CME

A Formidable Foe Is Sabotaging Your Results: What You Should Know about Biofilms and Wound Healing

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Learning Objectives: After reading this article, the participant should be able to: 1. Describe biofilm pathogenesis as it relates to problem wounds. 2. Understand the preclinical and clinical evidence implicating biofilm in problem wounds. 3. Explain the diagnostic and treatment challenges that biofilms create for problem wounds. 4. Demonstrate a basic understanding of emerging strategies aimed at counteracting these processes.

Summary: Biofilm represents a protected mode of growth for bacteria, allowing them to evade standard diagnostic techniques and avoid eradication by standard therapies. Although only recently discovered, biofilm has existed for millennia and complicates nearly every aspect of medicine. Biofilm impacts wound healing by allowing bacteria to evade immune responses, prolonging inflammation and disabling skin barrier function. It is important to understand why problem wounds persist despite state-of-the-art treatment, why they are difficult to accurately diagnose, and why they recur. The aim of this article is to focus on current gaps in knowledge related to problem wounds, specifically, biofilm infection. (*Plast. Reconstr. Surg.* 139: 1184e, 2017.)

The Centers for Disease Control and Prevention estimate that 65 percent of all human infectious disease is caused by bacteria with a biofilm phenotype, and the National Institutes of Health estimate that this number is closer to 80 percent.[1] Using this information, the collective toll of biofilm infection in the United States is estimated at 17 million infections and 550,000 deaths.[2] However, diagnosing biofilm infection is a significant clinical challenge. Definitive diagnosis requires visual confirmation of adherent bacteria encased in extracellular polymeric substance[3] using imaging methodologies that are not clinically available. In addition, biofilm-producing bacteria do not grow reliably in culture.[4–6] Thus, current clinical diagnostic tools are insufficient to identify biofilm infection. Polymicrobial infection consistent with biofilm infection is common in chronic cutaneous ulcers.[7] Chronic cutaneous ulcers are a major public health threat that affect 2 percent of the population in the United States and globally.[8] The estimated cost of caring for the 6.5 million people in the United States with chronic cutaneous ulcers is $50 billion per year.[9] Despite the prevalence, cost, morbidity, and mortality for patients, there have been no new pharmacologic treatments for chronic cutaneous ulcers approved by the U.S. Food and Drug Administration for over 10 years.[10] We posit that one of the reasons clinical trials have failed and problem wounds remain clinically challenging is occult biofilm infection.

BIOFILM BIOLOGY AND PATHOGENESIS

The formation of in vitro biofilm has been modeled into several stages (Fig. 1).[11] Initially, planktonic (free floating) bacteria reversibly attach to a surface in a monolayer, followed by irreversible attachment

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Related Video content is available for this article. The videos can be found under the "Related Videos" section of the full-text article, or, for Ovid users, using the URL citations published in the article.

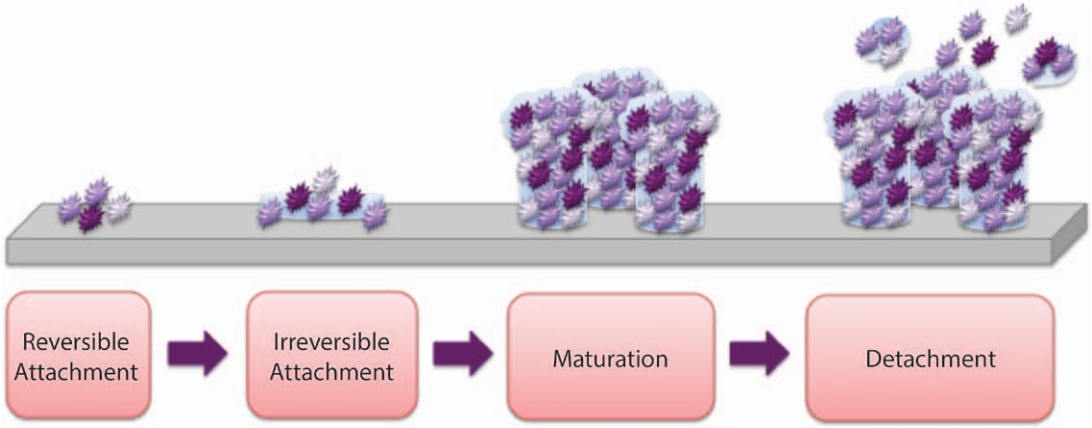
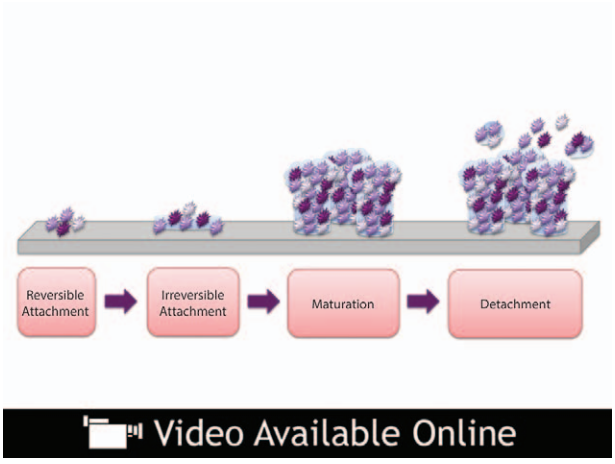


Fig. 1. Stages of biofilm development. Transient adhesion to a surface is followed by robust, irreversible attachment and the formation of microcolonies through clonal growth. Bacteria collectively communicate through a process known as quorum sensing to secrete extracellular polymeric substance to form a mature biofilm. Biofilm structures can be flat or mushroom-shaped. The last stage is characterized be a return to motility where either individual cells, or cells encased in small aggregates of the extracellular polymeric substance material, are sloughed. (Based on stages as described by Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as complex differentiated communities. *Annu Rev Microbiol*. 2002;56:187–209. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.)

and clonal growth into microcolony aggregates.[12] (**See Video, Supplemental Digital Content 1**, which reviews the stages of biofilm development and the criteria for diagnosing biofilm infection, and shows scanning electron microscopic images to demonstrate what a biofilm infection appears like. This video is available in the "Related Videos" section of the full-text article on PRSJournal.com or at ***http://links.lww.com/PRS/C139***.)

This process is mediated by large shifts in gene expression patterns that regulate phenotypic changes in the bacterium. *Pseudomonas aeruginosa* is capable of altering the expression of as many as 800 proteins during this process.[13] The initiation of a biofilm can occur within a matter of hours.[14] Bacteria within the biofilm communicate through a process known as quorum sensing, and collectively secrete a matrix composed of proteins, polysaccharides, and extracellular DNA called extracellular polymeric substance[15] that encases the microcolonies. A significant portion of the biofilm is composed of water channels, which function as a complex distribution system for oxygen and nutrients.[16,17] The biofilm proceeds through maturation phases, where mushroom-like structures develop.[18] Once the environment is no longer optimal for bacterial survival, as in the instance of nutrient exhaustion, bacteria either actively detach and disperse from the biofilm[11,19] or are detached by fluid shear forces and are separated from the larger structure in matrix-protected aggregates.[20]



Video. Supplemental Digital Content 1 reviews the stages of biofilm development and the criteria for diagnosing biofilm infection, and shows scanning electron microscopic images to demonstrate what a biofilm infection appears like. This video is available in the "Related Videos" section of the full-text article on PRSJournal.com or at ***http://links.lww.com/PRS/C139***.

The criteria proposed by Parsek and Singh to define biofilm infection (Fig. 2) are now widely accepted[3] (**see Video, Supplemental Digital Content 1**, ***http://links.lww.com/PRS/C139***). These include (1) adherence of infecting bacteria to a surface, (2) direct visual evidence that bacteria are encased in extracellular polymeric substance, (3) confinement of the infection to a particular location, and (4) demonstration of

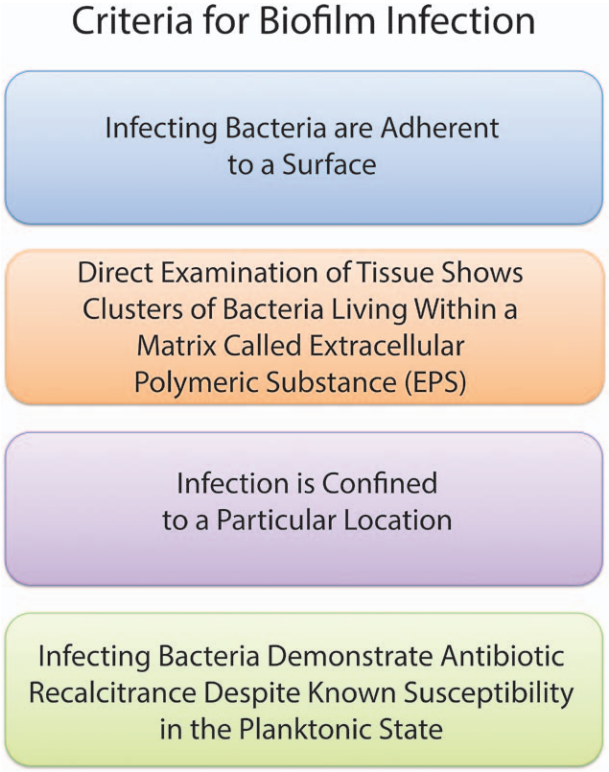


Fig. 2. Criteria for biofilm infection as described by Parsek and Singh (Parsek MR, Singh PK. Bacterial biofilms: An emerging link to disease pathogenesis. *Annu Rev Microbiol*. 2003;57:677–701).

antibiotic recalcitrance despite known susceptibility of the bacteria in the planktonic state. The fact that bacteria in a biofilm state are adherent and confined to a particular location indicates that they will not cause bacteremia or systemic manifestations of infection while in a biofilm state. Bacteria that are released from biofilm in a planktonic state can cause bacteremia; thus, the presence of a biofilm infection is not a benign finding and represents a potential risk of systemic infection. Any claims of biofilm infection or eradication should be held to the standard of meeting the four criteria of Parsek and Singh.[3]

CLINICAL EVIDENCE FOR BIOFILM INFECTION IN WOUNDS

An inciting injury or wound is not required for biofilm infection to develop. Biofilm can form in the context of a number of tissue types in the body, including wounds, the respiratory and sinus tracts, gastrointestinal mucosa, and on implantable and injectable materials. Biofilm is implicated in a number of disease states, including implant-associated infections, otitis media, cystic fibrosis, bacterial endocarditis, and infectious nephrolithiasis, in addition to osteomyelitis and problems wounds.[3,19,21–24]

The first evidence for biofilm formation in human wounds was described by James et al. and Bjarnsholt et al. in 2008.[23,25] The former used epifluorescent microscopy and scanning electron microscopy and observed biofilm formation in biopsy specimens from 30 of 50 problem wounds (60 percent).[23] Bjarnsholt et al. also provided evidence for biofilm in problem wounds using fluorescence in situ hybridization to observe *Pseudomonas aeruginosa* in the form of microcolonies, now known to be characteristic of biofilm.[25] Kathju et al. subsequently described the presence of biofilm, using confocal laser scanning microscopy and fluorescence in situ hybridization on retained sutures that were removed from a problem, nonhealing surgical-site infection.[26] Complete débridement and removal of the foreign suture material resulted in resolution of the wound. The same authors also demonstrated that biofilm contributes to wounds associated with mesh infections in the context of abdominal hernia repair.[27] Recently, Elgharably et al. evaluated the role of biofilm in sternal wound infections occurring after median sternotomy[5] (**see Video, Supplemental Digital Content 1**, ***http://links.lww.com/PRS/C139***). The authors prospectively enrolled six patients with sternal wound dehiscence and three patients without infectious complications undergoing repeated sternotomy for elective cardiac surgery. Using scanning electron microscopy and confocal laser scanning microscopy, staphylococcal biofilm infection was detected on the sternal wires of all six patients with wound dehiscence and none of the wires from the three patients without infectious complications (Fig. 3). Further work has demonstrated evidence for biofilm formation in burns,[28] diabetic and venous ulcers,[29–32] and malignant wounds associated with breast cancer.[33] It has been suggested that biofilm may contribute to oncologic transformation in the setting of gastrointestinal disease, but this has not been investigated in the context of cutaneous transformation (i.e., Marjolin ulcer).

THE CLINICAL CHALLENGES OF BIOFILM INFECTION

Biofilm presents specific challenges for plastic surgeons (Fig. 4) that render biofilm-infected wounds difficult to diagnose and difficult to treat.

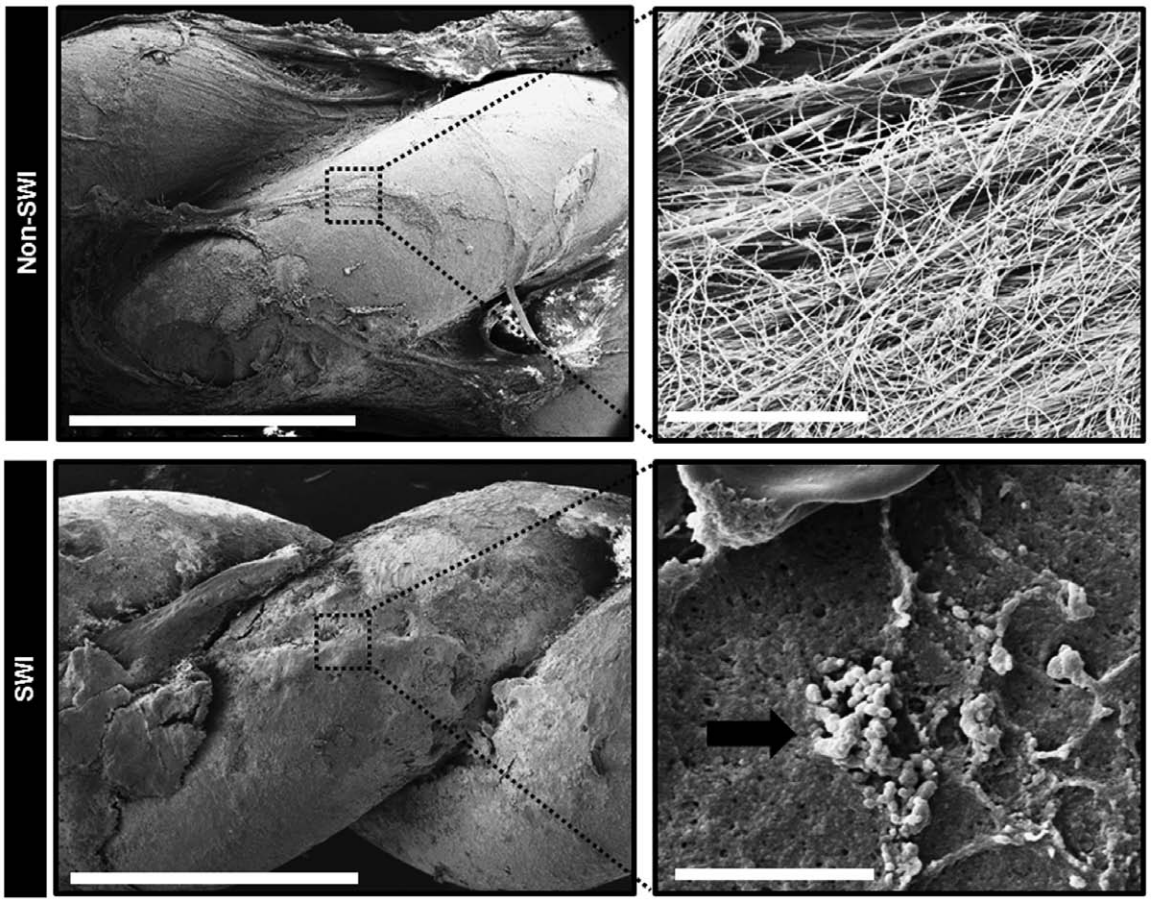


Fig. 3. Scanning electron microscopic detection of biofilm infection. Sternal wires were removed from patients undergoing elective sternotomy without wound infection (*non-SWI)* or with open sternal wounds caused by infection (*SWI*) and evaluated by scanning electron microscopy. Sternal wires removed from patients without infection have a patchy coating of proteinaceous strands with no bacteria seen on higher magnification. Wires from open sternal wounds caused by infection have a thick biofilm coating with evidence of bacterial cocci beneath biofilm (*arrow*). *Left*, original magnification, × 60 (*scale bar* = 1 mm); *right*, original magnification, × 10,000 (*scale bar* = 5 μm). (Reprinted with author permission from Elgharably H, Mann E, Awad H, et al. First evidence of sternal wound biofilm following cardiac surgery. *PLoS One* 2013;8:e70360.)

Biofilm Is Infrequently Detected by Routine Culture

Unlike infection caused by bacteria in a planktonic state, standard clinical culture techniques are insufficient to diagnose biofilm infection.[34–36] Elgharably et al. confirmed the presence of biofilm infection on sternal wires using scanning electron microscopy and confocal laser scanning microscopy, but of those six patients, only two had positive wound cultures that grew staphylococci. The other four patients had negative cultures (Table 1).[5] Emerging methods of molecular biofilm detection include nucleic acid amplification techniques, such as polymerase chain reaction, molecular detection of biofilm-associated molecules (such as quorum-sensing molecules), and fluorescent in situ hybridization to detect species-specific bacterial ribosomal DNA, among others.[37] Techniques that rely on the detection of bacterial elements alone are not sufficient to detect biofilm formation. For example, polymerase chain reaction may detect the presence of bacterial DNA, but this does not confirm that the bacteria detected are part of a biofilm structure. Thus, microscopic techniques (e.g., scanning electron microscopy), which directly visualize polymicrobial bacteria present in aggregates and indicate the extent of extracellular polymeric substance and biofilm formation on a surface, remain the gold standard for diagnosing biofilm infection.[37] Unfortunately, there are no routinely available techniques to diagnose biofilm infection in the clinical setting, and this is an important area for future research and development.

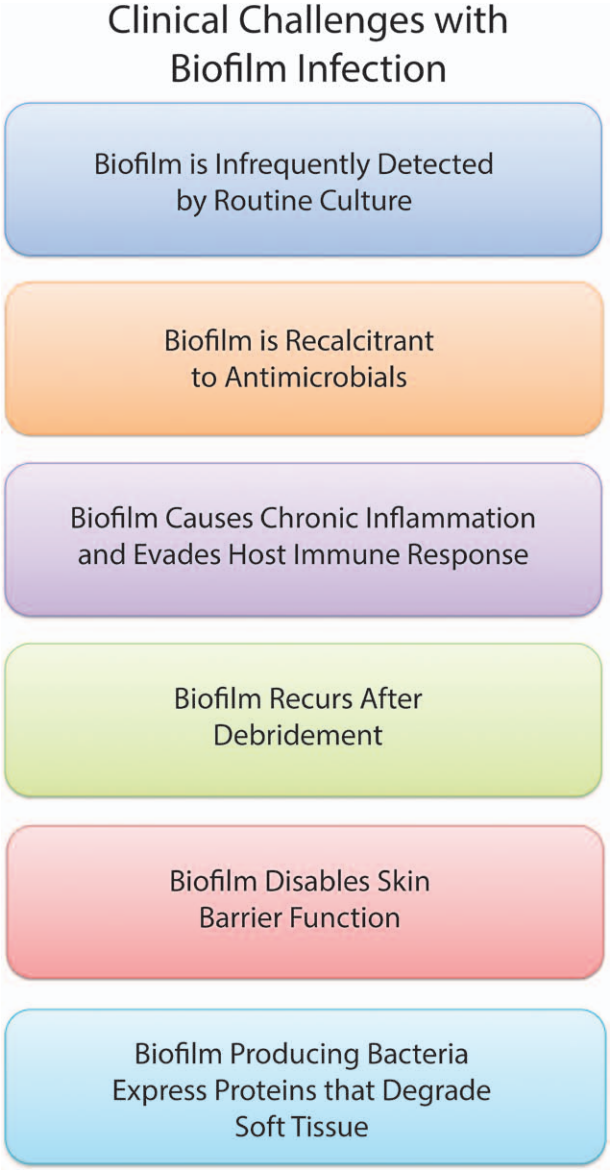


Fig. 4. The clinical challenges posed by biofilm infection.

Biofilm Creates Bacterial Recalcitrance to Antimicrobials

Biofilm is clinically problematic because it reduces the susceptibility of bacteria to antimicrobials. This includes topical antimicrobial dressings, such as silver-impregnated dressings.[6] There are two mechanisms that contribute to this recalcitrance: antimicrobial resistance and antimicrobial tolerance.[38,39] Antimicrobial resistance refers to the ability of bacteria to grow despite the presence of antibiotic because of inherent characteristics of the bacteria.[39] Examples of this include the expression of enzymes that degrade antibiotics, such as beta-lactamases, the expression of drug efflux systems that decrease the intracellular concentration of antibiotics, or through evolved mutations that modify the antibiotic's target. Resistance genes are thought to be more readily exchanged in the biofilm state because of the high concentration of extracellular DNA and the biofilm's structural stabilization that facilitates horizontal gene transfer.[3]

Antimicrobial tolerance, however, is the predominant mechanism for antimicrobial recalcitrance in the biofilm state.[39] Tolerance refers to the ability of bacteria to avoid cell death despite known susceptibility to the antibiotic because of the physical state of the bacterium. The slow growth rate of bacteria within biofilm decreases the efficacy of antibiotics that target rapid cell division, such as beta-lactam antibiotics that interfere with cell wall synthesis. Furthermore, biofilms do not simply act as diffusion barriers to antimicrobials. Distinct mechanisms exist that resist antimicrobial action. Macromolecules within extracellular polymeric substance, including extracellular DNA, are known to either bind antibiotics and interfere with their function, such as the aminoglycosides,[40–42] or provide physical barriers that protect bacteria from antibiotic exposure.[43] However, antimicrobial recalcitrance caused by the presence of biofilm is largely a reversible phenomenon and resolves when bacteria return to the planktonic state after biofilm disruption (e.g., following débridement).[44,45]

Biofilm Infection Evades Host Immune Response and Induces Chronic Inflammation

Nonhealing wounds contain elevated levels of proinflammatory cytokines and proteases and excessive neutrophils. Biofilms have evolved to be directly capable of inducing these changes by manipulating the host immune response. For example, *Pseudomonas aeruginosa* quorum-sensing molecules can directly induce expression of proinflammatory cytokines from host cells.[46] Biofilms release planktonic bacteria, lipopolysaccharide, quorum-sensing molecules and other exotoxins, and bacterial DNA into the local environment, resulting in recruitment of neutrophils to the wound site.[47,48]

Neutrophils are present in abundance in chronic wounds[49,50] but are rendered ineffective by biofilm in multiple ways. Reactive oxidants produced by phagocytic cells do not penetrate the extracellular polymeric substance. Furthermore, biofilm also prevents the appropriate clearance of neutrophils by macrophages.[13,51] Their subsequent aberrant degradation contributes to the release of proteases that are characteristically elevated in problem wounds and that negatively impact healing. Interestingly, some bacteria

Table 1. Demographic Characteristics of Patients ($n = 9$) and Sternal Wound Infection Status*

Subject	SWI	Age (yr)	Sex	BMI (kg/m^2)	Associated Medical Conditions	Procedure	Antimicrobial Therapy	Time Interval between Procedure and Débridement (wk)	Wound Culture	Blood Culture	Figure in Which Data Are Shown
SW001	Yes	60	M	34.7	CAD, DM, HTN, HLD, RF	CABG	Nafcillin, daptomycin	5	MSSA	N	3, *above*, *left*, 4, and 6
SW002	Yes	84	F	40.9	CAD, HTN, HLD, RD, COPD	Redo-MVR	Ertapenem	2	Negative	N	6
SW003	Yes	61	F	18	CAD, HTN, HLD, PVD	LVAD	Vancomycin, ciprofloxacin, sulfamethoxazole-trimethoprim, linezolid	12.1	No growth	N	7
SW004	Yes	54	M	27	DM, HTN, RD, OSA	CABG	Piperacillin-tazobactam, vancomycin	3.1	MRSA	N	3, *above*, *right* and 5
SW005	Yes	64	M	25.1	CAD, COPD, HLD, DM	PM, Repair of RV	Piperacillin-tazobactam, vancomycin, daptomycin	5.4	No growth	N	3, *above*, *left*
SW006	No	28	M	23.7	END, SEP	Excision scar					Not shown
SW007	Yes	43	F	41	HTN-P, HTN, RHD	MVR	Linezolid	9.2	No growth	MRSA	6
SW008	No	46	F	50.2	HTN-P, OSA, GERD,AKI	LRB					7
SW009	No	31	M	20.7	CGH, SVT	AVR					5

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SWI, sternal wound infection; BMI, body mass index, M, male, F, female; CAD, coronary artery disease; CGH, coronary heart disease; DM, diabetes mellitus; END, endocarditis; SEP, xxx; GERD, gastroesophageal reflux disease; HTN, hypertension; HTN-P, pulmonary hypertension; HLD, hyperlipidemia; RD, renal dysfunction; COPD, chronic obstructive pulmonary disease; PVD, peripheral vascular disease; OSA, obstructive sleep apnea; RHD, rheumatic heart disease; CABG, coronary artery bypass graft; MVR, mitral valve replacement; LVAD, left ventricular assisted device; PM, pacemaker; RV, right ventricle; N, negative; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; SVT, supraventricular tachycardia; AKI, acute kidney disease.

require the presence of these proteases to facilitate biofilm formation.[52] The benefit gained from host-immune manipulation is the formation of a parasitic relationship allowing for a sustained growth environment in the nonhealed wound and a sustained nutrient source through the influx of immune-induced protein-rich plasma exudate.

Biofilm Recurs after Débridement

In a preclinical porcine burn model of biofilm infection with *Pseudomonas aeruginosa*, standard-of-care débridement performed by a plastic surgeon was insufficient to eradicate biofilm. The authors observed a temporary decline in bacterial burden; however, biofilm can be regenerated by only a few remaining bacteria, and infection returned to predébridement levels after 48 hours. Moreover, new microcolonies were discovered within deeper tissue layers, after débridement, raising the concern that sharp débridement might result in inoculation into deeper tissue and persistent infection.[6] Indeed, pathogenic biofilms in tissues appear to be more likely to exist as semisolid microcolonies within tissue rather than strictly adherent to a wound surface, and are often located deeper within wounds.[18,29,30,38] A direct comparison between different débridement modalities, including sharp débridement, hydrosurgical débridement, or ultrasound-mediated disruption of biofilm, has not been performed to evaluate the efficacy of biofilm eradication and its potential effect on clinical outcomes.

Biofilm Disables Skin Barrier Function

The primary functions of skin include the following: thermoregulation, protection against evaporative water loss, and a barrier against pathogenic organisms. Biofilm compromises skin integrity by interfering with skin permeability, and negatively impacts the latter two functions.[6] Despite the fact that wounds may appear closed, it is now evident that gross visual inspection does not reflect the functional integrity of the skin. Transepidermal water loss provides a more objective and functional measurement of skin integrity than visual assessment alone.[53] Elevated transepidermal water loss is suggestive of increased skin permeability and potentially impaired function that not only allows egress of water, but can also allow bacteria to penetrate below the skin. Biofilm-producing strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* the two most common isolates from burn wounds,[54] induce human keratinocyte expression of micro-RNAs (miR-146a and miR-106b) that down-regulate the expression of tight junction proteins zona occludens-1 and zona occludens-2, causing elevated transepidermal water loss.[6] Tight junction proteins, along with gap and adherens junction proteins, are primarily responsible for skin barrier function in mammals.[55,56]

Biofilm-Producing Bacteria Express Proteins That Degrade Soft Tissues

Biofilm-producing bacteria are known to secrete ceramidase,[57–59] an enzyme that breaks down ceramide, further compromising skin integrity.[57] Ceramides are a major component of extracellular lamellar sheets present in the stratum corneum. They are a key component of the keratinization process in mammalian epidermis and function to maintain the permeability barrier and evaporative water loss functions of the skin.[60,61] When bacterial infection is in a biofilm form, it induces bacterial expression of proteases.[62–64] These bacteria-derived proteases can also activate host matrix metalloproteases[63,65–67] and stimulate neutrophil respiratory burst. The high numbers of neutrophils present in biofilm-infected wounds results in robust release of reactive oxygen species and host elastase that can degrade soft tissue. Thus, biofilm infection will promote tissue erosion. Clinical manifestations of this phenomenon are development of an open wound, such as sinus tracts or pressure ulcers, in areas overlying osteomyelitis because of biofilm infection. The diagnostic evaluation of a problem wound should include assessment for underlying osteomyelitis. Collectively, the inoculation of biofilm-producing bacteria into deeper tissues after débridement, with biofilm recurrence, the aggressive but dysfunctional immune response, the loss of skin barrier function, and biofilm-induced tissue degradation, all likely contribute to the high recurrence rate and flap and skin graft loss that is observed with problem wounds.[68–71]

THERAPEUTIC STRATEGIES

Case Scenario

The patient is a 17-year-old male wrestler with no significant medical history who underwent an orthopedic procedure for a ruptured patellar bursa. Postoperatively, the patient developed a surgical-site infection and subsequent nonhealing wound (**see Video, Supplemental Digital Content 1, *http://links.lww.com/PRS/C139***). Over the course of 5 months, the patient underwent removal of the patellar bursa and two attempts at skin grafting

without resolution. Wound biopsy specimens were obtained that demonstrated infection with methicillin-resistant *Staphylococcus aureus*. However, the wound did not respond to therapy with Bactrim (Hoffmann-La Roche, Inc., Basel, Switzerland), despite demonstrated sensitivity of the culture. At this point, the patient was referred to the senior author (G.M.G.) for evaluation (Fig. 5). Nonhealing wounds of this nature that are refractory to standard therapy are highly suggestive of occult biofilm infection. The patient responded to methicillin-resistant *Staphylococcus aureus* decolonization with 5 days of 4% chlorhexidine gluconate showers, 2% mupirocin applied to the nares and wound twice daily, oral Bactrim DS twice daily for 10 days, and serial wound débridements in the office.

This simple case highlights two important features of biofilm infection. First, bacteria do not require a compromised host/patient to establish biofilm infection. The capacity of the bacterial strain to produce biofilm can overcome innate immunity even in the healthiest patient. Second, débridement disrupts biofilm and the bacteria revert to a planktonic state.[13,72,73] During that time, they are susceptible to antibiotics. There is approximately a 48- to 72-hour window after sharp débridement and biofilm disruption before biofilm infection is reestablished.[6,73] A critical step in the management of problem wounds is to disrupt biofilm infection by débridement and then to take advantage of the therapeutic window by using topical and/or systemic antibiotics to eradicate the infection. Thus, the wound bed must be adequately prepared with débridement and eradication of biofilm infection before it can be closed either surgically or nonsurgically.

A novel method for addressing biofilm is the use of resorbable antibiotic-impregnated beads after débridement. In orthopedic surgery, biofilm represents a formidable challenge in the treatment of open extremity fractures and infected arthroplasties. Antibiotic-impregnated beads, placed into the wound after débridement, have been shown to elute high local concentrations of antibiotics, which can target biofilm much more directly than intravenous antibiotics.[74,75] They have also been shown to reduce infectious complications in open fractures[76] and diabetic foot ulcers.[77] Our case series below illustrates the application of this concept to the treatment of pressure ulcers with osteomyelitis.

Clinical Case Series

We have applied resorbable antibiotic beads into pressure ulcers after débridement and right before flap coverage for the past several years. We have compared the pressure ulcer recurrence rates at 12 months between patients who received antibiotic beads and those who did not. These patients were all treated by a single surgeon (G.M.G.) and received the same preoperative evaluations, surgical flaps for coverage, and the same postoperative care.

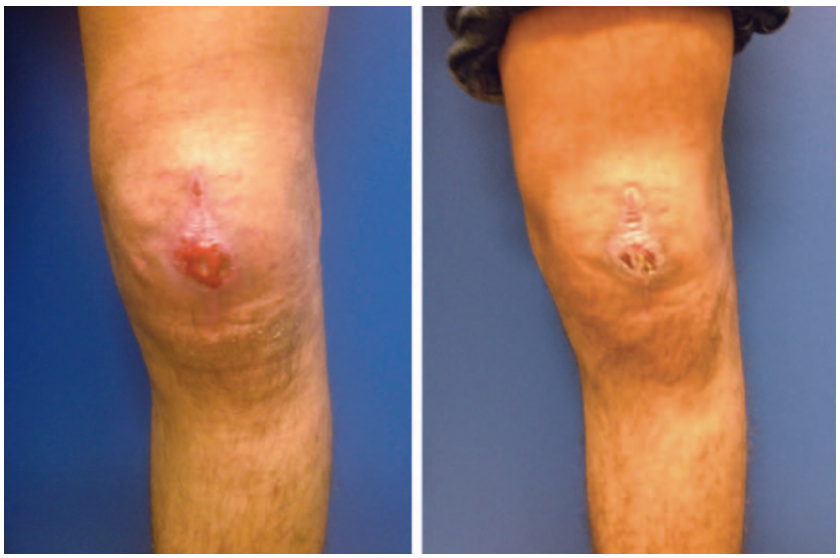

Fig. 5. Nonhealing postsurgical wound treated as a biofilm infection. Wound shown on presentation to the senior author (*left*) and 4 weeks later (*right*) after treatment with methicillin-resistant *Staphylococcus aureus* decontamination protocol and serial office débridements. The wound healed and did not recur.

In 104 patients who did not receive antibiotic beads, the recurrence rate at 12 months was 39.4 percent. In 16 sequential patients with focal osteomyelitis and pressure ulcers who received flap coverage with antibiotic beads, the recurrence rate at 12 months was 12.5 percent. The rate of pressure ulcer recurrence was compared between the two groups using chi-square analysis, with a value of $p < 0.05$ as a threshold for statistical significance. This difference was statistically significant ($p = 0.037$).

There is one U.S. Food and Drug Administration–approved antimicrobial wound dressing (Procellera; Vomaris, Inc., Tempe, Ariz.) that does not make therapeutic claims to treat biofilm but has been shown to inhibit biofilm formation in vitro.[78] This dressing works by generating a 1-V electrical field[79]; electrodynamic forces are known to disrupt biofilm formation.[80–83] There are many other potential therapeutic strategies for addressing biofilm infections that have recently been reviewed.[38] Areas of research include (1) topical application of agents that interfere with bacterial attachment such as medicinal honey or lactoferrin through iron sequestration; (2) quorum-sensing inhibitors such as synthetic furanones or medicinal honey; (3) lytic bacteriophage therapy, which uses viruses that are naturally destructive for bacteria; and (4) mechanical biofilm disruption through ultrasound-mediated or surgical débridement. At this time, there are no treatment interventions that have Level I evidence to support use in wounds and meet the Parsek and Singh criteria for biofilm infection and eradication.

CONCLUSIONS

Biofilm poses significant challenges for plastic surgeons. Biofilm disrupts normal wound healing by allowing bacteria to evade immune responses, prolongs inflammation, erodes tissues, and disables skin barrier function. It also complicates treatment options by causing antimicrobial recalcitrance and recurrence after débridement. There are no good ways to diagnose it; thus, treatment is initiated based on the clinical diagnosis alone. Although only recently described in the setting of chronic cutaneous ulcers and other problem wounds, the impact of biofilm is that it is common to *all* problem wounds regardless of cause, and occult biofilm infection may contribute to unexplained flap or graft loss. Understanding how biofilm affects the wound bed and using débridement and antimicrobial strategies to eradicate that infection will help plastic surgeons achieve better results when managing problem wounds.

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