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c0003 # Biofilms in wounds and wound dressing

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s0010 3.1 Introduction

p0010 The purpose of this chapter is to provide a primer on biofilms in wounds. Overall, it defines and describes key features of microbial biofilm, its impact on wound healing and wound healing strategies, and the prevalence and significance of microbial infection on human health. We briefly introduce several classes of biomaterials commonly used in the manufacturing of wound dressings, and key features of each that may influence microbial attachment and biofilm development. We also introduce several modifications to these materials that have been developed to kill or inhibit microbial growth and attachment that range from impregnation of the material with antimicrobial agents to chemical and structural modifications. We cover the concept of personalized biofilm-based wound care and introduce several types of debridement therapies that have been used to prepare the wound bed to optimize the functions and efficacies of antimicrobial therapies and wound dressings. Finally, we briefly discuss important considerations in the application of dressings to wounds and future trends in wound healing biomaterials, particularly with respect to advanced dressings that may incorporate indicators to determine the condition of the underlying wounds, such as reporters to detect the presence of microbial biofilm to improve wound management.

s0015 3.2 Infectious disease: microbial biofilm and human health

p0015 Although infectious disease is no longer the leading cause of death worldwide, with the advent of vaccines, antimicrobial therapies, and preventative practices, it is still the most common cause of death (approximately one-third) in low-income countries according to the World Health Organization (WHO).[1] Moreover, the accelerating emergence of antibiotic- and antiseptic-resistant microbial strains has further complicated treatment strategies. More than 30 years ago, antimicrobial resistance was generally associated with hospital-acquired infection; however, the reported incidence

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of community-acquired antimicrobial resistance has dramatically increased. WHO recently reported that the proportion of opportunistic pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* isolates that are resistant to commonly used antibacterial drugs exceeded 50% in many settings worldwide and that multidrug resistance has become more widespread, while the failure of last-resort antibiotics has become increasingly more common for some pathogens.[2] Unfortunately, the last completely new class of antimicrobial drugs was discovered almost 30 years ago as well. In their 2014 global report, WHO also called attention to the need to develop new diagnostics and antibiotics, while reducing antibiotic abuse and improving infection prevention and control to stay ahead of the emergence of antibiotic-resistant strains.[2]

p0020 Although microbial biofilm has been studied since the 1970s, it has only been in the last decade that its association with disease has become widely accepted and intensely studied. The appreciation of the extreme antibiotic and antiseptic tolerance exhibited by microorganisms under certain conditions and stages of growth (ie, bacterial biofilm, persister cells) independent of the presence and expression of antimicrobial resistance genes has lead to research to find ways to specifically remove microbial biofilm and prevent its development. Consequently, the preservation of antimicrobial drug efficacy is not the only problem that wound care providers face when creating effective treatment strategies. Fortunately, the consensus that use of antimicrobial drugs, particularly via systemic delivery, should be more judicious to preserve drug efficacy concurs with the consensus of research that indicates that systemic delivery of antimicrobial drugs is typically ineffective in eradicating biofilm infection and may in fact promote biofilm growth in tissue infections. Another significant outcome of research over the last decade has been the development of the microbial infection theory for chronic disease that suggests that chronic infection, particularly biofilm infection, is a risk factor for development or exacerbation of many types of noncommunicable diseases and conditions. For example, periodontal disease is a chronic inflammatory biofilm disease that has been associated with the development of several chronic systemic diseases such as vascular disease[3] and stroke,[4] which together were reported to be the cause of 17.5 million deaths in 2012 (30% of death worldwide).[1] Therefore understanding the function and interactions of biomaterials with microorganisms and how they may contribute or inhibit the development of biofilm is essential for proper application of these materials when developing safe and effective treatment strategies, not only for wound healing but also potentially as a consideration for preserving whole-patient health, particular for cases requiring long-term care.

s0020 3.3 Basic microbiology of planktonic and biofilm bacteria

p0025 Free-living microorganisms are defined here as microbial species that are not obligate intracellular pathogens and have all the genes required for replication encoded

in their DNA. Most microbial species (bacterial and fungal) are free-living single-cell organisms that take up nutrients from the environment, excrete waste, and reproduce by division (binary fission), as quickly as every 15 min, depending on the species and growth conditions. The exponential nature of microbial growth results in reproduction from a single cell to more than 10^5 cells within 16 generations, to over a million (10^6) cells within 20 generations (eg, less than 7 h for *E. coli*). The presence of 10^5 or more colony-forming units (CFU) per gram of tissue is considered clinically infected. An uncoated free-living bacterial cell generally has a net negative charge; its magnitude varies considerably with species and strain. The hydrophobicity of bacteria depends on factors ranging from the components of the cell wall (Gram-positive bacteria) or outer membrane (Gram-negative bacteria) to the types of extracellular polymeric substance (EPS) the microorganism secretes under various conditions.[5,6]

p0030 The physiological state of microorganisms is generally categorized as planktonic or biofilm. Microorganisms are commonly grown and studied under standard laboratory conditions and are in a planktonic state in broth cultures. The progression of planktonic growth is conventionally described as lag phase, initiating immediately after inoculation of growth media with cells, during which the cells recover and respond to their new environment. This lag phase is followed by the exponential (log) phase of growth wherein all the cells are regularly dividing at a constant rate that is dependent on the availability of nutrients and the environmental conditions (eg, temperature). This is followed by the stationary phase wherein the cells either stop dividing or the number of new daughter cells is balanced by the number of dying and lysing cells, depending on the microbial species. This phase results from exhaustion of nutrients and available space, combined with accumulation of growth inhibitory waste. The final phase of planktonic growth is the death phase wherein the number of viable cells decline, typically at an exponential rate.

p0035 Microbial biofilm is composed of structured communities consisting of one or more species. Growth and development of microbial communities is generally considered the natural end state of most free-living microorganisms found in the environment and on living hosts. The progression of biofilm development is conventionally described as an initiating attachment to a surface, and to each other, that transitions from a reversible to an irreversible state (Fig. 3.1). This is followed by stages of maturation starting with excretion of self-produced EPS that forms an extracellular matrix, and eventual development into communities of microcolonies that typically exhibit some type of architectural structure and cell-to-cell communication (ie, quorum sensing), with characteristics that are dependent on the species and strain of its constituents. This mature biofilm also develops some mechanism of dispersion that is believed to be either continuous or conditionally triggered (eg, environment stress or host signal), depending on the microbial species. This state facilitates dissemination of the microorganisms to distal sites. The nature of the surface to which specific species most commonly attach (eg, host tissue, medical devices, sediment) and successful development into mature biofilm are generally a consequence of adaptive evolution; however, most microorganisms are considered opportunistic, provided that nutrients required for growth are available and that they are able to survive all physical and chemical stressors that they are exposed to at a specific site.

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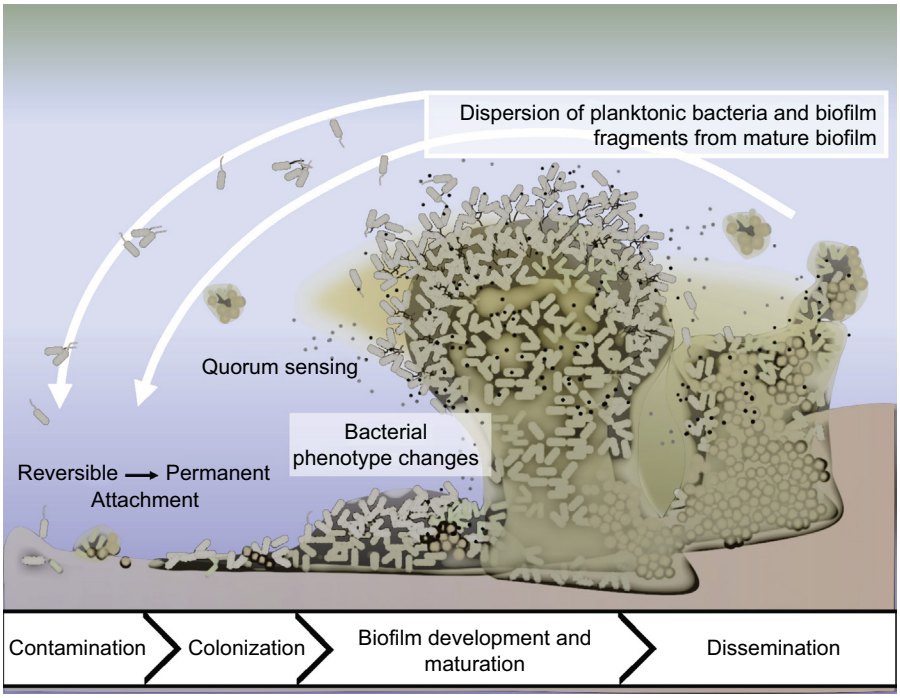

Figure 3.1 Stages of microbial biofilm development.

p0040 The key feature of microbial biofilm is its high tolerance to antibodies, antibiotics, antiseptics, and other antimicrobial agents at concentrations that effectively kill the same species when in a planktonic state. This characteristic is generally attributed to the reduced and altered metabolic state of the constituent microbial cells, and the presence of a protective EPS. Beyond the conventional definition of biofilm as irreversibly attached microorganisms, this definitive characteristic of high antimicrobial tolerance is the basis of a functional definition of fully mature microbial biofilm.

s0025 3.4 Biofilms in wounds

p0045 Prevention of infection is one of the primary goals of wound management of all wound types. Generally, the likelihood of infection increases with size, complexity, and/or time to heal. For example, according to current Centers for Disease Control and Prevention statistics of healthcare-associated infections (HAIs) occurring in acute care hospitals in the United States in 2011, 10% of HAIs (approximately 72,000) were inpatient surgical site infections. This value translates to approximately 0.14% of inpatient surgeries. The outcome of outpatient surgeries (more than 50,000,000 a year in the United States alone) is not expected to be much better. In contrast, it is hypothesized that one of the primary reasons many (possibly most) chronic wounds do not

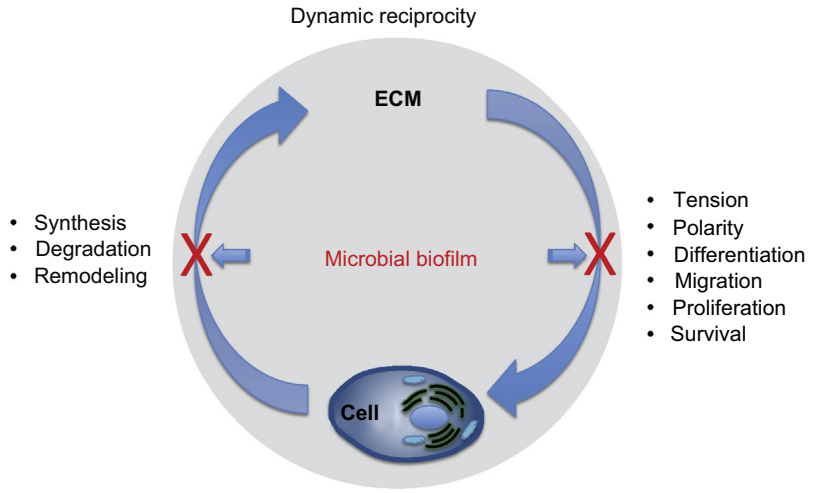

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Figure 3.2 Dynamic reciprocity in the wound microenvironment. Dynamic reciprocity between cells and the extracellular matrix (ECM), simplistically defined, is the process where cells synthesize, degrade, and remodel ECM components, while the ECM regulates cellular tension, polarity, differentiation, migration, proliferation, and survival.[7] The exact composition of the ECM varies by tissue and tissue state. The presence of microbial biofilm in the wound bed disrupts this process by inducing additional proinflammatory pressure, resulting in an overproduction of factors involved in ECM synthesis, degradation, and remodeling (eg, matrix metalloproteases and other enzymes). Microbial factors also directly impact cell migration, proliferation, and survival to various degrees, depending on the microbial species involved. Because of the nature of microbial biofilms,[8] even those containing species that are generally not considered pathogenic, they tend to persist despite the host immune response. Disruption (eg, microbial biofilm) or dysfunction (eg, tissue ischemia) of the normal process of dynamic reciprocity in the wound bed is the reason wounds do not heal.

heal is the presence of microbial biofilm infection (Fig. 3.2). This issue has become a major concern since the incidence of chronic wounds has grown, and it is expected to continue growing, primarily due to the growing age of the population, improved diagnosis, and increasing incidence of diabetes. More importantly, development of new and improved wound care products, particularly more cost-effective products and therapeutic devices, would improve wound healing outcomes for all wound types.

p0050 Polymerase chain reaction (PCR) identification of bacterial DNA in chronic wounds has detected a much larger spectrum of bacteria than had been detected using traditional culturing techniques (eg, ~60% of bacterial species in pressure ulcers were anaerobes). The bacteria residing in chronic wounds are polymicrobial in nature and are predictably contaminated with species found in the environment as well as with normal flora found on or in the body. Three important features of wound infections are: (1) targeting only one or a few species will allow surviving species to become dominant; (2) the microbial species found in an infected wound would be expected to continuously change, especially in response to external stress (eg, antimicrobial treatment); and (3) bacteria are located on the surface of wounds

as well as deeper in the wound bed, but they are likely to be species stratified based on nutritional and oxygen requirements, especially in chronic infections. Recent studies analyzing bacterial biofilms found in chronic infections (eg, chronic otitis media, cystic fibrosis, infected permanent tissue fillers, chronic wounds) point toward the presence of dominant monospecies biofilm aggregates within a multispecies background.[9–11] The prospect that relatively few species make up the majority of the microbial load in many wounds is not unexpected considering that these tissue types at these sites are normally sterile (ie, deep tissue), or poorly colonized when intact. There is no natural microbiome with an evolved ecology at these sites. During development of a microbial infection in a wound, opportunistic commensal bacteria, commonly found on or in humans, become pathogenic, and the site becomes contaminated with environmental species that are able to persist (eg, biofilm); thus, the etiology of an infected wound is typically undeterminable. Initial assessment of chronic wounds by scanning electron microscopy and light microscopy detected biofilm structures in ~60% of wounds.[12] Recent analysis has detected functional biofilms in ~90% of chronic wound samples biopsies/curettages [AU1] (our work, unpublished). Although all untreated wounds are quickly colonized with contaminating microorganisms and many studies suggest that most, if not all, chronic wounds are infected with polymicrobial biofilm, a review of published studies and a consensus of anecdotal evidence indicate that not all biofilm-infected wounds show all the classic clinical signs of an acute microbial infection (ie, redness, heat, swelling, pain).

s0030 3.5 Biofilm-based wound care

p0055 Two strategies have evolved that, when used together, have had a significant impact on wound healing, particularly chronic wound healing. They are based on three key suppositions. First, although there may be beneficial bacteria present on intact skin, evidence has not supported that there are any "good bacteria" in a wound; thus, the first strategy has been to identify and treat the whole spectrum of bacteria found in a wound. The future of microbial assessment in wound care will likely be the routine use of molecular-based techniques such as PCR and spectrometry. Second, all bacteria in a wound are likely capable of forming biofilm to some degree, and most, if not all, chronic wounds have some level of biofilm, which are typically greater than 100 times more resistant to antibiotics and antiseptics than they would be in a planktonic state. And third, many bacterial species often found in wounds typically double in population every 20–60 min; thus, the window of treatment opportunity in a contaminated wound is generally relatively small.[13] Furthermore, the antimicrobial-tolerant character of microbial biofilm negates the convention of 100,000 CFU per gram of tissue as the microbial load that results in infection pathology. These considerations lead to the second strategy of treating wound infections: recognize that it is essential to thoroughly remove contaminating bacteria and biofilm and to maintain an antimicrobial treatment and barrier regime to prevent redevelopment of biofilm, and to promote healing, while addressing other contributing factors. Following these guidelines has

led to the use of a combination of treatment methods to manage wound healing that we refer to collectively as personalized biofilm-based wound care.

s0035 3.6 Wound healing biomaterials: features, function, and impact on microbial biofilms

p0060 The majority of acute wounds that use advanced wound care products worldwide are major surgical wounds (approximately 100,000,000 inpatients a year worldwide).[14] Other wound types that typically require advanced wound care products are traumatic wounds (approximately 50,000,000 a year), medically treated burn wounds (approximately 6,000,000 a year), and chronic wounds.[14] Although development of chronic wounds is rare, there are approximately 4,500,000 pressure ulcers; 9,700,000 venous leg ulcers; and 10,000,000 diabetic ulcers that require treatment per year worldwide.[14] Chronic wounds are conventionally defined as wounds that do not show signs of healing after 4–6 weeks of treatment. Advanced wound care products and therapeutic devices are specifically designed to promote faster healing and to reduce the risk of infection or to treat infection if established. When comparing global incidence and prevalence data of acute and chronic wounds[15,16] to published market reports of leading manufactures, it seems that most acute wounds are not treated with advanced wound care products; however, their use has become more common, particularly in the treatment of chronic wounds. The development of advanced wound care products that show significant reduction in treatment cost by reducing cost of manufacturing and/or improving healing outcome would predictably increase use of these products.

p0065 A biomaterial is currently defined as a material that has been designed to be used in any therapeutic or diagnostic procedure to regulate the interactions of single or multiple components of living systems when applied alone or as part of a complex device (eg, wound dressing). When applying biomaterials to wounds, there are multiple factors associated with the chemical and physical characteristics and limitations of the biomaterial that must be considered. In addition, the biochemical, molecular, and physical characteristics of the wound and its microbial constituents must be considered to maximize and maintain optimal efficacy and function of the biomaterial. For example, the efficacies of some antimicrobial dressings, such as those containing silver, rely on moisture availability (either from wound fluid or applied) to maximize availability of the microbicidal agents. The release of silver for many silver-containing microbicidal/microbiostatic dressings is typically proportional to the rate of fluid uptake into the material. Silver-containing biomaterials should never be moistened or used in conjunction with saline due to precipitation, and thus inactivation of the silver ions. Additional factors that must be considered are the potential cytotoxic or inflammatory response that may be elicited by the biomaterial, which may depend on the type of wound being treated as well as the existence of additional health complications or predispositions in the patient that may impact wound healing. In the following sections, we introduce several classes of biomaterials used in the treatment of wounds and touch on wound bed preparation (ie, biofilm debridement) required for optimal

application of these biomaterials. We focus primarily on key characteristics of the biomaterial that impact its function and interaction with microbial biofilm and visa versa, irrespective of other host factors that impact wound characteristics and response to treatment (eg, ischemia, poor nutrition, cellular cytotoxic response).

p0070 Initial attachment of microbial biofilm is influenced by the biomaterial's hydrophobicity, roughness, porosity, charge, chemical composition, and surface free energy.[6,17] Different species of microorganisms have different surface adherent properties that influence their ability to attach to different kinds of biomaterials, which in turn impact the incidence of associated infections. For example, the incidence of HAIs associated with common synthetic polymer materials, particularly for medical devices such as catheters, is high (approximately one-quarter of reported HAIs[18]), and research to improve these generally hydrophobic materials is ongoing. Many comparative studies have been performed to determine the best polymer to use to reduce incidence of infection. However, there is no evidence to support use of one material type over another to prevent bacterial attachment for universal use. This is because there is tremendous variety inherent in microorganisms, even between strains of the same species. For example, studies have shown that *Staphylococcus epidermidis* attaches to Teflon better than to polyurethane, whereas *Pseudomonas aeruginosa* and *Staphylococcus aureus* attach to polyurethane better than to Teflon, and all three species are significant opportunistic pathogens.[19] The outcome of these early studies has been to provide some guidance for biomaterial selection for specific applications, particularly in cases where certain species of microorganisms are more problematic than others. Alternative avenues of study for improving the use of biomaterials have been investigated. One example is based on studies that have shown that microbial adherence to irregular and porous materials is higher than to dense and smooth, and particularly, regularly patterned surfaces, which has led to the development and use of devices with nonporous patterned surfaces.[20,21] Unfortunately, there are limitations on the efficacy of architectural modifications of the surface of these biomaterials on inhibiting biofilm growth. Many studies have also shown that biological fluids have components that significantly improve microbial attachment; importantly, microbial biofilm EPS functions to facilitate attachment to any surface type and has been shown in many studies to overcome physical barriers to attachment (eg, hydrophobicity, surface architecture) upon coating with organic material.[22,23] Furthermore, microorganisms quickly respond to characteristics of the surface type to which they are attaching by altering gene expression and the character of its excreted EPS to overcome these barriers.[23] Over the last decade, the focus of modifications of biomaterials made from these polymers seems to have shifted to minimizing microbial attachment and reducing microbial viability by various bactericidal mechanisms ranging from microbial cell lysis upon contact to materials that incorporate slow release of antimicrobial agents. The efficacy of these redesigned biomaterials for wound dressing applications has an advantage over medical device applications (eg, implants, catheters) in that dressings are used for a relatively short period; consequently, the goal for developing modifications for these materials is the inhibition of microbial attachment and biofilm development for 3–7 days.

t0010

Wound healing biomaterials

- Films and adhesives (eg, silicone, latex, rubber, vinyl, acrylics)
- Particulate polymer microparticles and beads (eg, hydrocolloids, hydrogels, cadexomer beads)
- Absorbent and/or three-dimensional synthetic polymer foam, fiber, mesh, and tissue scaffolds (eg, polyurethane, polystyrene, silicone, polymethylmethacrylate)
- Absorbent and/or three-dimensional biopolymer and natural fiber, mesh, and tissue scaffolds (eg, cotton, alginate, collagen, cellulose)

p0095 Films and adhesives are most commonly made of synthetic polymers and are primarily used as a biomaterial to cover and/or seal wounds, especially for minor and superficial wounds. They range from waterproof to water soluble and opaque to clear. Clear films are most commonly breathable (rather than occlusive) barrier dressings and can only be applied to dry skin. Thin films, whether breathable or occlusive, are often used as a secondary dressing over a primary dressing from one of the other three classes of dressing, particularly in open wound and burn wound applications; thus, direct contact is only made with the intact skin surrounding the wound. The function of this biomaterial, especially films, generally includes some degree of moisture barrier control and physical barrier protection from contamination by environmental microorganisms as well as protection from soiling and friction. These protective functions are generally considered to significantly outweigh the negative aspect of providing an additional surface for microorganisms to attach. Compared to other biomaterials with more surface area for microbial attachment (eg, absorbent dressings), this aspect is generally considered less problematic.

p0100 Particulate polymers are another class of biomaterial commonly used in wound dressings that are generally manufactured using a mixture of synthetic and biopolymer materials. They function to absorb wound exudate (eg, hydrocolloids) or to hydrate the wound bed (eg, hydrogels). Hydrocolloids and hydrogels both contain gel-forming agents (eg, carboxymethylcellulose, gelatin, starch), but they are distinguished by this difference in specific function and thus are applied to different wound types. Overall, these biomaterials are composed of stable superabsorbent materials that function to regulate and maintain a moist wound environment. In some cases, wound dressings categorized in this class based on function consist of polymers that have been covalently cross-linked to form more stable and controllable structures (eg, thin films, beads). This class of biomaterial is often coupled to, or impregnated with, antimicrobial agents to provide sustained slow release of the active ion/molecule. An excellent example is cadexomer iodine, which is comprised of absorbent water-soluble–modified starch polymer helical beads containing iodine (0.9%). The iodine is coupled to the polymer; thus, this biomaterial functions as an iodophor that releases iodine as it hydrates. Microorganisms are typically readily absorbed to hydrated particulate polymers such as hydrogels. Marketing strategies often cite this feature as an advantage for this biomaterial, suggesting that they reduce wound bioburden. Until the creation of modified hydrogels that have been shown to attract bacteria, primarily due to their

absorptive and particulate nature, and lyse bacterial cells on contact, due to stable highly positive charged surface properties,[24] hydrogels that were not impregnated with antimicrobial agents did not have this antimicrobial function. Rather, certain types of hydrogels (ie, pluronic polyols)[25] have been used in the laboratory to culture bacteria to provide a solid surface for microbial attachment and biofilm growth while retaining the ease of quantification that liquid culture provides.

p0105 Nongelling biomaterials designed to absorb wound exudate and promote wound healing come in a wide variety of forms and specialized functions. They can be divided into two broad classes of biomaterials: (1) absorbent synthetic polymer foam, fiber, and mesh and (2) absorbent biopolymer/natural fiber. Because of the absorbent nature of these biomaterials, microbial interaction is a major concern; therefore, modifications to these materials or combining antimicrobial agents and barriers with these materials is typical, especially for prolonged use (1–7 day) wound dressings. Short-term applications of these materials, such as wound cleansing and swabbing, generally do not incorporate antimicrobial functional modifications that significantly increase manufacturing costs. Although there are functional overlap and exceptions when comparing biomaterials made from biopolymers and synthetic polymers, generally, absorbent and three-dimensional (3D) biomaterials composed of biopolymers and natural fibers have the natural functional advantage of being biocompatible and biodegradable, while also generally being a more economical choice. Consequently, it is often the preferred biomaterial for treatment of difficult-to-treat and -heal wounds and chronic wounds requiring long-term management, particularly when there is no strong evidence that more expensive alternatives provide an advantage during treatment.

p0110 Biomaterials composed of biopolymers and natural fibers tend to naturally induce a cellular response ranging from altered cell proliferation and migration profiles of fibroblasts and keratinocytes to an inflammatory immune response, compared to most synthetic polymers in their native state. Since biopolymers and natural fiber materials are derived from biological sources (eg, plants, bacteria), differences in processing and manufacturing of these biomaterials tend to result in differences in purity, performance, and cellular response between dressings from different manufacturers and often between batches, which is more uncommon during manufacturing of synthetic polymer biomaterials. Furthermore, absorbent biomaterials composed of synthetic polymers are generally more amenable to tailored structural design and tend to have mechanical properties that provide a functional advantage over biopolymers and natural fibers, particularly for certain applications (eg, foams used in conjunction with negative pressure wound therapy with instillation).

p0115 Biomaterials that are applied as tissue scaffolds for tissue regeneration have been created using biopolymers (eg, collagen) and synthetics (eg, hydroxyapatite). Scaffolding biomaterials made of synthetic polymers are more amenable to tailored engineering; thus, many have been shown to have improved conductive properties in directing cell proliferation.[26] In contrast, biomaterials made of biopolymers such as collagen (the most abundant protein in vertebrates and essential for cellular adherence and migration) naturally contain functional groups that interact with cells.[26,27] This is a functional advantage for several wound treatment applications. For example, studies suggest that collagen dressings improve chronic wound healing by providing a

surrogate substrate for tissue-destructive proteases while stimulating cellular proliferation and migration. Elevated protease level in wounds has been shown to be a marker for development of chronic wounds.[28,29] Unfortunately, the ability to bind collagen is also a common virulence factor for many microorganisms (eg, *S. aureus*); therefore, this biomaterial is particularly suitable for microbial attachment and biofilm growth for many microbial species. Fortunately, biopolymers and natural fibers are often conducive to chemical modification to render the material less amenable to microbial attachment and growth. Although the options for modification are generally more limited for biopolymers than for most synthetic polymers (which can often be structurally modified to render them more antimicrobial), all of these materials have been used in the manufacturing of dressings that incorporate some type of antimicrobial agent.

t0015

Antimicrobial agents commonly incorporated in biomaterials	
Reactive chemicals	• Silver, iodine, hypochlorous acid, hydrogen peroxide
Cationic molecules	• Polyhexanide, chlorhexidine, poly(diallyldimethylammonium chloride)
Sugars and salts (hypertonic)	• Honey, sodium chloride, sucrose (granulated sugar)
Enzymes	• Nuclease, protease
Antibiotics	• Macrolides (erythromycin, clindamycin), aminoglycosides (gentamicin), tetracyclines (tetracycline, doxycycline, meclocycline), sulfonamide (sulfacetamide)

p0145 All of the antimicrobial agents introduced here are capable of killing microorganisms; however, the antimicrobial efficacy of each agent on a specific species of microorganism ranges from bactericidal (cell death) to bacteriostatic (growth inhibition), which is primarily dependent on the relative concentration of active/reactive ions or molecules.[30] Except for antibiotics, these agents are relatively nonspecific, have a broad spectrum of biocide activity, and are all effective biocides when applied at sufficient concentrations for sufficient periods of exposure.[30] The specificity of an antibiotic typically depends on its mechanism of killing and whether the target organism expresses genes to render it resistant. The ability to kill microbial biofilm for all the agents listed depends on its ability to penetrate the microbial EPS matrix and on other unique features of microbial biofilm (eg, lower metabolic rate) that render it more tolerant to the mechanism of killing of the antimicrobial agent. Studies suggest that these characteristics of biofilm are overcome by increasing the availability of the active biocide (ie, increased concentration) and/or sustained exposure for an extended period.

p0150 There are numerous naturally occurring, highly reactive chemicals that have been discovered to function as effective disinfectants and antiseptics throughout human history. Modern biochemistry research has led to development of improved formulations of these agents (eg, silver, iodine, hypochlorous acid, hydrogen peroxide) to maximize antimicrobial activity, while minimizing allergic reactions and toxicity.

Silver and iodine are two agents that are commonly incorporated in advanced wound dressings due to their stability and efficacy. These two agents are not commonly incorporated in the same wound product because like many agents in wound products, when combined, they may have negative interactions or inactive the active agent in one another.[31]

p0155 The hydrated form of molecular iodine has the highest antimicrobial potential, and its reactivity decreases with increased alkalinity and storage time.[32] Iodophors, composed of iodine complexed with organic compounds, are reported to be less allergenic due to slow release of free reactive iodine.[33,34] The most common iodophor in clinical use (eg, surgical scrub) is polyvinyl-pyrrolidone (povidone-iodine), with a concentration of available free iodine between 8% and 12% (w/v).[32] Historically, iodine has been considered cytotoxic, and its use in open wounds is commonly discouraged, except for severely infected wounds. Some evaluations report that iodine does not impair wound healing and is an effective antiseptic for wound care.[35] Newer biomaterials such as cadexomer iodine, designed to provide slow sustained release of iodine, have been approved for use in open wounds, and they have been reported to accelerate epithelialization of partial-thickness wounds in an in vivo porcine wound model.[36] Interestingly, the ability of the native cadexomer biomaterial alone to reduce *P. aeruginosa* biofilm infection was observed in an in vivo porcine wound study showing a significant reduction in *P. aeruginosa* microbial load (2.3 log) after 48 h of treatment compared to an untreated control.[36] This action is likely due to the contact dehydrative effect of the cadexomer beads, considering this species of bacteria is sensitive to dehydration. A similar result was observed upon application of native cadexomer beads with 24 h of exposure by using an ex vivo porcine skin mature biofilm model.[30] In contrast, complete *P. aeruginosa* biofilm kill was observed after 24 h of exposure when iodine was incorporated in this material to produce a particulate polymer functional iodophor biomaterial when applied using an ex vivo porcine skin mature biofilm model.[30] To date, iodine use is not associated with selection of resistant bacterial strains, neither in the clinic nor in microbial research studies designed to develop and select for resistant strains.

p0160 Silver-resistant bacteria have been reported since the 1950s, but such incidence is considered relatively rare.[32,37] The most common forms of silver currently used in advanced wound dressings and slow-release particulate polymer biomaterials are silver salts such as silver sulfadiazine as well as nanocrystalline silver-coated biomaterials. The primary antimicrobial mechanism of action of silver ions is thought to be deactivation of enzymes by binding to thiol groups as well as catalyzing reactions that result in the formation of disulfide bonds that alter protein structure and thus function. The silver ion concentration required to kill bacteria is still debated; however, the literature supports bactericidal activity at levels of parts per million.[37,38] One interesting molecular study conducted by Milliken & Company showed that regardless of the dressing type or the form of silver on the dressing, without organic loading, silver release in simulated wound fluid (142 mM NaCl, 2.5 mM $CaCl_2$) maintained an equilibrium concentration of only 0.5 ppm.[39] In contrast, with organic loading (5% bovine serum albumin) of the simulated wound fluid, the silver dressings were reported to release significantly greater amounts of silver, generally at levels considered to be

microbicidal, that varied with dressing and time (ie, 10 ppm to >80 ppm after 24 h of exposure).[39] The reported efficacy of slow-release silver dressings against microbial biofilm using ex vivo and in vivo porcine skin biofilm models were less that reported for slow-release iodine dressings. Evidence suggest that this observation is likely due to differences in sustained availability of the antimicrobial agent (ie, biomaterial design) rather that the mechanism of action of the ion itself.[40]

p0165 High-molecular-weight cationic molecules such as polyhexamethylene biguanide (PHMB) are broad-acting synthetic antimicrobial agents that are not absorbed and have no reported negative effects on wound healing.[34,41] Although susceptibly to these agents depends on the microorganism's membrane structure and make-up, selection and development of resistant strains from susceptible strains have never been demonstrated.[42–44] At concentrations commonly used in wound care materials, these cationic molecules (eg, PHMB, chlorhexidine, poly(diallyldimethylammonium chloride)) are generally considered noncytotoxic. The general mechanism of action of these cationic molecules is reported to be the induction of phase separation or the disruption of microbial membranes, leading to membrane dysfunction, leaking, and/or rupture.[41,45–47] Increasing concentrations of these cationic molecules, as well as length in the case of cationic polymers, generally correlate with increasing antimicrobial efficacy.[41,46] The protective function of biofilm EPS matrix is effective against these cationic molecules, particularly for the larger molecules that are less able to penetrate the matrix. Although the net charge of microbial biofilm depends on the species and soluble ions present, many EPS components of microbial biofilm are negatively charged at physiological pH and thus effectively bind these cationic molecules.

p0170 Sugar- and salt-based materials such as table salt (sodium chloride), granulated sugar (sucrose),[48] and honey are bactericidal and/or bacteriostatic, depending on the concentration of the agent and the target microorganism. Primarily through a mechanism of dehydration, the antimicrobial activity of these agents is maintained as long as hypertonic conditions are sustained throughout exposure. As the oldest known antimicrobial agent class used in human history, these abundant and naturally derived products are both inexpensive and nontoxic, and they have undergone periods of common and uncommon use in wound care applications. As a complex colloid, medical grade honey dressing is a functional biomaterial in its own right and has recently become a popular therapeutic dressing choice in wound care management. The low pH (~3.2–4.5) and high osmolarity of honey (~80%) has been historically used for its antimicrobial effect on bacteria and fungi.[49] Medical grade honey has also been shown to promote wound healing for some wound types by inducing autolytic debridement.[50] Medical grade honey dressing is most commonly manufactured with *Leptospermum* honey, which contains additional natural antimicrobial agents such as methylglyoxal (an α-oxoaldehyde that reacts with nucleotides and proteins).[51] Furthermore, honey is known for producing hydrogen peroxide when dissolved in water, proving a slow release of this oxidative microbicidal agent (~1 mmol/L).[52]

p0175 Although enzymes such a nucleases and proteases are not considered antimicrobial agents in the strictest sense, they have a detrimental effect on biofilm development.[53–55] These enzymes, depending upon their specificity, act by digesting constituents of the EPS matrix, thereby affecting both biofilm structure and susceptibility to host immune

factors and other compatible antimicrobial agents. They also assist in wound debridement, particularly proteases, and thus affect biofilm attachment, assisting in biofilm removal.[56] Nucleases have the added hypothetical potential of reducing incidence of microbial gene transfer to promiscuous microorganisms, which may potentially inhibit the spread of virulence factors to nonpathogenic strains.

p0180 Despite the many advances in design and the wide variety and growing use of commercially available advanced wound care biomaterials, particularly for specialized care and treatment of complex wounds, plain cotton fiber gauze is still the most commonly used dressing worldwide, primarily due to cost and convenience (eg, immediate availability), but partly due to education. Furthermore, the most common antimicrobial therapy used in wound management is supplementary application of topical antibiotics with these simple inexpensive dressings. Fortunately, some studies and clinical evaluations have led to the development of improved wound management strategies that include comprehensive identification of microbial constituents to optimize the efficacies of these generally more economical products. Although the biofilm-based wound care strategy previously introduced and defined has yet to become widely adopted, it is predicted to become the standard of care, particularly in wound care clinics. The future of wound care also includes research to improve the performance and cost-effectiveness of existing advanced wound care biomaterials; the development of new and innovative biomaterials; and the development of rapid, cost-effective diagnostic devices and procedures to detect and profile microbial biofilm and to assess the state of wound healing. The following sections cover these topics in detail.

s0040 3.7 Topical antibiotic combination treatments based on DNA identification of bacteria

p0185 As stated previously, biofilm is now well established to be present on the surface of chronic wounds[12] as well as on the surface of other chronic infections such as chronic rhinosinusitis,[57] cystic fibrosis,[58] otitis media,[59] ventilator-associated pneumonia,[60] and atherosclerosis.[61] Biofilm is present in chronic infections[62] and seems to drive the behavior of these chronic infections. Chronic infections are infections that persist, wax and wane (episodic) in their symptoms, and are incompletely responsive to antibiotics, only to reemerge when the antibiotics are withdrawn.[63] This tolerance to antibiotics is a hallmark of biofilm mode of growth.[64]

p0190 Because the individual bacteria within an infectious biofilm express up to 800 different genes compared to if the same species of bacteria grew as a single cell (planktonic), biofilm phenotype bacteria are quite different than those we encounter in the microbiology laboratory.[65] This different phenotype (different gene expression) for the constituent bacteria of biofilm is what makes them unsuited to grow in a clinical culture.[66] That is, the biofilm cells are alive, yet they do not readily grow under the nutrients and laboratory conditions of a routine clinical culture (viable but not cultivable). This leads to most chronic infections being poorly characterized by a clinical microbiology culture.

p0195 Wounds usually have high microbial diversity[67] that limits the usefulness of clinical microbial cultures even more. Over half the time the dominant species of bacteria in a chronic wound sample is not identified by standard clinical culture techniques.[68] Rather, minor components that grow more easily under laboratory conditions are amplified giving the clinician a misleading result. This has led to many studies determining that by using clinical cultures to diagnose the bacteria on chronic wounds there is no improvement in outcome.[69,70] There is still considerable controversy as to whether more detailed knowledge of the wound bioburden provided by molecular methods will improve wound healing outcomes.[71]

p0200 Molecular methods which use the bacteria's DNA to identify the different species and quantitate each species' contribution to the biofilm are much more clinically useful. The microbes' mode of growth, whether planktonic or biofilm, does not affect the analysis. The quantitative component to molecular diagnostics allows the clinician to reevaluate the wound biofilm to determine whether treatment strategies have been effective. This allows real-time feedback to adjust treatment strategies.

s0045 3.8 Polymerase chain reaction and sequencing

p0205 Antibiotics are significantly limited by the properties of biofilm. By multiple colony defenses, biofilm is tolerant to antibiotics up to 500–1000 times the concentrations that can be achieved systemically.[72] Therefore, systemic antibiotics at best can only suppress the biofilm for the duration of the antibiotics, with the biofilm reemerging once antibiotics are withdrawn. This is the pattern observed in the treatment of most chronic infections.

p0210 To avoid the problem of systemic antibiotic tolerance, topical antibiotics have become a mainstay in treating accessible biofilm infections. For example, the gold standard of managing otitis media with tubes,[73] otitis external infections,[74] and ophthalmologic infections[75] is the use of topical antibiotics. Yet, there has been pushback in the use of topical antibiotics in wounds.[76] There is a fear of producing antibiotic resistance or sensitizing the patient to antibiotics, the review by Kennedy and Jones shows these complications are much less with topical use of antibiotics compared to systemic antibiotics.[77] It should be pointed out that 70% of patients with chronic wounds will receive at least one course of systemic antibiotics[78] and patients with chronic wounds receive systemic antibiotics for coinfections (eg, urinary tract infection, upper respiratory infection). This exposes the wound biofilm to low intermittent doses of systemic antibiotics, which is exactly the driver for antibiotic-resistant organisms.

p0215 Studies have demonstrated that in ear and eye infections the use of topical antibiotics for over 30 years in one clinic did not lead to resistance of microorganisms.[79] This may be because topical antibiotics at high concentrations are much less likely to lead to resistance than subtherapeutic doses of systemic antibiotics. Also, systemic use of antibiotics exposes more tissue to the agent, leading to more host allergic complications than local topical use.

p0220 The use of topical antibiotics which can achieve high concentrations has other advantages. Most genetic resistance information (eg, mecA cassette, Extended-spectrum β-lactamases) confers resistance to an antibiotic at levels of 2–16 times the minimal inhibitory concentration. Therefore, vancomycin-resistant *Enterococcus* or vancomycin-intermediate *S. aureus* can easily be managed by the high concentrations achieved by topical vancomycin. In fact, methicillin-resistant *S. aureus* becomes more sensitive to higher concentrations of aminoglycosides; for example, at 0.1% gentamicin (marginal),[80] at 0.3% tobramycin (−3 log in 90 min),[81] and at 1% amikacin (100% of 47 multidrug-resistant *S. aureus* were sensitive).[82] Also, antibiotics that rapidly become ineffective systemically, such as rifampin, can maintain their potencies topically.[83]

p0225 It has become clear in the management of biofilm that appropriate antibiotics challenging the biofilm at high concentrations for long durations maximally suppress the biofilm, thus mitigating the barrier that biofilm can exert on wound healing. By accurately identifying and quantitating the microbes composing the wound biofilm and then formulating a personalized group of antibiotics to target the constituents of the biofilm, wound healing outcomes improve.[84] This demonstrates that wound biofilm is a barrier to wound healing. This also shows that for the present topical antibiotics have a significant role to play in the management of wound biofilm.

s0050 3.9 Biofilm debridement

p0230 Removal of mature microbial biofilm from wounds generally cannot be successfully performed by simple application of one of the biomaterials we have previously introduced. As stated, studies have shown that effective treatment of infected wounds include complete removal of existing microbial biofilm combined with application of antimicrobial wound therapies and dressings that may function as barriers to prevent contamination while promoting healing. Successful removal of existing biofilm typically requires some method of debridement, again, often in conjunction with antimicrobial treatment, which depends on the type and complexity of the wound. Therefore, a brief overview of simple biofilm debridement methods has been included in this chapter. We do not cover chemical debridement (eg, enzymatic) or more complex types of wound therapies that have been shown to reduce microbial biofilm such as negative pressure wound therapy with instillation of antimicrobial solutions.[85]

t0020

Nondressing approaches to reduce/remove wound biofilms
• Sharp debridement (bedside vs surgical) • Ultrasonic cleansing/debridement • Larval debridement

p0250 Sharp debridement is achieved by the removal of necrotic or nonviable tissue from the wound bed with sharp surgical instruments such as scalpels, “pick ups,” forceps,

and curettes. Although the clinician cannot see the biofilm structures within the wound, sharp debridement is one method of physically removing biofilm structures that are presumed to be residing in the necrotic tissue. In bedside sharp debridement, the necrotic tissue is typically attended to by first discerning where the nonviable tissue adheres to the viable tissue and then removing it at this junction. This is performed by physicians, physicians' assistants, and certified wound specialists, including nurses (depending on state or country regulations) in an ambulatory clinic or inpatient bedside location. The use of this technique may be limited by a patient's pain/tolerance of this procedure or by coagulopathies (patients on anticoagulant therapy), as well as how tightly adherent the necrotic tissue may be to the wound bed or other important structures (such as tendon). In surgical sharp debridement, a surgeon performs an excisional surgical procedure in an operating theater. The necrotic tissue is typically removed to include a margin of healthy tissue. This procedure, although not limited by a patient's pain (anesthesia may be more readily available), may be limited by coagulopathies or nearby delicate structures such as blood vessels. Another concern is that studies indicate that when there is biofilm present in the wound bed, there is only a short window of time (no more than 3 days) after sharp debridement before a mature bacterial biofilm structure will completely regenerate itself.[13,86]

p0255 Device-assisted mechanical cleansing/debridement methods have been developed primarily for the purpose of reducing the level of pain the patient experiences relative to the pain typically associated with surgical sharp debridement. For example, contact and noncontact (nonthermal) ultrasonic debridement devices have been developed that have been shown to be effective in assisting wound cleansing/debridement,[87,88] and they have shown promise in removing microbial biofilm from tissue. Data suggest that with the addition of antimicrobial solutions, ultrasonic debridement generates a synergistic effect in reducing microbial biofilm load when compared to use of these devices with saline or compared to simple application (eg, lavage) of the antimicrobial solutions alone (unpublished work).[89]

p0260 Larval debridement therapy (LDT, medicinal maggots) is considered a medical device by the US Food and Drug Administration, but it may be considered a pharmaceutical in some other countries such as the United Kingdom. Regardless, LDT may be one of the most effective methods of removing bacterial biofilm in wound beds. Ex vivo experiments conducted by us in 2012 demonstrated that within 24–48 h, sterilized medicinal green bottle fly larvae (*Lucilia sericata*) were able to remove 100% of mature bacterial biofilm grown on porcine explants (Fig. 3.3).[90] Additional studies demonstrate these larvae secrete powerful enzymes[91] which effectively and rapidly break down nonviable tissue which the maggots then ingest (including bacteria). To date, there has been no other product or material tested in our laboratories which has demonstrated this level of biofilm eradication. A systematic review of LDT conducted by Sun et al.[92] suggests medicinal maggots improve chronic wound healing outcomes. Clinically, LDT has certain limitations. Aesthetically, some patients or caregivers may not be able to tolerate LDT. Larval debridement therapy may not be appropriate for wounds that are deep in body cavities (maggots need air to breathe), wounds which cannot be completely off-loaded, or wounds with fistulas and/or exposed blood vessels.[90]

f0020

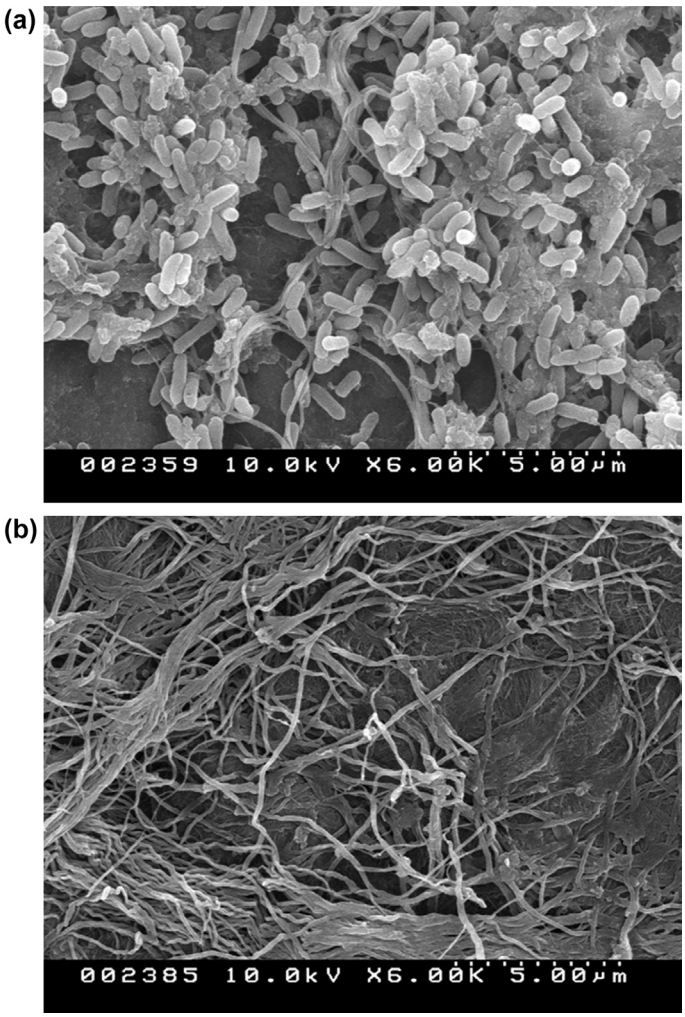

Figure 3.3 *Pseudomonas aeruginosa* (PAO1) 3-day mature biofilm growth on porcine explant before larvae (a) and after 24 h of LDT (b).

s0055 ## 3.10 Discussion and future trends

p0265 A critical consideration when analyzing log reduction of microbial biofilm load when reviewing published efficacy data of antimicrobial agents is the recovery rate of biofilm.[13] For example, 1-log reduction from 1×10^9 to 1×10^8 is a loss of 9×10^8 bacteria or can be expressed in simple mathematical terms as 90% reduction. However, this is an insignificant reduction in a biological sense in wounds when bacteria such as *P. aeruginosa* (PAO1) has an average generation doubling time of ~40–50 min; thus, full recovery could be achieved in less than 3 h. Alternatively, a 5-log reduction of PAO1 biofilm would require at least 17 generations to recover

(~12 h). At this level of antimicrobial efficacy against bacteria/biofilms attached to skin wounds, multiple applications of nonspecific microbicide treatment would be feasible and likely effective against this species. We propose that a dressing with greater than 5-log reduction in bacterial biofilm is most likely an effective antimicrobial dressing. A dressing with greater than 3-log reduction in bacterial biofilm may be an effective antimicrobial dressing for wounds with a low microbial bioburden, or as a barrier dressing. Dressings containing antimicrobial agents showing less than 3-log reduction are likely better used as barrier dressings. It is important to understand that the application of barrier dressings to prevent microbial contamination of properly prepared wound beds, whether it is achieved by physical of biochemical means, has considerable value in wound care and has been shown to reduce the incidence of infection.[93]

p0270 In addition to developing advanced wound dressings that are more effective in preventing the formation of bacterial biofilms or killing/dispersing established biofilms on wound beds, future dressings will likely incorporate the capability to provide real-time, point-of-care information about the status of the wound bed. For example, dressings may be developed that incorporate the ability to assess the pH of different regions of a wound bed by using a visual "pH strip" color indicator that would indicate whether the pH of a region is abnormally (detrimentally) too high or too low. Similarly, dressings might be developed that incorporate a very thin membrane layer that contacts the surface of the wound bed and nonspecifically binds all the various molecules exposed on the wound bed surface. Initial studies have demonstrated that staining the "molecular map" membrane with cationic dye molecules that ionically bind to the highly negatively charged matrix of biofilms creates a "biofilm map" of the wound surface that clinicians could use to guide sharp debridement of the wound (and confirm removal of surface biofilm after debridement). Alternatively, advanced wound dressings, sometimes referred to as "smart dressings," could incorporate a membrane coated with a very thin layer of a common protease substrate, such as collagen or gelatin that would contact the surface of a wound bed. Regions of a wound bed with elevated levels of matrix metalloproteases or elastase would degrade the thin film of substrate, resulting in clear zones of the membrane that would indicate regions that need debridement. These examples of a molecular map of a patient's wound bed would help to select regions that need debridement and could help in selecting the most appropriate postdebridement therapies. Thus, it seems very likely that major new developments will occur in the next few years in the design and functionality of dressings that will advance the field of wound care.

s0060 References

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Non-Print Items

Abstract
In the last decade, the association of microbial biofilm with delay or nonhealing of wounds has become widely accepted and intensely studied. The primary characteristics of microbial biofilm that have lead to this focus are its ability to quickly regenerate; its extremely high tolerance to antimicrobial agents; and its ability to compromise would healing, even when present at microbial loads well below levels traditionally considered infected and when classic clinical signs of infection are absent. Biomaterials used in wound management have been developed with target applications ranging from reducing microbial load to functioning as physical barriers to microbial contamination, with future applications looking toward the incorporation of diagnostic features. Other key considerations are inhibition of development of biofilm on the biomaterial itself and best practices for wound bed preparation. This chapter touches on these topics and summarizes the significance of microbial biofilm in wound care.

Keywords: Biofilms, Biomaterials, Dressings, Wounds.