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Anti-Biofilm Strategies and the Need for Innovations in Wound Care

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Abstract

With an aging and obese population, chronic wounds such as diabetic ulcers, pressure ulcers, and venous leg ulcers are of an increasingly relevant medical concern in the developed world. Identification of bacterial biofilm contamination as a major contributor to non-healing wounds demands biofilm-targeted strategies to treat chronic wounds. While the current standard of care has proven marginally effective, there are components of standard care that should remain part of the wound treatment regime including systemic and topical antibiotics, antiseptics, and physical debridement of biofilm and devitalized tissue. Emerging anti-biofilm strategies include novel, non-invasive means of physical debridement, chemical agent strategies, and biological agent strategies. While aging and obesity will continue to be major burdens to wound care, the emergence of wounds associated with war require investigation and biotechnology development to address biofilm strategies that manage multi-drug resistant bacteria contaminating the chronic wound. The article presents some of the recent patents related to anti-biofilm strategy in wound care.

Keywords

Bacterial biofilm; wound care; therapy

INTRODUCTION

While quality of life has vastly improved with advances in medicine and nutrition in the developed world, the related rise in obesity and the aging population threaten to reverse these advances and place a major burden on the already overwhelmed healthcare system. Chronic wounds such as diabetic foot ulcers, pressure ulcers, and venous leg ulcers have contributed to a steep rise in medical costs [1]. In the United States alone, an estimated $58 billion in medical costs is associated with chronic complications that afflict nearly 18 million diabetics [2]. Current standard of care for non-healing wounds consists of transiently effective systemic and topical antibiotic treatment often followed by amputation. An estimated 14–24% of diabetic patients in the United States will undergo amputation [1] and must suffer the resultant co-morbidities. There is clearly a need for targeted, effective therapy for the rising problem of chronic wounds.

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CONFLICT OF INTEREST

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Chronic and acute wounds initially progress through the same stages of healing [3]. In normal wound healing, the wound will progress through hemostasis, inflammation, granulation, epithelialization, and maturation; however, in the chronic wound, resolution of inflammation does not occur and the wound remains in a persistent state of “acute” inflammation [4]. Typical of unresolved inflammation, the chronic wound is characterized by prolonged expression of the inflammatory cytokines interleukin-1 [IL-1] and tumor necrosis factor-alpha [TNF-α] [5, 6]. While many factors may contribute to the abnormal condition of the chronic wound, it has become increasingly clear that bioburden is a significant barrier to normal wound healing [7]. That bioburden includes both devitalized tissue and colonizing microorganisms, including bacteria organized into biofilms (Fig. 1).

Biofilms are structured communities of microorganisms, organized into microcolonies, adhered to a surface, and exhibiting phenotypic heterogeneity [8]. In the chronic wound, bacterial contamination develops into colonization, and devitalized tissue provides the surface to which biofilms adhere. Unlike planktonic bacteria, bacterial biofilms consist of about 80% extracellular polymeric substances [EPS] consisting of polysaccharides, proteins, and nucleic acids and about 20% bacterial cells [9]. Additionally, bacterial biofilms are typically multi-species [8]. In the chronic wound, non-healing is dependent on the bacteria successfully establishing biofilm growth [8, 10, 11]. It has been well demonstrated that the establishment of bacterial biofilm in the wound is the major reason for the failure of acute wound treatment and development of chronic, non-healing wounds [4, 12–14]. In the United States alone, the National Institutes of Health estimates that 80% of microbial inflections could be characterized as biofilms [4, 15]. In animal chronic wound models, biofilm formation in the wound is associated with unregulated inflammation and delayed or altered wound healing [16]. Clearly, therapies addressing wound healing must account for the presence of contaminating biofilm and directly incorporate strategies that target bacterial biofilm.

Treatment of bacterial biofilm in the wound is complicated by the very character of the biofilm mode of growth including increased resistance of the biofilm to traditional antimicrobial treatments and host immune defense [10, 15, 17–19]. Multiple characteristics of the biofilm contribute to this resistance. Traditional antibiotics have been designed against planktonically-grown bacteria and treat metabolically active bacteria; however, bacteria in a biofilm are metabolically different from planktonic bacteria [18] and within the biofilm, the metabolic activity of the bacteria can change significantly with highly metabolically active cells at the surface of the biofilm and metabolically inert cells deep within the biofilm [15, 20]. Furthermore, mixed species biofilms have complementary metabolic strategies for obtaining nutrients and for degradation of host immune molecules [21–24]. Further protection of the biofilm is provided by the physical barrier of the EPS surrounding the bacterial cells [9, 15] and by the presence of metabolically inactive persister cells deep within the biofilm [25]. Finally, the multispecies biofilm community creates an efficient platform for the exchange of drug resistance genes [26, 27]. In designing novel therapies for wounds, it is necessary to consider and account for the unique ability of the biofilm to resist treatment.

The present review aims to provide a perspective on the emergence of novel wound treatments designed to address the presence of bacterial biofilm contamination in the wound. There are many wound treatment strategies both in development and out on the market and it is beyond the scope of this review to comprehensively cover all these strategies, rather it is the intention of this review to provide a sampling of biofilm targeted strategies for wound healing.

CURRENT STANDARD OF CARE

Current standard of care includes, but is not limited to, the use of antiseptics and both topical and systemic antibiotics in combination with wound dressings that may or may not be designed with anti-biofilm characteristics. While topical antiseptics can be bactericidal [28], antiseptics can damage host cells such as fibroblasts and keratinocytes and may thus interfere with the normal wound healing process [29]. While not necessarily designed to be anti-microbial, wound dressings help reduce bacterial load and acute infection rates [30, 31]. The physical presence of the wound dressing, regardless of whether the dressing is designed with anti-biofilm characteristics, does appear to inhibit microbial colonization in the wound by eliminating pockets of open space at the wound surface [32, 33]; however, the physical interaction between the wound bed, the wound dressing, and colonizing bacteria remains an area of limited research.

As mentioned above, current antibiotics may have little long-term effect at preventing or treating the established biofilm as most of these antibiotics are designed to target metabolically active bacteria and dormant cells within the bacterial biofilm are unresponsive [9]. However, in the case of deep tissue wounds, systemic antibiotics are warranted to prevent systemic bacterial invasion and sepsis [28]. Conversely, when treating or preventing the establishment of bacterial biofilms within the wound, system antibiotics have been found to be only 25–32% efficacious [34, 35]. Furthermore, use of systemic antibiotics is problematic with ischemic wounds because of the lack of sufficient circulation at the wound site [28]. Although current standard of care clearly has some role in wound treatment, there is a need for more tools in the wound care toolbox.

BIOFILM CONTROL STRATEGIES IN WOUND CARE

Physical Strategies of Biofilm Management

Bioburden in the wound consists of both contaminating biofilm and devitalized tissue; removal of this bioburden not only reduces the contaminating bacteria, but also revitalizes the host immune defenses. Sharp physical debridement of the wound bed significantly reduces the microorganisms in the wound bed, removes devitalized host tissue, and is thus a vital step in biofilm control in wound care [36–38]. Standardization of physical debridement will greatly enhance the quality of wound care. To that end, recent patents on wound debridement have been submitted. For example, a method, device, and kit for lesion debridement was filed in 2005 [39]. Sharp debridement can be very painful and comprehensive debridement is dependent on the clinician; therefore, alternative methods of debridement have recently shown some interest. For example, pulsed electrical fields have been used to disaggregate bacterial biofilm [40] and use of acoustic shock waves to eradicate

or prevent biofilm formation has been submitted for patent [40] as well as use of ultrasound for debriding wounds [41]. Pulsed ultrasound for biofilm disruption has been supported *in vitro* and its use in patients has been associated with decreased bioburden; however, a direct demonstration of the efficacy of ultrasound debridement has not yet been achieved *in vivo* [42, 43]. While these methods are promising as non-invasive means of debridement, their efficacy has yet to be proven in the clinic.

Chemical Strategies of Biofilm Management

***Ionic Silver—**Use of ionic silver has become increasingly popular in the wound care industry and there are many wound dressings on the market that contain silver either covalently bound or as nanocrystaline particles. The large variation in silver content, silver release, and antibacterial activity between various silver containing dressings make identifying the most efficacious dressing for a wound condition difficult. Although silver dressings have been demonstrated as effective against biofilms *in vitro* [44, 45], there remains some debate as to whether enough ionic silver is released from silver containing dressings into the wound bed in order to treat biofilms present in the chronic wound [46]. Regardless of the variation on the market, ionic silver has been demonstrated to be bactericidal in very low concentrations and to be efficacious against multiple species of pathogenic bacteria [47, 48]. Use of silver containing materials against biofilms has been patented for use with medical devices [49]. Of recent concern, is the potential for damage to host keratinocytes with the use of high silver-containing wound dressings [50]

***Iodine—**Iodine is a naturally occurring, though unstable, chemical element that has been used as a disinfectant for acute wounds for many years. While commonly used, the long-term antimicrobial efficacy of iodine remains debatable and as an anti-biofilm strategy concerns about the chemical stability of iodine remain. Of further concern is the potentially toxic effect of iodine on host cells [29, 51]. To address concerns of chemical stability, elemental iodine has been complexed with polyvinylpyrrolidone [PVP] to get providone-iodine [PVD-I]. Use of providone-iodine has been demonstrated as microbicidal on *Staphylococcus epidermidis* biofilms *in vitro* [52] and may damage the host cells less than elemental iodine [53]. Use of providone-iodine in a composition for managing bacterial biofilm has been patented [54] in addition to an older patent using providone-iodine for wound-healing preparations [55]. To make water-soluble iodine, cadexomer iodine is produced by a reaction of dextran with epichlorhydrin and iodine. While cadexomer iodine has been demonstrated as effective as part of the comprehensive treatment of venous leg ulcers [56], more recently it has been demonstrated to be directly microbicidal against *Staphylococcus aureus* biofilms *in vitro* [57]. Although iodine has been around for quite a while, the efficacy of iodine against bacterial biofilm remains to be established *in vivo*.

***Gallium—**Gallium is a chemical element that does not occur in nature as elemental gallium, but rather as the gallium [III] salt. Because gallium is very similar to iron on an atomic scale, gallium ion can localize and interact with biological systems dependent on iron [III]. The bactericidal character of gallium is thought to result from gallium disruption of iron[III]-dependent biological processes in bacteria including respiration [58]. Gallium nitrate is FDA approved for clinical use to treat hypercalcemia associated with tumor

metastasis to bone [59], and has been shown to interfere with biofilm development [28]. More recently, gallium has been investigated as an anti-biofilm strategy in cystic fibrosis and has been used effectively against biofilms comprised of the major cystic fibrosis pathogen *Pseudomonas aeruginosa* [60]. Recent patents have been filed claiming use of gallium against oral biofilms [61], use against antibiotic resistant pathogens [62], and use for coating medical devices to prevent biofilm formation [63]. Use of gallium as a topical wound treatment strategy hold promise; however, more research is necessary considering the pharmacokinetics of gallium [64].

***EDTA—**Ethylenediaminetetraacetic acid [EDTA] is a polyamino carboxylic acid that chelates metal ions such as calcium[II] and iron[III]. EDTA has been used as an antibacterial strategy for over forty years and acts as a microbicide primarily through the ability to chelate iron and interfere with iron[III]-dependent biological pathways in bacteria [65]. While EDTA has been used extensively in the clinic to treat lead and heavy metal poisoning [66], more recently EDTA has been used therapeutically for coronary heart disease [67]. Disodium EDTA was demonstrated to inhibit *Staphylococcus epidermidis* attachment to medical catheters *in vitro* over twenty years ago [68]; however, more recently tetrasodium EDTA showed a broad spectrum inhibitory effect against *in vivo* generated biofilms attached to catheters [69, 70]. Finally, incorporation of EDTA into a wound gel enhanced the efficacy of the gel against *Pseudomonas aeruginosa* biofilms [71]. Because of its observed antimicrobial properties, use of EDTA as part of an antiseptic composition for use against biofilms has been patented [72]. Although EDTA has been used medically for years, concerns remain regarding the effect of EDTA on the host [73].

***General Biocides—**While bactericidal activity cannot be disregarded in anti-biofilm strategies, the EPS characteristic of the biofilm plays an important role in biofilm resistance to antimicrobials. No matter how effective an anti-microbial is at killing bacteria, it will be virtually useless if it is unable to penetrate into the biofilm. Therefore, dispersion or disaggregation of biofilms by chemically removing the EPS renders the biofilm more susceptible to antibiotic treatment [15]. Quaternary ammonium compounds are a general biocide that increases biofilm cell susceptibility to antimicrobial peptides and antibiotics by chemically changing the biofilm structure [74]. Another general biocide is the bismuth thiols, which suppress bacterial exopolysaccharide expression in *Klebsiella*, *Staphylococcus*, and *Pseudomonas* sp. [75]. This ability to enhance the susceptibility of bacterial biofilm to antimicrobials makes the bismuth thiols an interesting biofilm control strategy and therefore patent submission has been made on the use of bismuth thiols for medical products [76]. Although EPS dispersal is an intriguing strategy for biofilm management in the wound, combined treatment with an antimicrobial would also be necessary to inhibit bacterial contamination into the wound.

Biological Agent Strategies of Biofilm Management

***Honey—**Honey has been used medicinally by indigenous cultures for hundreds, perhaps thousands of years; however, recent investigations into the use of honey as a treatment for wounds suggest that honey may indeed have antimicrobial and pro-healing effects. Killing of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms *in vitro* was successfully

demonstrated with two types of honey, Sidr and Manuka honeys. As has been observed for many antimicrobials, efficacy against planktonic culture was notably better for both honeys when compared to efficacy against biofilm culture [77]. Honey may also mediate pro-healing effects on host cells as *in vitro* studies with monocytic cell lines found that honey enhances release of inflammatory cytokines associated with innate immunity [78, 79]. Although it remains unclear as to how honey mediates biofilm control, it has been suggested that key mechanisms may include changes in osmotic potential and activity of phytochemicals [80]. Regardless of the mechanism of action, the recent, successful use of honey to treat wounds has lead to patent submission of a honey-based wound dressing [81].

***Lactoferrin—**Lactoferrin is an iron chelating protein found in most bodily fluids, but concentrated in milk [82]. Lactoferrin has been determined to be effective at killing both planktonic and biofilm bacteria [83, 84]. Additionally, lactoferrin has been demonstrated *in vitro* to prevent *Pseudomonas aeruginosa* biofilm formation by preventing bacterial adhesion to a surface, the essential first step in biofilm formation [85]. Iron[III] binding by lactoferrin can have a bacteriostatic effect on bacteria by depriving the cells of this essential nutrient [86], but may also contribute to destabilization of the bacterial membrane [84]. In cultures with planktonic cells, lactoferrin has also been demonstrated to bind the lipopolysacchride component of the bacterial outer membrane causing membrane permeablization and cell death in Gram-negative bacteria [83]. Because of its multifaceted nature, lactoferrin is broad spectrum and has been used effectively to control *in vitro* biofilms consisting of periodontal pathogens [87], cystic fibrosis-associated pathogens [88], and atopic skin-associated pathogens [89]. In the clinic, lactoferrin has been used successfully as part of a comprehensive treatment regime to manage biofilm-associated chronic rhinosubusitis [90] and biofilm-associated ischemic wounds [36]. Because of the demonstrated efficacy of lactoferrin against biofilm-forming pathogens, patents describing the use of lactoferrin in treating wounds have been submitted [91, 92].

***Xylitol—**Xylitol is a sugar alcohol found in a limited number of fruits and vegetables [93]. The majority of research concerning the antimicrobial properties of xylitol has been centered on oral biofilms [94]. For example, xylitol inhibited biofilm growth in a six species oral biofilm model [95] and inhibited expression of metabolizing enzymes required for surface adhesion of carriogenic streptococci [96]. Accordingly, a patent has been filed on inventions that make claims on the use of xylitol with oral products [97]. Use of xylitol to treat oral biofilms lead to the fortuitous discovery that oral use of xylitol reduces the incidence of recurrent otitis [98]. Subsequent use of xylitol in the nasal passage demonstrated that the xylitol could inhibit bacterial adhesion to the nasal mucosa and lead to the patent submission for a xylitol-based nasal spray [99]. Additionally, treatment of atopic dry skin with xylitol was effective in preventing colonization by *Staphylococcus aureus* [100] possibly through inhibition of bacterial glycolysis [101]. Xylitol was also recently demonstrated to be mildly efficacious against wound colonizing *Pseudomonas aeruginosa* biofilms *in vitro* [84] and patent claims have been filed on the use of xylitol to prevent or control biofilms in chronic wounds [91].

***Dispersin B—**Dispersin B is a naturally occurring N-acetylglucosa-minodase produced by the periodontal pathogen *Aggregatibacter actinomycetemcomitans* [102]. Although the endogenous role of Dispersin B is debatable, it has been demonstrated to inhibit biofilm formation [103–105]. Principally studied for use in dentistry, Dispersin B disseminates bacterial biofilm by targeting the EPS and degrading the biofilm community structure [106]. Specifically, Dispersin B hydrolyzes glycosidic linkages in the polysaccharide of the EPS to destabilize the biofilm framework [107–111]. These investigations led to the submission of a patent for the use of Dispersin B to detach bacterial and fungal biofilms [112]. Although use of Dispersin B is unlike other biofilm strategies in that the intention is not to kill the bacteria but rather to break up the structure of the biofilm, this enzymatic disruption of the would be beneficial in combination with a microbicidal agent for managing bacterial biofilm contamination within the wound.

***Bacteriophages—**Bacteriophages are viruses that infect bacteria and have recently become of renewed interest to the field of biofilm control due to their ability to lyse bacteria [113]. Bacteriophages are highly abundant and can be isolated from anywhere bacteria exist: from the soil to the human intestine [114]. Because bacteriophages only infect bacteria, therapy with bacteriophages targets sights of bacterial colonization [115]. Use of bacteriophages to control systemic bacterial infections has been successful with treatment of meningitis and septicemia [116], and bacteriophages have been demonstrated to be effective against biofilms both through disruption of the EPS and lysing of the biofilm-associated bacterial cells [117]. This suggests that use of bacteriophages to manage colonizing bacteria in the wound may prove both safe and efficacious; however, there remains concern that bacterial lysis by the bacteriophages will release endotoxin into the wound resulting in non-specific and unrestrained activation of innate immunity and the inflammatory response. Despite potential concerns regarding the patenting of living organisms, the medical use of bacteriophage to inhibit biofilm formation has been submitted as a patent [118].

***Quorum Sensing Inhibitors—**Quorum sensing by bacterial species allows for coordinated gene expression based on the density of the bacterial population. Inter-and intra-species bacterial communication and genetic synchrony through quorum sensing appear to play an important role in biofilm formation [119]. Because of this apparent requirement for quorum sensing in biofilm development, inhibition of quorum sensing has developed into an area of biofilm management strategy [120]. Additionally, patents have been filed in which the invention is either a compound designed to inhibit bacterial quorum sensing [121] or a series of synthetic ligands intended to act either as agonists or antagonists to bacterial quorum sensing networks [122]. The Gram-negative bacterial quorum sensing system primarily consists of networks mediated by acylhomoserin lactone and has been identified in more than seventy species [123]. Therefore, patent filing has occurred for the use of synthetic ligands designed to modulate quorum sensing mediated through the acylhomoserin lactone pathway [124]. Other signaling molecules found in Gram-negative bacteria include autoinducers [2 and 3], diffusible signal factor, and cyclic dipeptide; however, autoinducer 2 [AI-2] has best been demonstrated as required for mixed species biofilm formation [125]. To manage bacterial biofilms, the use of halogenated furanone compounds has been investigated [126]; however there is some concern with the use of furanone compounds due

to the potential for host damage [127]. Autoinducer-2 analogues have also been patented for regulating bacterial growth and pathogenesis including biofilm formation [128]. Although use of quorum sensing inhibitors may prove efficacious for management of bacterial biofilms in wounds, their efficacy and safety *in vivo* remains to be substantiated.

CURRENT & FUTURE DEVELOPMENTS

Although the epidemic increase in obesity and the comorbidities associated with the development of obesity-related diabetes must remain a major area of research and technology development, another area of tremendous importance in wound care has recently emerged. In modern combat, wounds that once would have been fatal are now being survived, but at a great cost to the wounded. Combat wounds are distinct from diabetic wounds and particularly challenging in part because of high contamination, the devitalized nature of the wounds, and delays to medical treatment. On the other hand, chronic war wounds are similar to chronic civilian wounds in that a major barrier to healing is mediated by the presence of bacterial biofilm, in particular multi-drug resistant Gram-negative bacteria [16, 129]. Not only are soldiers and war wounded civilians receiving wounds in the field, but are also at high risk of developing hospital-associated infections [130]. Unfortunately, war continues and regardless of the political and social complications of war, comprehensive treatment of the war wounded is an essentially moral responsibility for the biomedical discipline. Clearly, there is a need for cost effective, highly stable, biofilm targeted strategies to manage wounds received on the field and developed in hospitals.

Part of the difficulty of treating war wounds is the highly recalcitrant character of bacterial biofilm and the development of multi-drug resistance; therefore, there is a need for medical biotechnology that targets these issues. In order to manage the development of resistance, biofilm targeted strategies would best be designed with multiple targets of bacterial inhibition. A patented example of the use of combined treatment for managing bacterial biofilm in wounds is the use of lactoferrin and xylitol [91]. While both lactoferrin and xylitol are naturally occurring antimicrobials, bacteria have evolved mechanisms of resistance to the use of either of these antimicrobials alone [131–133]; however, when used in combination, lactoferrin and xylitol are significantly more efficacious against establish biofilm *in vitro* than when used alone [84]. Because lactoferrin and xylitol have been utilized by nature to combat bacteria, the use of these antimicrobials in combined therapy provides a cost-effective, easily obtained, biofilm-targeted strategy for treatment of both civilian chronic wounds and the war wounded. In conclusion, more strategies are needed in which integrated, biofilm-targeted therapies are used to manage the presence of bacterial biofilm in the wound.

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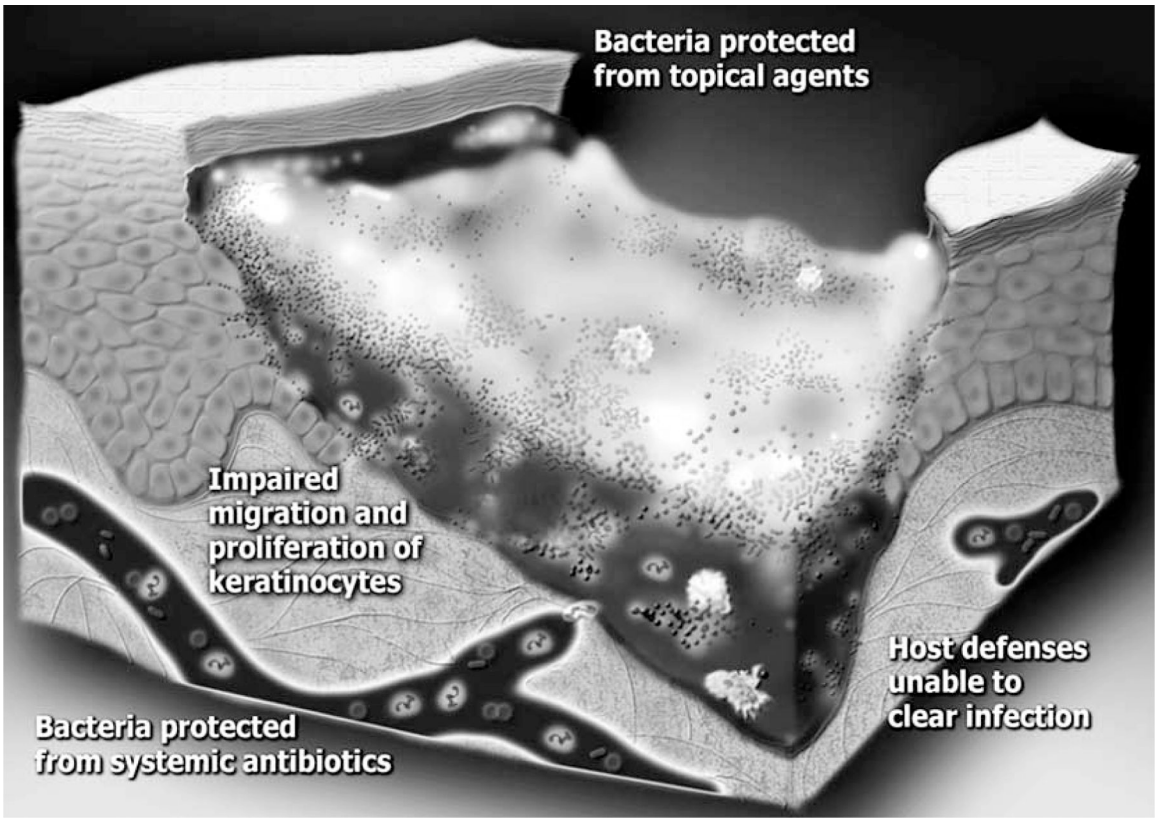

Fig. (1). The biofilm hypothesis as it applies to chronic wounds.

In a chronic wound, bacterial colonization of the wound bed progresses to biofilm formation. The infection persists because microorganisms in biofilms are resistant to killing by systemic antibiotics, topical antiseptics, and components of the host immune system. The presence of biofilm impairs the normal wound healing process resulting in a chronic wound.

Table 1.

Biofilm Control Strategies for Wound Care and Associated Example Patents

Biofilm Control Stategy	Control Agent	Example Patent
Physical	Manual debridement	US20070135706 (**2007**)
	Pulse electrical field	US20070239073 (**2007**)
	Ultrasound debridement	US20080183109 (**2008**)
Chemical	Ionic silver	US20040131698 (**2004**)
	Iodine	US20020037260 (**2002**)
		US4844898 (**1989**)
	Gallium	US20070231406 (**2007**)
		US20080241275 (**2008**)
		US20060018945 (**2006**)
	EDTA	US20040110841 (**2004**)
	Bismuth thiols	US20080181950 (**2008**)
Biological	Honey	US20056956144 (**2005**)
	Lactoferrin	US20070116750 (**2007**)
		US20080318834 (**2008**)
	Xylitol	US5536511 (**1996**)
		US5719196 (**1998**)
		US20016258372 (**2001**)
	Dispersin B	US20077294497 (**2007**)
	Bacteriophage	US20090191254 (**2009**)
	Quorum sensing inhibitor	EP1475092 (**2004**)
		US20060052426 (**2006**)
		US20080312319 (**2008**)
		US20036559176 (**2003**)