as quality of life, cost effectiveness and provider preferences. The author hopes that over time this information will become more available and accessible as biofilm management in chronic wounds becomes more widely recognized and studied. The author also hopes that implementation of these recommendations at local facilities will help to show effectiveness and be a useful way to generate improvements in future updates. The author anticipates that an effective schedule for updating these recommendations will be every five years.

**Conclusion**

Bacterial biofilms have only recently, over the past decade or so, begun to be recognized and studied as present within and contributing to non-healing in chronic wounds. Evidence is slowly building around treatments to manage biofilm in chronic wounds. A multi-faceted approach that addresses accurate diagnosis, effective debridement and appropriate topical therapies will be essential to managing biofilms and promoting improved outcomes with wound healing.
References


Figure A: Literature Review, article inclusion and exclusion

Initial search results:
446 articles

Excluded articles:
204 – Biofilms not in chronic wounds
67 – Biofilm physiology, not treatment
11 – Biofilm model development
36 – Possible future treatments
12 – Wound care/dressings
15 – Editorials/letters to the editor
5 – Abstract unavailable

Articles for complete review:
96

Diagnostic Testing
21 articles
(Cultures, molecular diagnostics)

Wound Bed Preparation
26 articles
(Debridement, Maggot therapy, ultrasound)

Topical Management
55 articles
(Silver, Iodine, Honey, NPWT, lactoferrin, xylitol, etc.)
Figure B: Recommendations for management of biofilms in chronic wounds – algorithm

**Initial wound assessment:**
Biofilm suspected

**Diagnostic testing:**
Standard swab cultures – not recommended
Molecular diagnostics - suggested

**Wound Bed Preparation**
- **Cleansing:** Per facility protocol, no specific recommendation.
- **Debridement:** Regular debridement strongly recommended. Method and frequency based on wound characteristics.

**Optional Wound Bed Preparation**
- Noncontact, Low-frequency Ultrasound
- Maggot therapy

**Application of Topical Therapies**
*Use of therapies in combination may be effective & is suggested*

- Silver
- Iodine
- Honey
- Lactoferrin
- Xylitol
- Lactoferin
- Xylitol
- Negative Pressure Wound Therapy (NPWT)

Topical antibiotics – use cautiously; molecular diagnostics to guide therapy is suggested
Polyhexamethylene Biguanide may be used with caution.

**Evaluate effectiveness of therapy**
Continue if effective, change to alternative therapy if wound not improving
Table A: Summary of Grading of Evidence

<table>
<thead>
<tr>
<th>Grade of Recommendation</th>
<th>Implication</th>
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<tbody>
<tr>
<td>Strong</td>
<td>Benefits clearly outweigh the risks or benefits. The recommendation is applicable to most patients</td>
</tr>
<tr>
<td>Weak</td>
<td>The benefits of treatment may balance closely with risk and burdens. The choice of action may vary depending on patient circumstance or values, and alternative approaches may likely be better.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quality of Evidence</th>
<th>Implication</th>
</tr>
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<tbody>
<tr>
<td>High (Grade A)</td>
<td>Evidence is from randomized controlled trials that were well-performed and the results are consistent. Evidence from further research is unlikely to affect the risk versus benefit estimates concerning the recommendation.</td>
</tr>
<tr>
<td>Moderate (Grade B)</td>
<td>The evidence is based on randomized, controlled trials that have limitations such as methodological flaws. May also include other research designs if the evidence is strong. (-) indicates research designs other than randomized controlled trials that have methodological flaws or limitations</td>
</tr>
<tr>
<td>Low (Grade C)</td>
<td>Evidence based on randomized, controlled trials with serious limitations or flaws, observational studies or clinical experience. (+) indicates high-quality observational or <em>in vitro</em> studies. (-) indicates clinical experience, expert review or <em>in vitro</em> studies with limitations</td>
</tr>
<tr>
<td>Low (+)</td>
<td></td>
</tr>
<tr>
<td>Low (-)</td>
<td></td>
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</table>
### Table B: Recommendations for the Management of Biofilms in Chronic Wounds

#### Diagnostic Testing
- **Standard swab cultures are not recommended for diagnosis of bacterial biofilms in chronic wounds.**
  - Strength of recommendation = Weak
  - Level of evidence = Low (+)
- **Molecular diagnostic techniques are suggested for the diagnosis of bacterial biofilms and identification of organisms present.**
  - Strength of recommendation = Weak
  - Level of evidence = Low (+)

#### Wound Bed Preparation

**Cleansing**
- Follow facility protocol for wound cleansing. Preparations of hypochlorous acid in buffered saline solution and topical polihexanide may help to decrease biofilm, and are suggested as options.
  - Strength of recommendation = Weak
  - Level of evidence = Low

**Debridement**
- Regular debridement is recommended; suggested methods include sharp debridement and hydrosurgery. Frequency and method to be determined by clinician based on wound characteristics.
  - Strength of recommendation: Strong
  - Level of evidence = Low (+)
- **Low-frequency ultrasound suggested as optional treatment to reduce biofilm.** Follow manufacturer guidelines.
  - Strength of recommendation = Weak
  - Level of evidence = Low
- **Maggot therapy suggested as an alternative method of debridement when appropriate and available.**
  - Strength of recommendation = Weak
  - Level of evidence = Low

#### Topical Management
- **Topical silver is recommended; effectiveness can be related to form of silver used.**
  - Strength of recommendation = Weak
  - Level of evidence = Moderate
- **Iodine-containing dressings are suggested, although not recommended for prolonged treatment.**
  - Strength of recommendation = Weak
  - Level of evidence = Low
- **Topical antibiotics may be used cautiously; selection based on results of molecular diagnostic testing is suggested.**
  - Strength of recommendation = Weak
  - Level of evidence = Low
- **Polyhexamethylene biguanide is cautiously suggested for biofilm prevention; monitor for lack of effect.**
  - Strength of recommendation = Weak
  - Level of evidence = Low
- **Medical-grade honey preparations in gels or dressings are suggested for biofilm treatment.**
  - Strength of recommendation = Weak
  - Level of evidence = Low
- **Lactoferrin is suggested as an option for treatment of biofilms; may be combined with other therapies such as silver and xylitol for increased effect.**
  - Strength of recommendation = Weak
  - Level of evidence = Low
- **Xylitol is suggested as an option for biofilm treatment; may be combined with other therapies such as silver and lactoferrin.**
  - Strength of recommendation =Weak
  - Level of evidence = Low
- **Negative pressure wound therapy is suggested as an option for wound management with biofilm; may be combined with other therapies such as silver and instillation of antimicrobials.**
  - Strength of recommendation = Weak
  - Level of evidence = Low
### Table C: Summary of Grading of Evidence – Diagnostic Testing

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Level of evidence</th>
<th>Key Points</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirketerp-Moller et al., 2008</td>
<td>Observational</td>
<td>Low (+)</td>
<td>PNA-FISH results differ from swab culture; <em>P. aeruginosa</em> was detected more frequently using PNA FISH</td>
<td>Analysis: samples from 22 chronic wounds using PNA FISH and standard cultures</td>
</tr>
<tr>
<td>Leake et al., 2009</td>
<td>Observational</td>
<td>Low (+)</td>
<td>qPCR and pyrosequencing identified pathogens that were not found on culture and results were available sooner than standard culture</td>
<td>Analysis of 43 wound samples using qPCR, pyrosequencing and standard culture</td>
</tr>
<tr>
<td>Rhoads et al., 2012</td>
<td>Observational</td>
<td>Low (+)</td>
<td>16S ribosomal DNA sequencing detected 85% of bacteria detected by culture; culture detected 15.7% of aerotolerant bacteria; cultures were more likely to detect aerotolerant bacterial species with a higher relative abundance.</td>
<td>Samples from 51 chronic wounds were tested using standard culture and molecular diagnostics</td>
</tr>
<tr>
<td>Siddiqui &amp; Bernstein, 2010</td>
<td>Expert opinion</td>
<td>Low (-)</td>
<td>Review of quantitative vs. semi quantitative culture techniques</td>
<td></td>
</tr>
<tr>
<td>Wolcott &amp; Dowd, 2008</td>
<td>Expert opinion, case study</td>
<td>Low</td>
<td>qPCR results differed from traditional culture results; qPCR showed <em>P. aeruginosa</em></td>
<td>Single case study of qPCR use</td>
</tr>
<tr>
<td>Wolcott et al., 2010a</td>
<td>Expert opinion</td>
<td>Low (-)</td>
<td>Standard culture techniques can be inaccurate and biased towards bacteria easily grown in laboratory conditions.</td>
<td></td>
</tr>
<tr>
<td>Wolcott, Cox &amp; Dowd, 2010</td>
<td>Retrospective</td>
<td>Moderate (-)</td>
<td>The use of molecular diagnostics with “biofilm-based wound care” (frequent debridement with selective biocides, antibiofilm agents and targeted antibiotics) resulted in increased healing rates and reduced healing times for venous, diabetic and pressure ulcers.</td>
<td>IT consultant performed review of patient EMR data; compared findings before and after implementation of molecular diagnostics. Study over two years (2007-2009), reviewed over 1000 patients.</td>
</tr>
</tbody>
</table>
**Table D: Summary of Grading of Evidence – Wound Bed Preparation**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study design</th>
<th>Level of evidence</th>
<th>Key points</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black &amp; Costerton, 2010</td>
<td>Expert opinion</td>
<td>Low (-)</td>
<td>Discussion of sharp debridement, hydrosurgery and chemical debridement.</td>
<td></td>
</tr>
<tr>
<td>Blueman &amp; Bousfield, 2012</td>
<td>Expert review</td>
<td>Low (-)</td>
<td>Larval debridement is effective against <em>S. aureus</em>, <em>P. aeruginosa</em> and MRSA</td>
<td></td>
</tr>
<tr>
<td>Brown et al., 2012</td>
<td>In vitro</td>
<td>Low</td>
<td>Maggot secretions were able to remove <em>P. aeruginosa</em> biofilm over 24 hours</td>
<td>Model: wound slough/eschar on agar plates</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Analysis: crystal violet based assay, ultraviolet transillumination, CLSM</td>
</tr>
<tr>
<td>Crew et al., 2012</td>
<td>In vitro and Case study</td>
<td>Low</td>
<td>Hypochlorous acid was bactericidal against <em>S. aureus</em></td>
<td>Model: CDC reactor, Calgary Biofilm Device</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Analysis: CLSM</td>
</tr>
<tr>
<td>Hubner et al., 2010</td>
<td>In vitro</td>
<td>Low</td>
<td>PHMB was as effective as chlorhexidine in reducing bacterial metabolism and biofilm amount.</td>
<td>Model: microtitre plate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Analysis: CFU count, photometer mass measurement, CLSM</td>
</tr>
<tr>
<td>Kaehn, 2010</td>
<td>Expert opinion</td>
<td>Low (-)</td>
<td>Polihexanide recommended as broad-spectrum antiseptic, including against biofilm.</td>
<td></td>
</tr>
<tr>
<td>Karau et al., 2010</td>
<td>In vitro</td>
<td>Low</td>
<td>Quostic debridement (low frequency ultrasound) decreased <em>P. aeruginosa</em>, <em>S. epidermis</em> and <em>S. aeruginosa</em> biofilms</td>
<td>Model: CDC reactor</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>Analysis: CFU counts</td>
</tr>
<tr>
<td>Kim &amp; Steinberg, 2012</td>
<td>Expert opinion</td>
<td>Low (-)</td>
<td>Excisional debridement, MIST, US, hydrojet recommended for biofilm removal and disruption.</td>
<td></td>
</tr>
<tr>
<td>Nusbaum et al., 2012</td>
<td>In vivo porcine</td>
<td>Low</td>
<td>Sharp debridement and hydrosurgery showed statistically significant reduction in MRSA counts on days 0, 2, 9 and 21 compared to no debridement.</td>
<td>Analysis: CFU counts, pathologist review</td>
</tr>
<tr>
<td>Study</td>
<td>Type</td>
<td>Instr.</td>
<td>Treatment</td>
<td>Model</td>
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<tr>
<td>van der Plas et al., 2008</td>
<td>In vitro</td>
<td>Low</td>
<td>Maggot excretions/secretions degraded <em>S. aureus</em> and <em>P. aeruginosa</em> biofilms; larger amounts of excretions were needed for effect against <em>P. aeruginosa</em>.</td>
<td>Model: microtitre plates</td>
</tr>
<tr>
<td>Wolcott &amp; Rhoads, 2008</td>
<td>Retrospective, single-center</td>
<td>Low</td>
<td>The use of biofilm-based wound care (including a lactoferrin/xylitol gel) resulted in improved healing frequency compared to a previously published study.</td>
<td>Not all patients received the same treatment. Other therapies used in combination included silver, iodine and antibiotics</td>
</tr>
<tr>
<td>Wolcott et al., 2010b</td>
<td>In vitro, in vivo, and clinical longitudinal</td>
<td>Low (+)</td>
<td>Sharp debridement resulted in a significant decrease in biofilm resistance to gentamycin for 24 hours; resistance increased to original levels by 72 hours</td>
<td>Model: Drip flow reactor; fresh porcine skin explants; mouse surgical wound excision; clinical debridement of 3 patients. Analysis: CFU counts</td>
</tr>
</tbody>
</table>
### Table E: Summary of Grading of Evidence – Topical Management

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study design</th>
<th>Level of evidence</th>
<th>Key points</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammons et al., 2009</td>
<td>In vitro</td>
<td>Low</td>
<td>Combination therapy with xylitol and lactoferrin significantly decreased <em>P. aeruginosa</em> biofilm viability.</td>
<td>Modified CDC flow reactor model. Viability quantification using in situ epiflourescence.</td>
</tr>
<tr>
<td>Ammons, Ward &amp; James, 2011</td>
<td>In vitro</td>
<td>Low</td>
<td>Combination therapy with lactoferrin, xylitol and silver was significantly more effective than hydrogel against biofilms of <em>P. aeruginosa</em>.</td>
<td>Model based on colony biofilm and drip-flow models. Quantification of bacterial growth based on incubation of recovery plates and counting of colony forming units.</td>
</tr>
<tr>
<td>Beele, Meuleneire, Nahuys &amp; Percival, 2010</td>
<td>Prospective randomized open-label</td>
<td>Moderate (-)</td>
<td>Silver alginate/carboxymethylcellulose dressing showed statistically smaller wound surface area over 4 weeks than controls using a non-silver calcium alginate dressing.</td>
<td>Sample size 36 patient; 24 in silver group, 12 in non-silver. Determination of biofilm presence based on clinical signs and symptoms, not diagnostic testing.</td>
</tr>
<tr>
<td>Bjarnsholt et al., 2007</td>
<td>In vitro</td>
<td>Low</td>
<td>Silver is effective against mature <em>P. aeruginosa</em> biofilms; strength of silver concentration influences effectiveness.</td>
<td>Continuous-culture once-through flow chamber model. Measurement using CLSM.</td>
</tr>
<tr>
<td>Bradley &amp; Cunningham, 2013</td>
<td>Expert review</td>
<td>Low (-)</td>
<td>Negative pressure wound therapy with instillation may prevent biofilm formation.</td>
<td></td>
</tr>
<tr>
<td>Davis et al., 2008</td>
<td>In vivo laboratory</td>
<td>Low</td>
<td>Mupirocin and Neosporin have reduced efficacy against biofilm <em>S. aureus</em> compared to planktonic bacteria.</td>
<td>Model: partial thickness porcine wounds Analysis: light microscopy, SEM, epiflourescent microscopy</td>
</tr>
<tr>
<td>Dowd et al., 2009</td>
<td>In vitro</td>
<td>Low</td>
<td>Xylitol inhibited <em>P. aeruginosa</em> biofilm growth; salicylic acid, farnesol and erythritol had varying effects.</td>
<td>Model: Lubbock chronic wound biofilm Analysis: quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>Hammond et al., 2011</td>
<td>In vitro</td>
<td>Low</td>
<td>Mupirocin, triple antibiotic ointment and gentamycin significantly reduced biofilm</td>
<td>Model: cellulose discs on agar plates Analysis: CFU count and</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Effectiveness</td>
<td>Summary</td>
<td>Model/Analysis</td>
</tr>
<tr>
<td>----------------------------</td>
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</tr>
<tr>
<td>Hill et al., 2010</td>
<td>In vitro</td>
<td>Low</td>
<td>Cipro and flucoxacin were not effective against <em>P. aeruginosa</em> and <em>S. aureus</em> biofilms; iodine based dressings were effective against 7-day biofilms, 2 out of 6 silver dressings had effect against 3 day biofilms, but not against 7 day biofilms.</td>
<td>Model: constant depth film fermenter. Analysis: SEM, EPS staining with epifluorescent microscopy</td>
</tr>
<tr>
<td>Kostenko et al., 2010</td>
<td>In vitro</td>
<td>Low</td>
<td>Effectiveness of silver dressing against biofilm related to base material used; hydrophilic base materials had diminished efficacy over time. Bacteria that survived silver treatment were susceptible to topical antibiotics, whereas untreated bacteria were resistant to the same antibiotics.</td>
<td>Model: Serum-coated MBEC device used, with daily transfers of protein-rich medium. Treatment length was 7 days. Analysis: CSLM and energy-dispersive x-ray spectrometry. <em>MRSA, P. aeruginosa, &amp; E. coli</em></td>
</tr>
<tr>
<td>Lenselein &amp; Andriesse, 2011</td>
<td>Cohort observational</td>
<td>Low</td>
<td>Wounds treated with PHMB dressings showed increased granulation, decreased yellow tissue and decreased pain after 24 weeks.</td>
<td>Sample size 28 patients, no control group, high drop-out rate.</td>
</tr>
<tr>
<td>Lipp et al., 2010</td>
<td>In vitro</td>
<td>Low</td>
<td>Silver and PHMB dressings had statistically fewer bacteria (<em>S. aureus</em> and <em>P. aeruginosa</em>) than non-antimicrobial controls; silver was significantly more effective against <em>P. aeruginosa</em>; neither was able to prevent biofilm formation.</td>
<td>Model: colony drip-flow reactor Analysis: CFU count, SEM, epifluorescent microscopy</td>
</tr>
<tr>
<td>Merckoll et al., 2009</td>
<td>In vitro</td>
<td>Low</td>
<td>Honey was bactericidal against <em>MRSA, MRSE, K. pneumoniae</em> and <em>P. aeruginosa</em>.</td>
<td>Model: microplate Analysis: photometric optical density measurement</td>
</tr>
<tr>
<td>Ngo, Vickery &amp; Deva, 2011</td>
<td>In vitro</td>
<td>Low</td>
<td>Topical negative pressure therapy significantly reduced biofilm at 2 weeks; reduction more significant when</td>
<td>Model: Biofilm coupons using agar base and flow chambers. Analysis: CSLM and</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Level of Evidence</td>
<td>Methodology</td>
<td>Findings</td>
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</tr>
<tr>
<td>Percival &amp; Cutting, 2009</td>
<td>Expert opinion</td>
<td>Low (-)</td>
<td>Recommends use of debridement in combination with antimicrobial agents such as iodine, ionic silver and honey.</td>
<td>Plate counting.</td>
</tr>
<tr>
<td>Percival, Bowler &amp; Woods, 2008</td>
<td>In vitro</td>
<td>Low</td>
<td>Silver hydrofiber was able to achieve total bacterial kill in 48 hours with biofilms of <em>P. aeruginosa</em>, <em>E. cloacae</em> and <em>S. aureus</em>.</td>
<td>Model: Chambered cover glass slides Analysis: CLSM</td>
</tr>
<tr>
<td>Thorn &amp; Greenman, 2009</td>
<td>In vitro</td>
<td>Low</td>
<td>Iodine dressings showed rapid initial antimicrobial effect that later waned and allowed regrowth of <em>S. aureus</em>. Silver had a more gradual effect against <em>S. aureus</em> and <em>P. aeruginosa</em>.</td>
<td>Model: Flat-bed perfusion Analysis: environmental SEM</td>
</tr>
<tr>
<td>Thorn et al., 2009</td>
<td>In vitro</td>
<td>Low</td>
<td>Silver and iodine were both effective against <em>P. aeruginosa</em> and <em>S. aureus</em>; iodine was more efficacious after 24 hours</td>
<td>Model: flat-bed perfusion biofilm Analysis: serial dilution and CFU count</td>
</tr>
<tr>
<td>Wolcott &amp; Rhoads, 2008</td>
<td>Retrospective, single-center</td>
<td>Low</td>
<td>The use of biofilm-based wound care (including a lactoferrin/xylitol gel) resulted in improved healing frequency compared to a previously published study.</td>
<td>Not all patients received the same treatment. Other therapies used in combination included silver, iodine and antibiotics</td>
</tr>
<tr>
<td>Wolcott, Cox &amp; Dowd, 2010</td>
<td>Retrospective</td>
<td>Moderate (-)</td>
<td>The use of molecular diagnostics with “biofilm-based wound care” (frequent debridement with selective biocides, antibiofilm agents and targeted antibiotics) resulted in increased healing rates and reduced healing times for venous, diabetic and pressure ulcers.</td>
<td>IT consultant performed review of patient EMR data; compared findings before and after implementation of molecular diagnostics. Study over two years (2007-2009), reviewed over 1000 patients.</td>
</tr>
<tr>
<td>Woods et al., 2012</td>
<td>In vitro</td>
<td>Low</td>
<td>Growth of <em>S. aureus</em>, <em>P. aeruginosa</em> and <em>Cl. Perfringens</em> was significantly reduced with nanocrystalline silver dressing; PHMB dressing was not effective.</td>
<td>Model: colony drip-flow reactor Analysis: light microscopy; CFU count</td>
</tr>
</tbody>
</table>
Abbreviations:
CFU = colony forming units; CLSM = confocal laser scanning electron microscope; EMR = electronic medical record; EPS = extracellular polymeric substance; IT = information technology; MRSA = methicillin-resistant *S. aureus*; MRSE = methicillin-resistant *S. epidermis*; PHMB = polyhexamethylene biguanide; PNA FISH = peptide nucleic acid fluorescence in situ hybridization; qPCR = quantitative polymerase chain reaction SEM = scanning electron microscopy;