Chapter 2
Chronic Wound Colonization, Infection, and Biofilms

Klaus Kirketerp-Møller, Karen Zulkowski, and Garth James

2.1 Introduction

The term "chronic wound" is generally accepted, but yet no simple definition has been agreed upon. A mechanistic definition such as "those not following normal wound healing trajectory" have been proposed but the most common definition have been "ulcers (wounds) older than 3 months of age". Indeed, some ulcers have been present for years. One to two percent of the population in the developed countries will experience a chronic wound in their lifetime (Gottrup 2004). It is expected that the number of chronic wounds will increase worldwide due to the increase of lifestyle diseases, such as diabetes, obesity, and cardiovascular diseases. It is estimated that 246 million people had diabetes worldwide in 2007 and this is expected to increase to 380 million people by the year 2025 (Diabetes Atlas).

Chronic wounds cause a significant burden to healthcare systems as well as morbidity and mortality to mankind. In Denmark, it has been estimated that the prevalence of non-healing wounds is about 1% of the population. The prevalence and incidence of ulcers are similar to elsewhere in the industrialized world. The total expenses for treatment of wounds are estimated to be approximately 2–3% of the total budget of the health care system in Denmark (Ayello et al. 2004, Gottrup 2004) which matches the percentages from the European Community and probably United States of America as well. Notably in the United States there has been an increase of hospital stays for pressure ulcers by approximately 80% from 1993 to 2006. The 2007 Medicare data indicated that the development of a pressure ulcer increased a person's hospital costs by $43,180. This adds up to 11 billion dollars a year in increased health care cost in the United States. According to the American Diabetes Association (ADA), 17.9 million Americans are diagnosed with either type I or type II diabetes, which is a major cause of premature mortality and disability. Additionally, it has been estimated that there are 6.2 million undiagnosed cases of diabetes in the United States. Often a chronic wound is the first manifestation of diabetes complications. Roughly 15% of people living with diabetes are estimated to

K. Kirketerp-Møller (✉)
Koege University Hospital, DK-4600 Koege, Denmark
e-mail: kkm@dadlnet.dk

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develop lower extremity ulcers, and 14–24% of people with a lower extremity ulcer eventually suffer an amputation (ADA). The annual cost for treating diabetic foot ulcers has been predicted to be around $20,000 per patient. This amounts to billions of dollars spent on diabetic foot ulcers annually in USA alone. Diabetes and, therefore, diabetic foot ulcer complications are growing at double digit rates and have the potential of becoming even a more devastating epidemic. Despite efforts directed at prevention, the rate of amputation in patients with DM continues to rise. Novel treatment strategies are required to reverse the rising trend in the rate of amputation. Focusing on healing these wounds will improve human health as well as reducing healthcare cost.

Interest in the potential association of biofilms in the pathology of chronic wounds has been demonstrated by the recent influx of publications referencing the key words "biofilm" and "chronic wound" (Fig. 2.1). Increasing evidence from these studies suggests that biofilms may play a role in wound chronicity. Microscopic evaluation of specimens from chronic wounds often indicates the presence of biofilms (Fig. 2.2). However, the role of bacteria and biofilms in wound healing is poorly understood. The following chapter will describe chronic wounds, and current treatments as well as review evidence that biofilms may be a major barrier to wound healing.

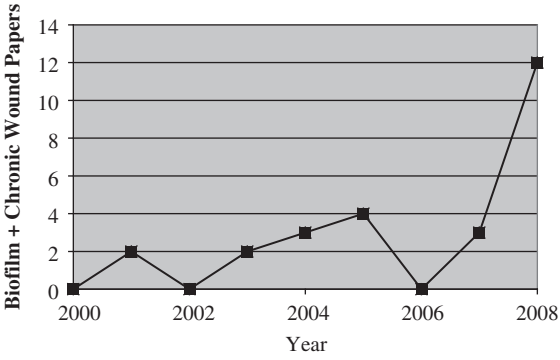

Fig. 2.1 Number of papers, by year, in the Institute for Scientific Information Web of Knowledge database (www.isiwebofknowledge.com) indexing to the combination of keywords "biofilm" and "chronic wound"

2.2 Types of Chronic Wounds

Chronic wounds are traditionally divided into three major groups: venous leg ulcers, diabetic foot ulcers, and pressure ulcers. Additionally there are other subgroups such as ischemic ulcers, cancer ulcers, and inflammatory ulcers. Common for all these groups are that all have underlying predisposing factors for origin and persistence.

2.2.1 Venous Leg Ulcers

Venous leg ulcers are the most common ulcers of the lower leg. Persons with venous ulcers often have lower leg edema and a history of previous phlebitis, tired aching legs, and a minor traumatic event (e.g., bumping the leg). Most venous

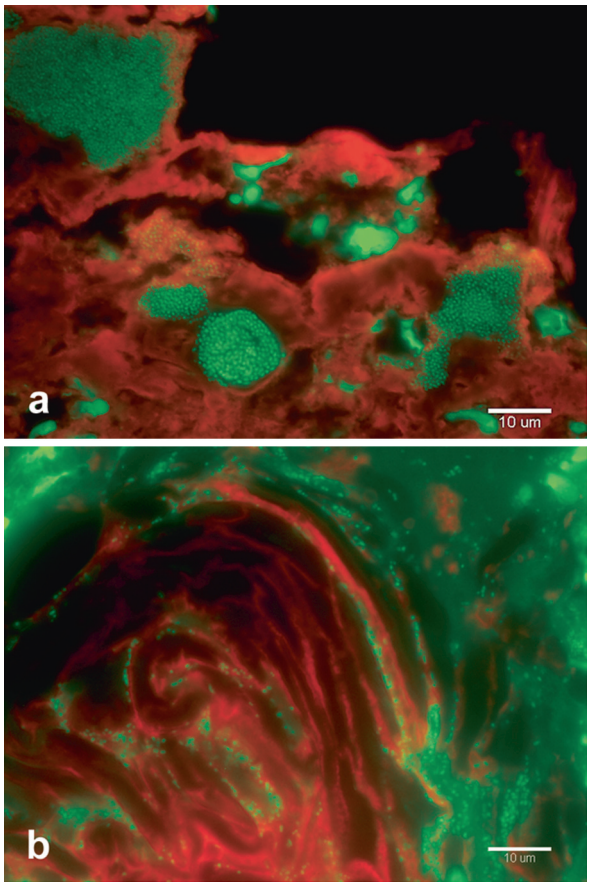

Fig. 2.2 Confocal scanning laser micrographs showing clusters of cocci that formed biofilms in a specimen from a venous leg ulcer specimen (**a**) and rod-shaped bacteria that formed biofilm in a specimen from a diabetic foot ulcer (**b**). Wound tissue was formalin fixed, processed, and embedded in paraffin. Sections (3–5 um) were then deparaffinized and stained with Molecular Probes' ViaGram™ Red+ Bacterial Gram Stain and Viability Kit following their fluorescent staining protocol. The kit includes SYTOX® Green nucleic acid stain, which stains DNA (Gram+ and Gram– DNA and host DNA) and Texas Red®-X dye-labeled wheat germ agglutinin (WGA), which stains host extracellular tissue and also selectively binds to the surface of gram-positive bacteria . Thus, small areas of scattered green staining indicate bacterial cells while the larger green regions are host cells. The host tissue was stained red. Microscopy by Dr. Kelly Kirker

ulcers are located on the medial malleolus and the skin surrounding the wound is hyper-pigmented.

2.2.2 Pressure Ulcers

Pressure ulcers are a localized injury to the skin and underlying tissue, usually over a bony prominence as a result of pressure or pressure in combination with friction and shear. They usually occur in patients who are immobilized either temporarily or

permanent especially with concomitant impaired sensation. Pressure ulcers are also called decubitus ulcers, bedsores, or pressure sores.

2.2.3 Diabetic Ulcers

This term is frequently used to describe ulcers that occur at the bottom of the feet in persons with diabetes mellitus. The direct cause of these ulcers is pressure. Peripheral neuropathy, structural foot deformities, or limited range of motion in the foot may increase pressure and contribute to the development of these ulcers. Diabetics often have ischemia as well, and the term "neuro-ischemic foot" is often used. The neuropathic foot is characterized by dry atrophic skin, deformity, and limited range of motion. The sensory function is often impaired and leaves the foot susceptible to injury.

2.2.4 Neuropathic Ulcers

The most common cause of neuropathy is diabetes. However, other causes include alcohol or drug abuse, hereditary defects, and some metabolic diseases or malnutrition. The frequency of neuropathy increases with age and is not uncommon in the age group of 80+ years.

2.2.5 Traumatic Ulcers

Although almost all other ulcers have a history of minor trauma, the term traumatic ulcer is used for ulcers that occur in patients who do not have a prior history of predisposing factors such as diabetes, venous or arterial vascular disorder, or immune-incompetence. Normally traumatic ulcers heal uneventfully but the size, necrotic tissue, contamination, or local edema may disturb healing. This may also include skin tears which are common in elderly persons. Skin tears may provide an ideal site for biofilm formation.

2.2.6 Arterial Ulcers

Peripheral vascular occlusive disease, which often presents as intermittent claudication, can also lead to slow-to-heal wounds. Many of the risk factors are similar to those for coronary artery disease. As with venous ulcers, the direct cause of an arterial ulcer is often a minor bump or bruise. Arterial ulcers often have a "punched out" appearance and there are signs of impaired tissue perfusion, such as pale skin and diminished or absent pedal pulses. However, arterial wounds often heal after successful revascularization or bypass of the arterial blockage.

2.3 Wound Treatment

Ideally the wounds are treated according to their origin, i.e., pressure ulcers are treated with off-loading and ambulation, venous ulcers with compression therapy,

and diabetic ulcers with off-loading and management of ischemia. However, in our experience, even referrals to tertiary centers have not received this treatment and have often been on several courses of antibiotic treatment, and numerous different dressings have been applied. The lack of recognition of underlying predisposing factors leads to insufficient treatment and delays or even prevents healing. This includes restoration of the patient's health status in general if at all possible.

2.3.1 Local Wound Treatment

Removal of necrotic or devitalized tissue is perhaps the most important part of local treatment. Several wound treatment regimens include this in their concept as in TIME (Tissue, Infection or Inflammation, Moisture balance and Edge effect) (Falanga et al. 2008, Rhoads. et al. 2008, Wolcott et al. 2009) and others (Werthen et al. 2004).

Removal of devitalized tissue minimizes the bioburden of the wounds by decreasing the presence of bacteria, reducing the hypoxic part of the wound and diminishing the local inflammatory reaction.

Moisture balance is an important aspect of wound healing and this is the main purpose of modern dressings. It has been demonstrated using occlusive and semi-occlusive dressings that a moist wound environment speeds epithelialization (Eaglstein and 1978) and collagen synthesis (Alvarez et al. 1983). However, occlusive dressings can also promote the growth of pathogens (Mertz and Eaglstein 1984), particularly anaerobic bacteria (Marshall et al. 1990). In contrast to wounds where moisture retention is necessary, highly exudative wounds require moisture removal. This has been achieved using absorbent dressings and more recently Negative Pressure Wound Therapy (NPWT).

A large variety of wound dressings are available; some of the most common types are shown in Table 2.1. Selection of a wound dressing should be based on several factors including (a) exudate amount, (b) presence of necrotic tissue, (c) bacterial burden, (d) presence of undermining or tunneling, and (e) providing protection from the environment. Dressings may also help with wound pain. Dressing choice can change as the wound progresses or deteriorates. Recently a number of antimicrobial wound dressings have become available. These dressings contain various antimicrobial agents including PHB (Wolcott and Rhoads 2008a) and silver (Percival et al. 2008). A number of non-dressing wound therapies are also currently used. Negative Pressure Wound Therapy (NPWT) is the application of a controlled level of sub-atmospheric pressure to a wound at 50–175 mm Hg (intermittent or continuous). The pressure is generated by a portable programmable pump. The suction effect is applied to the entire interior surface of a clean wound through open-cell polyurethane or polyvinyl alcohol (PVA) foam or most recently gauze, either impregnated with antimicrobial agents or plain. The fluid from chronic wounds may have a detrimental effect on the wound and its surroundings. Trengove et al. (1996a) demonstrated increased levels of cytokines and metalloproteases in chronic wounds compared to acute wounds. These and other factors likely contribute a negative

Table 2.1 Common wound dressings

Dressing type	Description
Foam	Inert material which is hydrophilic and non-adherent, modified polyurethane foam
Transparent film	Polyurethane and polyethylene membrane film coated with a layer of acrylic hypoallergenic adhesive. Moisture vapor transmission rates (MVTR) vary
Hydrocolloid	Gelatin, pectin, carboxymethylcellulose in a polyisobutylene adhesive base with polyurethane or film backing. Hydrophilic colloid particles bound to polyurethane foam
Hydrogel	Water or glycerin-based, non-adherent, cross-linked polymer. May or may not be supported by a fabric net, high water content, and varying amounts of gel-forming material (glycerin, co-polymer, water, propylene glycol, and humectant)
Calcium alginate	Calcium sodium salts of alginic acid (naturally occurring polymer in seaweed). Nonwoven composite of fibers from a cellulose-like polysaccharide which acts via an ion exchange mechanism, absorbing serous fluid or exudate and forming a hydrophilic gel which conforms to the shape of the wound
Silver	Topical silver has broad spectrum antimicrobial activity that has been shown to be effective against many antibiotic-resistant wound pathogens and can be added to a variety of composite dressings
Honey	Use of or addition of certain honeys as a part of a variety of composite dressings. Honey provides a hypertonic environment thought to assist with microbial control

effect on healing and excess amounts of wound fluid should be removed by the dressing.

Other non-dressing therapies include growth factors and skin grafting. Skin grafts may be of the persons own skin or tissue engineered skin. Hyperbaric oxygen is also considered a useful wound therapy.

2.3.2 Debridement

The removal of necrotic tissue and wound exudates is recognized as an important aspect of wound care. Debridement strategies range from occlusive dressings, which provide a moist environment for digestion by endogenous enzymes, to surgical removal of tissue. Wolcott and Rhoads (2008b) have stressed the importance of frequent (weekly) debridement as an important aspect of their biofilm-based wound care regimen. They used surgical debridement for non-healing ischemic leg ulcers and ultrasonic debridement as an adjunct therapy. Maggot larvae have also been used for wound debridement. Not only do the maggots digest and consume necrotic tissue and bacteria, but they also produce antimicrobial secretions (Bexfield et al. 2008, Huberman. et al. 2007). However experimental research on maggot excretions has shown different effects on biofilm formation in *Staphylococcus aureus* and *P. aeruginosa* (van der Plas et al. 2008). At least in the beginning of biofilm formation the maggot excretions seem to enhance the biofilm formation

in *P. aeruginosa*, but not in *S. aureus* (Leid et al. 2002a, van der Plas et al. 2008). Indeed the experience in Copenhagen Wound Healing Center is that maggot debridement in heavily *P. aeruginosa* colonized wounds was ineffective. Experimental research (Andersen et al. 2010) have shown that a Quorum Sensing dependent virulence factor from *P. aeruginosa* is capable of killing maggots. Debridement of chronic wounds likely plays an important role in biofilm control by not only removing the biofilm but also necrotic tissue which can harbor subsequent biofilm re-growth. Additionally, debridement can increase blood and oxygen supply to the wound bed.

2.4 Bacteria and Wounds

The role of bacteria in wound healing has been debated over the years. Some have suggested that bacteria may play a beneficial role in normal wound healing and wounds will heal despite the presence of large numbers of microorganisms (Edwards and Harding 2004). Nonetheless, the detrimental effects of specific pathogens, such as *Clostridium perfringes* and *Streptococcus pyogenes,* have been well recognized. These are typically invasive bacteria that are not normal members of the human skin microflora. In contrast, *S. aureus*, which is part of the microflora of many humans, commonly also causes wound infections. In cases of acute wound infection, wound cleansing, dressings, and systemic antibiotic therapy often provide an effective cure by killing or inhibiting the growth of bacteria thereby allowing the immune system to clear the infection.

The clinical definition of wound infection includes pain, erythema, edema, heat, and purulent exudates (Robson et al. 1990). Attempts have also been made to correlate the quantity of bacteria in a wound to infection. Robson et al. have suggested that quantitative bacterial counts from tissue biopsy samples of $<10^5$ CFU/gram indicate wound infection. Lower tolerance for specific organisms, such as haemolytic *Streptococci* is accepted due to higher virulence.

There has been some controversy over the method used to quantify wound bacteria, with some arguing for a simple swab culture (Bill et al. 2001) and others advocating tissue biopsy specimens (Robson et al. 1990). Biopsy specimens likely provide the most complete specimen through the depth of the wound, including bacteria invading the tissue. However, superficial specimens are less invasive and less difficult to collect, handle, and analyze, and may provide useful information regarding the quantity and types of microorganisms within a wound (Bowler et al. 2001). Furthermore, a swab culture may provide a specimen from a larger surface area of the wound. In our research, we have analyzed specimens obtained during sharp debridement of chronic wounds (Dowd et al. 2008, James et al. 2008) which provided a tissue specimen without involving a non-routine procedure. However, processing these specimens required specialized handling and analysis, and this approach is likely unpractical for routine clinical microbiology. In another work, Kirketerp-Moller et al. (2008) found discrepancy between clinical culturing and detection of bacteria with PNA-FISH and confocal light microscopy. *P. aeruginosa*

was detected in several cases where culturing failed, despite the fact that *P. aeruginosa* is considered easy to culture. In Fazli et al. (2009), it was shown that *S. aureus* is more likely to be at the surface of the wounds and that *P. aeruginosa* resides deeper in the tissue. This again adds fuel to the debate whether to use swabs or biopsies in detection of presence of bacteria.

Traditionally, wound microbiology has been described in three phases: contamination, colonization, and infection. Contamination refers to the presence of bacteria that are not multiplying, whereas colonization refers to bacteria which are growing within the wound but not causing tissue damage. Bacteria causing tissue damage and clinical signs of infection, discussed above, indicate an infected wound. The concept of "critical colonization" has also been used to describe bacteria that are growing within the wound and, while not causing classical clinical symptoms of infection, are adversely affecting wound healing (Edwards and Harding 2004).

The polymicrobial nature of wound communities has long been recognized. Various bacteria can be introduced into a wound from exogenous (soil and water) and endogenous (skin, saliva, urine, and feces) sources. As discussed above, most of these organisms are contaminants that do not multiply within the wound and even bacteria that do multiply are not considered "infective" unless they cause detrimental effects. This is particularly the case for bacteria considered commensal skin organisms, such as Corynebacteria and coagulase-negative Staphylococcus. However, the interactions of multiple microbial populations in chronic infections is poorly understood. In a study of 52 patients by Trengove et al., bacterial diversity was correlated with wound chronicity (Trengove et al. 1996b). Selective media were used for culture of six groups of bacteria; no particular group was associated with delayed healing but wounds that yielded four or more groups had a significantly higher proportion (42%) that failed to heal.

Over the past several years molecular-based methods have been increasingly applied in skin and wound microbiology research. Molecular studies of wound microbiology have revealed very diverse bacterial communities. These studies involved polymerase chain reaction (PCR) amplification of the bacterial gene encoding the small ribosomal subunit RNA (16S). This gene contains both highly conserved and variable regions of DNA, which enables bacterial speciation and forms the basis of bacterial phylogeny. Pioneering studies by Davies et al. (2001, 2004) and Hill et al. (2003) used PCR of 16S DNA along with denaturing gradient gel electrophoresis and clone libraries for culture-independent analysis of the microflora of chronic venous leg ulcers. More recent studies have used additional molecular methods including metagenomics for the evaluation of chronic wound microflora (Dowd et al. 2008, Frank et al. 2009, Price et al. 2009). The results of these studies indicate that chronic wounds contain diverse polymicrobial communities and similar community features, such as the presence of strictly anaerobic bacteria, even though the studies were from diverse geographic regions. However, Kirketerp-Moller et al. (2008) found only a few polymicrobial colonies in a study of venous leg ulcers. Thus, the prevalence and importance of polymicrobial biofilms in chronic wounds remains unclear.

2.5 Immune Response to Biofilms

In-vitro studies have demonstrated the ability of human leukocytes to penetrate *S. aureus* biofilms (Leid et al. 2005) (see also Chapter 15). In a murine model of acute wound infection, Akiyama et al. found that antimicrobial efficacy against *S. aureus* biofilms was significantly better in normal mice than those depleted of leukocytes (Akiyama et al. 2002) (see also Chapter 16). They suggested that the primary mechanism of antimicrobial action in normal mice was invasion of PMNs into the biofilm. For *P. aeruginosa* biofilms, production of the extracellular polysaccharide, alginate, protected them from IFN-γ mediated phagocytosis by human leukocytes (primarily monocytes) (Leid et al. 2005, Vuong et al. 2004). Similarly, polysaccharide intercellular adhesin (PIA) protected *Staphylococcus epidermidis* against phagocytosis and killing by polymorphonuclear leukocytes (PMN) (Gardner et al. 2001, Vuong et al. 2004). Thus, extracellular polysaccharides seem to be an important factor in biofilm resistance to phagocytosis. Furthermore, PIA also protected *S. epidermidis* against the antibacterial peptides, cathelicidin/hCAP18, human b-defensin 3 and dermcidin (Vuong et al. 2004). In addition to limiting the effectiveness of innate immune factors, *P. aeruginosa* biofilms cause killing of PMN through the production of rhamnolipids (Jensen et al. 2007). *S. aureus* is also capable of producing leukotoxins, including the Panton-Valentine leukocidin associated with severe cutaneous infections.

Chronic wounds often show increased levels of proinflammatory cytokines including IFN-α, IFN-γ, TNF-α, and interleukin 1. These cytokines are commonly produced in response to bacterial virulence determinants, such as lipopolysaccharides, peptidoglycans, and DNA. Chronic wounds also have high levels of matrix metalloproteinases (MMPs) including collagenases and gelatinases that are produced by PMN (Tarnuzzer and Schultz 1996) and correspondingly low levels of tissue inhibitors of matrix metalloproteinases (Yager and Nwomeh 1999). It has been suggested that these chronic wound characteristics are due to the presence of biofilms (Bjarnsholt et al. 2008).

In addition to the primarily innate immune mechanisms discussed above, *S. aureus* biofilms also can elicit adaptive immune responses, which may result in the development of biofilm-specific diagnostics and possibly vaccines (Leid et al. 2002b).

2.6 Biofilm Formation in Acute Wounds: In Vivo Models

Biofilm formation in wounds has been demonstrated in vivo using both murine and porcine models (see also Chapter 16). Akiyama et al. used neutropenic mice to evaluate *S. aureus* and *Streptococcus* biofilm formation within incisional wounds (Akiyama et al. 1996, Akiyama et al. 2002, 2003). The mice were treated with cyclophosphamide to inhibit leukocytes because normal mice were found to quickly clear inoculated bacteria with a strong PMN response. Biofilms were identified using light microscopy (LM) and electron microscopy (SEM) as well as confocal

scanning laser microscopy (CSLM). Aggregated clusters of bacteria were apparent. Polysaccharide extracellular polymeric substance (EPS) surrounding the bacteria was imaged after staining with ruthenium red (EM) and FITC-labeled concanavalin A (ConA, CSLM). Schaber et al. also used a murine model to evaluated biofilm formation by *P. aeruginosa* in burn wounds. They demonstrated that *P. aeruginosa* rapidly colonized burn wounds and formed biofilms primarily around blood vessels. Again the criteria used for biofilm classification was the presence of cell clusters and EPS. In addition to the general polysaccharide stain, ruthenium red, the presence of alginate was specifically detected using an anti-alginate antibody. Of all in vivo models developed to date, porcine skin structure and wound healing is the most similar to humans. Davis et al. demonstrated the formation of *S. aureus* biofilms and EPS on excisional wounds in pigs and further showed that planktonic bacteria recovered from the wounds were more susceptible to topical antimicrobial agents than the biofilm bacteria recovered from the same wounds (Davies 2003). All of the in vivo models described above utilized acute wound models. Further development is required to establish an in vivo model of chronic wounds. Nonetheless, these studies clearly demonstrate that bacteria readily form biofilms in animal models of wound infection. It should also be noted that, with the exception of anti-alginate antibody, they used general carbohydrate stains to identify EPS. Similar methods have been used in studies of human specimens. Due to the wide variety of carbohydrate residues associated with mammalian cells and tissues, it is impossible to determine whether the EPS is of bacterial or mammalian origin. As this material forms the matrix encapsulating the biofilm cells in vivo this should be considered an important part of the biofilm regardless of origin. Furthermore, incorporating host components into the EPS could help biofilms evade the immune system.

2.7 Evidence of Biofilms in Human Wounds

It had been speculated as early as 2001 that bacteria colonizing human chronic wounds exist as biofilm communities. Akiyama et al. used safranin, ConA, and immunofluorescent staining with CSLM to study and demonstrate the presence of *S. aureus* biofilms in specimens collected from patients with the skin diseases bullous impetigo, atopic dermatitis, and pemphigus foliaceus (Akiyama et al. 2003). Kirketerp-Møller et al. evaluated specimen wounds of 22 patients suspected of *P. aeruginosa* colonization (Kirketerp-Moller et al. 2008). They used PNA FISH and anti-alginate antibodies and found that *P. aeruginosa* existed as biofilms rather than single cells in these wounds. James et al. microscopically evaluated specimens from 50 chronic wounds and 16 acute wounds for the presence of biofilms and found biofilms in 60% of the chronic wounds and only 6% of the acute wounds (James et al. 2008).

2.8 Clinical Implications of Chronic Wound Biofilm

The recognition of bacterial biofilm in chronic wounds may give us the opportunity to explain many of the characteristics of the chronic wound. It may explain why chronic wounds does not heal despite adequate treatment of underlying condition

(Bjarnsholt et al. 2008) and it gives us new paths of research that may lead to new treatments.

The bacterial biofilm communities interfere with the human immune system in numerous ways. This interference facilitates establishment of further bacterial communities and inflammation of the chronic wound, and prevents healing. It is tempting for the clinician to start antibiotic treatment, but in case of established, mature biofilm this treatment often has only temporary effect on both inflammation and healing. In addition the clinician has to rely on the results from a swab or biopsy, which rarely reflects all specimens present in the wound. The bacteria in biofilm are up to 1000 times less susceptible to antibiotics (Bjarnsholt et al. 2007), and MIC is not reached in the chronic wound fluid. Even silver treatment, as incorporated in several wound dressings, has limited effect in biofilm in vitro (Falanga 2000, Wolcott and Rhoads 2008b). With this in mind the clinician should exercise restraint in admission of antibiotics. Administering antibiotics favors biofilm capable bacteria and promotes resistance to the administered antibiotic. Mechanical removal of wound debris and even granulation tissue is an effective way of diminishing the bacterial load and is an important part of treatment protocols as TIME and others.

Even in the case of extensive surgical debridement in combination with split skin transplant the presence of *P. aeruginosa* prior to surgery seams to influence the healing (Hoegsberg et al., unpublished results). This indicates that the bacteria reside deep in what is thought to be normal tissue, probably protected in biofilm.

From our point of view the recognition and acceptance of bacterial biofilm in chronic wounds already have changed wound care. “Biofilm managing strategies” have been made, but none have yet proved to be more effective than others or even better than “Best Practice.” But the rapid change reflects the need for new and more efficient treatment regimens and the research in biofilm may provide wound care specialists with new ways to heal the wounds.

2.9 Future Aspects

Understanding bacterial biofilm communities will provide us with knowledge to design new treatments for chronic wound patients. These treatments might work alone or act together in helping the host immune defense system to fight pathogens in the chronic wound. There could be several ways to do this. See Table 2.2.

Table 2.2 Future treatment options

Biofilm formation	Turn off biofilm production
Quorum sensing	Manipulating QS
Antibiotic resistance	QS Manipulation
	New biofilm penetrating drugs
Biofilm disrupting agents	New agents have to disrupt the biofilm in order to reach bacteria residing deep in the wound
Mechanical debridement	Surgery
	Ultra sound assisted surgery

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References

Akiyama H, Huh WK, Fujii K, Yamasaki O, Oono T, Iwatsuki K (2002) Confocal laser microscopic observation of glycocalyx production by Staphylococcus aureus in vitro. J Dermatol Sci 29(1):54–61

Akiyama H, Kanzaki H, Tada J, Arata J (1996) Staphylococcus aureus infection on cut wounds in the mouse skin: experimental staphylococcal botryomycosis. J Dermatol Sci 11(3):234–238

Akiyama H, Morizane S, Yamasaki O, Oono T, Iwatsuki K (2003) Assessment of Streptococcus pyogenes microcolony formation in infected skin by confocal laser scanning microscopy. J Dermatol Sci 32(3):193–199

Alvarez OM, Mertz PM, Eaglstein WH (1983) The effect of occlusive dressings on collagen synthesis and re-epithelialization in superficial wounds. J Surg Res 35(2):142–148

Andersen AS, Joergensen B, Bjarnsholt T et al (2010) Quorum-sensing-regulated virulence factors in Pseudomonas aeruginosa are toxic to Lucilia sericata maggots. Microbiology 156:400–407

Ayello EA, Dowsett C, Schultz GS, Sibbald RG, Falanga V, Harding K, Romanelli M, Stacey M, TEot L, Vanscheidt W (2004) TIME heals all wounds. Nursing 34(4):36–41

Bexfield A, Bond AE, Roberts EC, Dudley E, Nigam Y, Thomas S, Newton RP, Ratcliffe NA (2008) The antibacterial activity against MRSA strains and other bacteria of a <500 Da fraction from maggot excretions/secretions of Lucilia sericata (Diptera: Calliphoridae). Microbes Infect 10(4):325–333

Bill TJ, Ratliff CR, Donovan AM, Knox LK, Morgan RF, Rodeheaver GT (2001) Quantitative swab culture versus tissue biopsy: a comparison in chronic wounds. Ostomy Wound Manage 47(1):34–37

Bjarnsholt T, Kirketerp-Moller K, Jensen PO, Madsen KG, Phipps R, Krogfelt K, Hoiby N, Givskov M (2008) Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen 16(1):2–10

Bjarnsholt T, Kirketerp-Moller K, Kristiansen S, Phipps R, Nielsen AK, Jensen PO, Hoiby N, Givskov M (2007) Silver against Pseudomonas aeruginosa biofilms. APMIS 115(8):921–928

Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14(2):244–269

Davies D (2003) Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2(2):114–122

Davies CE, Hill KE, Wilson MJ et al (2004) Use of 16S ribosomal DNA PCR and denaturing gradient gel electrophoresis for analysis of the microfloras of healing and nonhealing chronic venous leg ulcers. J Clin Microbiol 42:3549–3557

Davies CE, Wilson MJ, Hill KE et al (2001) Use of molecular techniques to study microbial diversity in the skin: chronic wounds reevaluated. Wound Repair Regen 9:332–340

Dowd SE, Sun Y, Secor PR, Rhoads DD, Wolcott BM, James GA, Wolcott RD (2008) Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol 8, 43

Eaglstein WH Mertz PM (1978) New methods for assessing epidermal wound healing: the effects of triamcinolone acetonide and polyethelene film occlusion. J Invest Dermatol 71(6): 382–384

Edwards R, Harding KG (2004) Bacteria and wound healing. Curr Opin Infect Dis 17(2):91–96

Falanga V (2000) Classifications for wound bed preparation and stimulation of chronic wounds. Wound Repair Regen 8(5):347–352

Falanga V, Brem H, Ennis WJ, Wolcott R, Gould LJ, Ayello EA (2008) Maintenance debridement in the treatment of difficult-to-heal chronic wounds. Recommendations of an expert panel. Ostomy Wound Manage Suppl:2–13

Fazli M, Bjarnsholt T, Kirketerp-Moller K et al (2009) Nonrandom distribution of Pseudomonas aeruginosa and Staphylococcus aureus in chronic wounds. J Clin Microbiol 47:4084–4089

Frank DN, Wysocki A, Specht-Glick DD, Rooney A, Feldman RA, St Amand AL, Pace NR, Trent JD (2009) Microbial diversity in chronic open wounds. Wound Repair Regen 17(2):163–172

Gardner SE, Frantz RA, Troia C, Eastman S, MacDonald M, Buresh K, Healy D (2001) A tool to assess clinical signs and symptoms of localized infection in chronic wounds: development and reliability. Ostomy. Wound Manage 47(1):40–47

Gottrup F (2004) A specialized wound-healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. Am J Surg 187(5A):38S–43S

Hill KE, Davies CE, Wilson MJ, Stephens P, Harding KG, Thomas DW (2003) Molecular analysis of the microflora in chronic venous leg ulceration. J Med Microbiol 52:365–369

Huberman L, Gollop N, Mumcuoglu KY, Breuer E, Bhusare SR, Shai Y, Galun R (2007) Antibacterial substances of low molecular weight isolated from the blowfly, Lucilia sericata. Med Vet Entomol 21(2):127–131

James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J, Costerton JW, Stewart PS (2008) Biofilms in chronic wounds. Wound Repair Regen 16(1):37–44

Jensen PO, Bjarnsholt T, Phipps R, Rasmussen TB, Calum H, Christoffersen L, Moser C, Williams P, Pressler T, Givskov M, Hoiby N (2007) Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by Pseudomonas aeruginosa. Microbiology 153(Pt 5):1329–1338

Kirketerp-Moller K, Jensen PO, Fazli M, Madsen KG, Pedersen J, Moser C, Tolker-Nielsen T, Hoiby N, Givskov M, Bjarnsholt T (2008) Distribution, organization, and ecology of bacteria in chronic wounds. J Clin Microbiol 46(8):2717–2722

Leid JG, Costerton JW, Shirtliff ME, Gilmore MS, Engelbert M (2002b) Immunology of Staphylococcal biofilm infections in the eye: new tools to study biofilm endophthalmitis. DNA Cell Biol 21(5–6):405–413

Leid JG, Shirtliff ME, Costerton JW, Stoodley AP (2002a) Human leukocytes adhere to, penetrate, and respond to Staphylococcus aureus biofilms. Infect Immun 70(11):6339–6345

Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK (2005) The exopolysaccharide alginate protects Pseudomonas aeruginosa biofilm bacteria from IFN-gamma-mediated macrophage killing. J Immunol 175(11):7512–7518

Marshall DA, Mertz PM, Eaglstein WH (1990) Occlusive dressings. Does dressing type influence the growth of common bacterial pathogens? Arch Surg 125(9):1136–1139

Mertz PM, Eaglstein WH (1984) The effect of a semiocclusive dressing on the microbial population in superficial wounds. Arch Surg 119(3):287–289

Percival SL, Bowler P, Woods EJ (2008) Assessing the effect of an antimicrobial wound dressing on biofilms. Wound Repair Regen 16(1):52–57

Price LB, Liu CM, Melendez JH, Frankel YM, Engelthaler D, Aziz M, Bowers J, Rattray R, Ravel J, Kingsley C, Keim PS, Lazarus GS, Zenilman JM (2009) Community analysis of chronic wound bacteria using 16S rRNA gene-based pyrosequencing: impact of diabetes and antibiotics on chronic wound microbiota. PLoS One 4(7):e6462

Rhoads DD, Wolcott RD, Percival SL (2008) Biofilms in wounds: management strategies. J Wound Care 17(11):502–508

Robson MC, Stenberg BD, Heggers JP (1990) Wound healing alterations caused by infection. Clin Plast Surg 17(3):485–492

Tarnuzzer RW, Schultz GS (1996) Biochemical analysis of acute and chronic wound environments. Wound Repair Regen 4(3):321–325

Trengove NJ, Langton SR, Stacey MC (1996a) Biochemical analysis of wound fluid from nonhealing and healing chronic leg ulcers. Wound Repair Regen 4(2):234–239

Trengove NJ, Stacey MC, McGechie DF, Mata S (1996b) Qualitative bacteriology and leg ulcer healing. J Wound Care 5(6):277–280

van der Plas MJ, Jukema GN, Wai SW, Dogterom-Ballering HC, Lagendijk EL, van, Gulpen C, van Dissel JT, Bloemberg GV, Nibbering PH (2008) Maggot excretions/secretions are differentially effective against biofilms of Staphylococcus aureus and Pseudomonas aeruginosa. J Antimicrob Chemother 61(1):117–122

Vuong C, Voyich JM, Fischer ER, Braughton KR, Whitney AR, Deleo FR, Otto M (2004) Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. Cell Microbiol 6(3):269–275

Werthen M, Davoudi M, Sonesson A, Nitsche DP, Morgelin M, Blom K, Schmidtchen A (2004) Pseudomonas aeruginosa-induced infection and degradation of human wound fluid and skin proteins ex vivo are eradicated by a synthetic cationic polymer. J Antimicrob Chemother 54(4):772–779

Wolcott RD, Rhoads DD (2008a) A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care 17(4):145–2, 154

Wolcott RD, Rhoads DD (2008b) A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care 17(4):145–2, 154

Wolcott RD, Kennedy JP, Dowd SE (2009) Regular debridement is the main tool for maintaining a healthy wound bed in most chronic wounds. J Wound Care 18(2):54–56

Yager DR, Nwomeh BC (1999) The proteolytic environment of chronic wounds. Wound Repair Regen 7(6):433–441

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