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Significance: Biofilms have been implicated in a variety of wound complications.
Recent Advances: Research has confirmed that biofilms form in wounds of patients experiencing delayed healing and may be a precursor to infection.
Critical Issues: Research into the strength of this association is still in its infancy. Is biofilm formation a cause of these complications, a step toward them, or a signal that unresolved factors injuring tissue or delaying healing are setting the stage for biofilm formation, infection, and healing delay? To qualify biofilms for use in informing clinical practice decisions, biofilm characteristics supporting those decisions need standardized definitions and valid evidence that they predict or diagnose healing or infection outcomes. Literature searches of relevant terms reviewed biofilm definitions and validation of their role in predicting and diagnosing delayed wound healing or infection.
Future Directions: Further research is needed to provide a rapid accurate technique to identify and characterize biofilms in ways that optimize their validity in diagnosing or screening patient risk of infection or delayed healing and to inform clinical decisions. This research will help validate biofilm's capacity to support wound care clinical practice decisions and establish their importance in guiding clinical practice.

SCOPE AND SIGNIFICANCE

Evidence indicates that biofilms exist in a majority of chronic wounds.[1,2] While developing evidence-based wound infection guidelines, the Association for the Advancement of Wound Care Guideline Task Force observed evidence gaps supporting biofilm-based clinical decisions to diagnose, screen, or treat wounds at risk of delayed healing or infection.[3] There is no point-of-care tool to confirm the wound biofilm presence. Research to date has focused primarily on methods to manage biofilm, while preclinical research has examined its impact on wound healing. Research needs to clarify risk factors for biofilm formation and its impact on optimizing management practices in nonhealing and/or infected wounds.

TRANSLATIONAL RELEVANCE

Translational research[4–6] has shown that when biofilm is deliberately introduced into controlled wound models *in vivo*, infection may ensue, healing is delayed, and interventions to remove wound biofilm reverse this delay.[7] In subjects with comorbidities, wound healing

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has been observed to be delayed more so than in wild-type subjects,[8–10] suggesting interactions between host factors and microbial bioburden. Therefore, further translational research is needed to ascertain what host–environment–organism interactions are required for wound biofilms to develop and compromise wound infection status and healing progression.

CLINICAL RELEVANCE

Observation of biofilms in complex chronic wounds is difficult, requiring a trained clinical eye if visible[11–13] and expensive, highly technical microscopes if microscopic.[1,2] However, biofilms can readily be visualized on the surface of long-term urinary catheters and this has been directly associated with an increased infection rate.[14] Verifying catheter biofilm as a primary source of urinary tract infection relies on clinical signs of infection, direct visual observations of catheter biofilm, and urine analysis. Similarly, by better understanding the clinical signs of wound biofilm[15] and utilizing future point-of-care tools to confirm wound biofilm, management practices and patient care can be optimized.

BACKGROUND

Biofilm is defined as "a structured community of microbial cells enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface."[16] The role of biofilm as a precursor, a signal, or a cause of delayed wound healing or progressive infection interacting with host, environment, or microbial variables (Fig. 1) needs more scientific and clinical clarification to provide full support for evidence-based clinical practice to improve wound outcomes. While it is accepted that further translational research and clinical studies designed to investigate factors and relationships between biofilm presence and wound recalcitrance are warranted, the current knowledge base is sufficient to ensure that patients benefit from biofilm-based wound management practices in the interim.

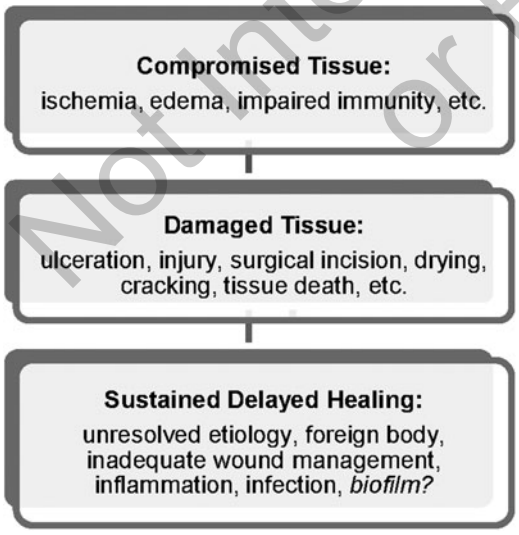

Figure 1. Biofilm in perspective of factors involved in delayed wound healing.

Objective

To critically review the literature to determine the clinical usefulness of wound biofilm in accurately guiding decisions as well as identify the research needed to improve infection and wound healing outcomes.

DISCUSSION

Methods

Search and selection process: to assess the validity, reliability, and efficacy of wound biofilm in guiding wound care decisions, authors searched the MEDLINE, CINAHL, and Scopus literature databases from 1966 to January 10, 2014, for articles containing the term "biofilm" combined with the terms "wound infection" or "wound healing." Additional searches combined the last two terms with "sensitivity," "specificity," or "predict" to capture studies validating sensitivity or specificity of biofilms in promoting delayed wound healing or clinical infection or positive or negative predictive validity used clinically on admission to practice to identify individuals at risk of delayed healing or infection. Abstracts were read and searched for the terms "sensitivity," "specificity," "reliability," or "predict."

Any study was included if it objectively documented wound biofilm's reliability and/or validity for predicting infection or diagnosing or predicting documented delayed healing. Clinical signs and symptoms were used to define the outcome "wound infection" rather than a biopsy microbial burden of $\geq 10^5$ colony forming units. The latter is often isolated from healing wounds without clinical infection signs (false-positive results) and often not isolated from early clinical infections.[17,18] Consequently, biopsy was not used as a standard for screening or diagnosing clinically recognized wound infection. This is important because false positives could result in unnecessary antibiotic use placing patients at future risk of harboring antibiotic-resistant strains of organisms. Case reports, abstracts of meetings, review articles, and non-English language articles were excluded as well as studies that did not contain objective characterization of one or more

clinical signs of wound infection (increased pain, heat, purulent exudate, odor, or swelling[19]) or unexplained delayed healing for chronic wounds.

Results

A CONSORT diagram of biofilm evidence found in the literature search is presented in Figure 2.

Definitions

The literature search found the following definitions of biofilm:

- Structured community of microbial cells enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface.[16]
- A complex microbial community, consisting of bacteria embedded in a protective matrix of sugars and proteins, known to form on the surface of medical devices and in wounds.[20]
- A coherent cluster of bacterial cells embedded in a biopolymer matrix, which compared with planktonic cells, shows increased tolerance to antimicrobials and resists the antimicrobial properties of the host defense.[21]
- Communities of bacteria attached to a surface, embedded in a self-produced extracellular polysaccharide matrix.[22]

A composite operational definition of wound biofilm consistent with currently available literature is: communities of surface-attached or self-attached microorganisms, embedded within a hydrated matrix of extracellular polymeric substances (or slime), which provides protection against antimicrobial agents and host defenses.

State of the science

Biofilm and wound infection. Costerton first described the link between biofilm and infections,[22] and Hall-Stoodley *et al.* have recently revisited diagnostic guidelines for biofilm-associated infections.[23] Based on their clinical and scientific observations in wounds, Bjarnsholt *et al.* hypothesized that *Pseudomonas aeruginosa* biofilm is prevalent in wound infections and is a causal factor in wound chronicity, particularly deeper in the wound bed.[24] While this theory has been challenged by others who consider wound biofilm to be problematic due to its multispecies nature—and initially more surface-associated[25]—the notion that biofilm can cause wound infections is generally accepted.[26] Host defenses are less effective against biofilm bacteria than they are against planktonic bacteria, and biofilm bacteria are more tolerant to antiseptics and antibiotics than planktonic bacteria.[27] The combination of compromised host defenses, unresolved tissue damage, and the stubborn tolerance of biofilm to external antimicrobial agents places subjects with chronic wounds and biofilm at particularly high infection risk. Biofilm progression in local tissue is likely to be associated with local inflammatory activity and the production of microbial toxins that causes tissue damage[28]; surface-associated biofilm may shed biofilm fragments or planktonic cells that can seed adjacent tissues and surfaces.[47,48]

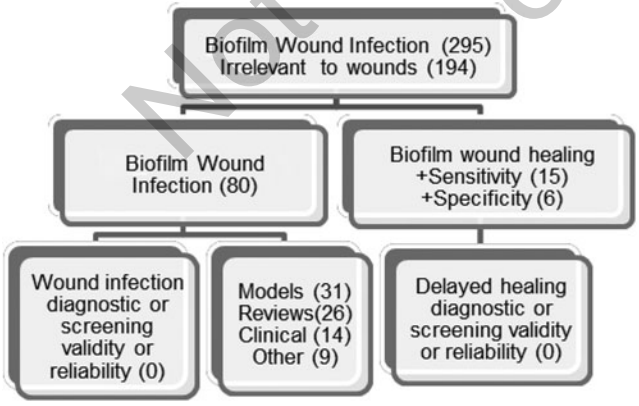

Figure 2. CONSORT diagram of MEDLINE, CINAHL, and Scopus biofilm search results (number of citations found).

Biofilm and wound healing. A growing body of *in vivo* evidence now exists that implicates biofilm in delayed wound healing.[4] Various animal models are providing insight into the ways in which biofilms can delay healing. The multispecies biofilm has been shown to be more pathogenic than single species,[29] and different species exhibit different levels of biofilm virulence.[30] Elgharably *et al.* provided the first direct evidence of biofilm involvement in human deep sternal wound infection.[49] It has been demonstrated that biofilm impairs key healing processes such as the inflammatory immune response, granulation tissue formation, and epithelialization.[5] Biofilm has been shown to tolerate antibiotics and topical antiseptics in isolation, although multimodal strategies to suppress biofilm were more effective.[7]

Biofilm and moisture. While moisture is essential for optimal wound healing, poor moisture/exudate control within a wound environment is likely to encourage the development of biofilm.[11,12] Historically, the appropriate use of moisture-retentive dressings (*e.g.*, hydrocolloids) has been shown to significantly reduce the likelihood of infection and healing time in randomized controlled trials compared to gauze-dressed clinical acute[31] and chronic

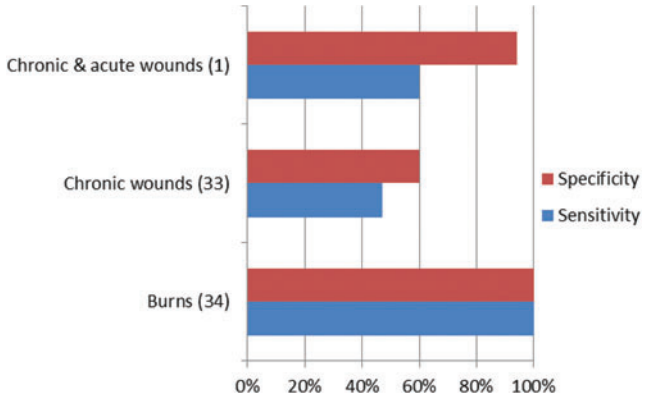

Figure 3. Diagnostic validity of wound biofilm.[1,33,34] Biofilm currently lacks the positive and negative predictive validity to support clinical screening decisions needed to improve wound healing or infection outcomes. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

wounds.[32] Consequently, moisture balance is essential to optimize the wound environment for healing and minimize the opportunity for biofilms to develop.

Diagnostic validity of wound biofilm

Three small studies explored diagnostic validity of biofilm presence in mixed chronic wounds[1,33] or burns[34] experiencing delayed healing. As may be expected given the early stage of biofilm research, no studies were found reporting reliability or predictive or validity of any aspect of biofilm development for screening chronic or acute wounds likely to (a) become infected or (b) experience delayed healing (Fig. 3). A study by Wolcott and Rhoads reported 77% of 190 subjects with critical limb ischemia (peri-ulcer $TcPO_2 < 20$ mmHg) healed during a 5-year trial after receiving at least five episodes of biofilm-based wound care.[35] This was reduced to 67% healed in the 79 subjects who followed the

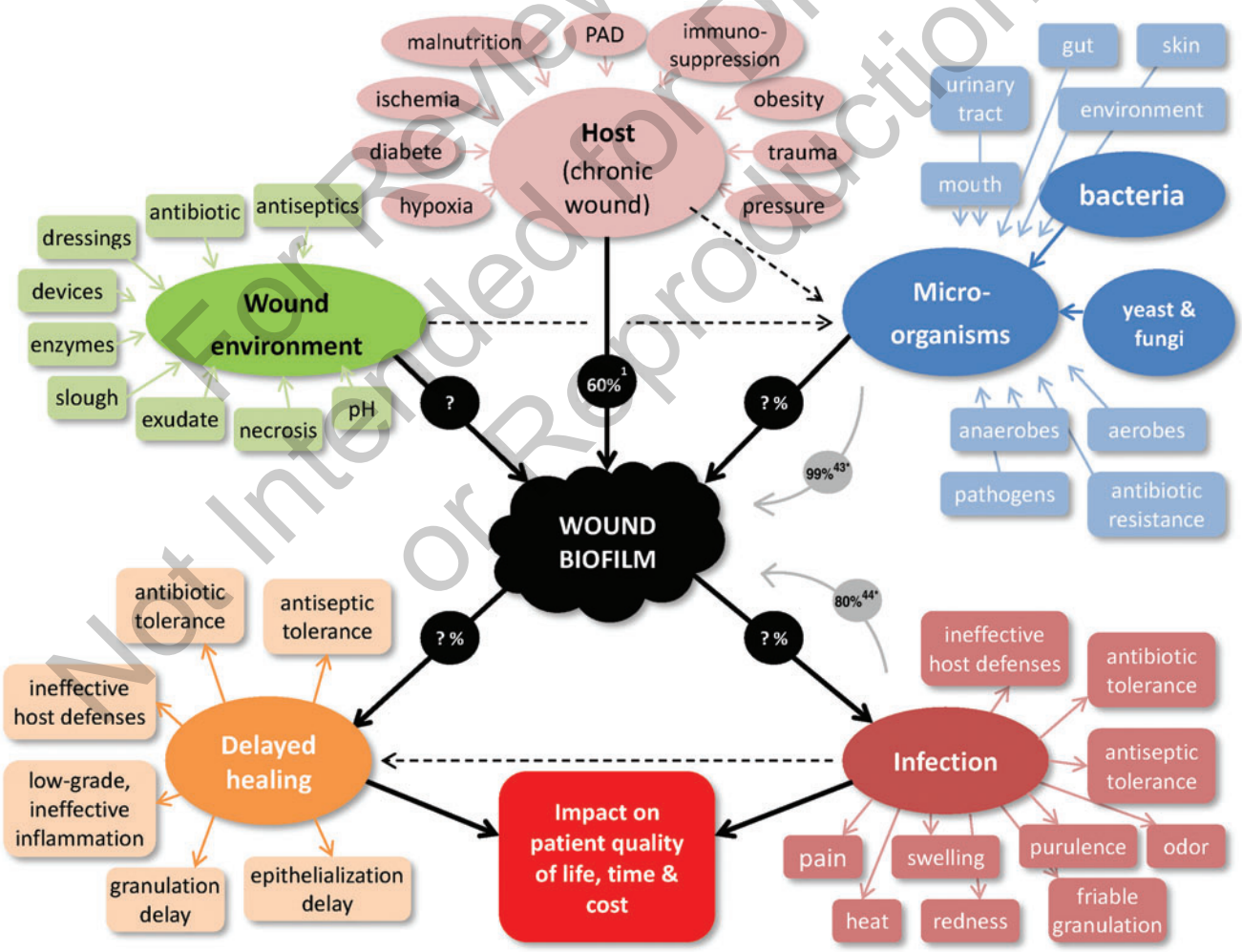

Figure 4. Mind map exploring the links between wound biofilm and factors that lead to its development and the effects it has on the wound and ultimately, the patient quality of life. *See references 43 and 44. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

protocol with both diabetes and osteomyelitis, underscoring the importance of host and wound environment variables, in addition to biofilm, in delaying healing.

Lessons learned

Wound biofilm has been documented as emerging in acute wound drains within 2 h after surgery[36] in damaged tendons[37] and in chronic[1,2,33,34] and acute[1,49] wounds. However, there is still a need for further evidence-based knowledge in improving clinical wound or patient outcomes. In addition to urinary tract infection research,[14] wound biofilm research may learn from parallels of early research on catheter-related bloodstream infections (CR-BSI), which determined that biofilm formation on catheters is a precursor of CR-BSI that is established for longer than 10 days.[38,39] Despite there being no method to detect catheter biofilms, local and systemic signs of infection[40] are used in conjunction with paired quantitative blood cultures to provide the highest accuracy of biofilm-related CR-BSI.[41] Wound biofilm research may benefit by following a similar trajectory in the absence of a simple, rapid, reliable biofilm detection method.

Detection of wound biofilm may empower wound care providers to avert infection before its clinical signs manifest such as elevated temperature, suppuration, increasing wound pain, erythema or edema, or unexplained healing delay. Wounds that do not heal despite rigorously applied standards of care may alert healthcare providers to examine the wound for direct or indirect signs of biofilm[15] or inert foreign matter such as sutures or gauze fibers that may act as a biofilm substrate.[42] Healthcare providers may therefore use nonhealing, local temperature elevation, or suspected low-grade inflammation as indirect clinical indicators of wound biofilm,[15] thus informing care while avoiding false positives leading to unnecessary antibiotic use. Future biofilm detection may enhance screening or diagnostic validity or reliability of current tools that alert clinicians to impending wound infection or healing delay.

A mind map has been considered to explore the complex interrelated causes of delayed healing and infection, and to

TAKE-HOME MESSAGES

What we know about wound biofilm

- Biofilms can form in chronic wounds[1,2,33,34] and, less frequently, in acute wounds.[49]
- Biofilm presence means that stubborn communities of microorganisms are established that are tolerant to antibiotics, antiseptics, and inflammatory processes.
- Biofilm development has been shown to delay healing[15] and can lead to infection.[26]
- Addressing biofilm requires management of host and environmental factors that led to their development (Fig. 4) as well as wound cleansing, debridement, or antimicrobial use.
- Biofilm can be visualized using a variety of microscopy techniques, including fluorescent staining with confocal microscopy and peptide nucleic acid fluorescent *in situ* hybridization,[2,45] scanning electron microscopy,[1] and light microscopy in conjunction with gram staining.[46]
- There is growing evidence for how best to recognize biofilms in clinical wounds based on wound recalcitrance, recurring infection, ineffectiveness of antibiotics, and increasing or excessive wound fluid.[11,12,15,50]
- Diagnostic confirmation of the presence of biofilm in a wound will be useful in supporting decisions that improve clinical outcomes in wounds that do not appear to be healing normally, while conserving limited clinical and economic resources.
- All local and underlying causes of delayed wound healing should be addressed to reduce the likelihood of biofilm-related delayed wound healing or infection.

Opportunities for wound research on biofilms

- What do clinicians need to know about biofilm to improve wound care outcomes?
- What is its importance in clinical wound management as a screening or diagnostic tool or as a signal to address more rigorously the factors that led to biofilm development?
- How can clinicians use biofilm information to improve patient and wound outcomes beyond those obtained using currently available diagnostic or screening tools?
- Under what circumstances is clinical biofilm development not likely to delay healing or lead to infection? Knowing this may prevent unnecessary treatment.
- Point-of-care biofilm detection tools to facilitate biofilm visualization and optimal wound management.
- Establishment of optimal techniques for identifying biofilms in recalcitrant wounds and optimizing their predictive validity to inform clinical decisions.
- What sort of patient assessments or local wound therapies can reliably prevent delayed healing or progression to infection associated with biofilm formation?
- How do biofilms interact with slough, necrotic tissue, sutures, or other foreign bodies? Do they underlie or potentiate infection or protect from host immune attack?
- Compare autolytic with other debridement methods in quality randomized clinical trials (RCTs) to strengthen evidence for debriding wound biofilms and the compromised tissue on which they thrive.
- Compare methods of managing wound biofilms in RCTs to find which methods have the greatest efficacy, safety, and cost effectiveness for managing biofilm.
- Continue to develop *in vitro* and *in vivo* wound biofilm models that reflect the clinical situation in nonhealing wounds.

help identify host and wound environmental factors that increase the predictive and diagnostic validity of biofilms for early screening of patients at risk of delayed healing or wound infection (Fig. 4). The capacity of biofilm formation to predict delayed healing may increase in subjects with conditions such as impaired arterial or venous circulation or diabetes. Wound surface biofilm may serve as an early signal to alert clinicians that the wound has diverted off its normal healing path before a reduced rate of wound contraction is noticed after 2–4 weeks of treatment. Figure 4 highlights interrelated variables that can be used as a guide to generate an effective approach to optimize screening and diagnostic value of wound biofilm. Detection of early (young) biofilms may identify at-risk patients and enable intervention before recalcitrance and/or infection become a problem.

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Abbreviations and Acronyms

AAWC GTF = Advancement of Wound Care Guideline Task Force
CR-BSI = catheter-related bloodstream infections
RCT = randomized clinical trial