Biofilms made easy

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Introduction

This article describes what biofilms are and the important roles they appear to play in disrupting wound healing. In addition, it discusses potential interventions aimed at removing/reducing biofilms and preventing their reformation in wounds.

Authors: Phillips PL, Wolcott RD, Fletcher J, Schultz GS
Full author details can be found on page 5.

What are biofilms?

Biofilms are complex microbial communities containing bacteria and fungi. The microorganisms synthesise and secrete a protective matrix that attaches the biofilm firmly to a living or non-living surface[1].

Biofilms are dynamic heterogeneous communities that are continuously changing[2]. They may consist of a single bacterial or fungal species, or more commonly, may be polymicrobial, ie contain multiple diverse species[3,4]. **At the most basic level a biofilm can be described as bacteria embedded in a thick, slimy barrier of sugars and proteins.** The biofilm barrier protects the microorganisms from external threats.

How are biofilms relevant to wounds?

Biofilms have long been known to form on surfaces of medical devices, such as urinary catheters, endotracheal and tympanostomy tubes, orthopaedic and breast implants, contact lenses, intrauterine devices (IUDs) and sutures[5,6]. They are a major contributor to diseases that are characterised by an underlying bacterial infection and chronic inflammation, eg periodontal disease, cystic fibrosis, chronic acne and osteomyelitis[2,5,7].

Biofilms are also found in wounds and are suspected to delay healing in some. Electron microscopy of biopsies from chronic wounds found that 60% of the specimens contained biofilm structures in comparison with only 6% of biopsies from acute wounds[8]. Since biofilms are reported to be a major factor contributing to multiple chronic inflammatory diseases, it is likely that almost all chronic wounds have biofilm communities on at least part of the wound bed.

How do biofilms form?

Stage one: reversible surface attachment

Microorganisms are commonly perceived to be free-floating and solitary (ie planktonic). However, under natural conditions most microorganisms tend to attach to surfaces and eventually form biofilms[1,9] (Figure 1). The initial attachment is reversible.

Stage two: permanent surface attachment

As the bacteria multiply, they become more firmly attached (sessile) and differentiate, changing gene expression patterns in ways that promote survival[6,9]. This is usually the result of a type of bacterial communication known as quorum sensing[10] (see glossary page 5).

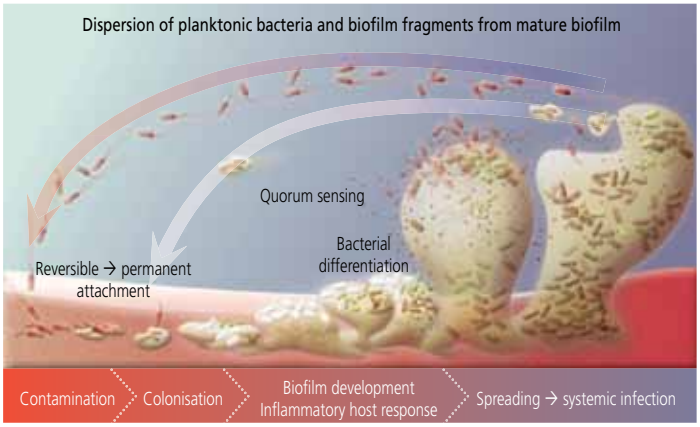
Stage three: slimy protective matrix/biofilm

Once firmly attached, the bacteria begin to secrete a surrounding matrix known as extracellular polymeric substance (EPS)[11]. This is a protective matrix or 'slime'. Small bacterial colonies then form an initial biofilm[2,6].

The exact composition of EPS varies according to the micro-organisms present, but generally consists of polysaccharides, proteins, glycolipids and bacterial DNA[2,9,11]. Bacterial DNA released by living or dead bacteria is thought to provide an important structural component for biofilm EPS matrix[12]. Various secreted proteins and enzymes help the biofilm to become firmly attached to the wound bed[9].

Fully mature biofilms continuously shed planktonic bacteria, microcolonies and fragments of biofilm, which can disperse and attach to other parts of the wound bed or to other wounds, forming new biofilm colonies[5,6].

Figure 1 Schematic representation of polymicrobial biofilm formation (adapted from[13])



Biofilms made easy

Living in the mixed microbial communities typical of biofilms allows microorganisms to share their individual 'skills and abilities' for the survival of the group[14,15]. This gives them many protective advantages.

How quickly do biofilms form?

Experimental laboratory studies[16,17] have shown that planktonic bacteria, eg *Staphylococci*, *Streptococci*, *Pseudomonas* and *Escherichia coli*, typically:

- **attach within minutes**
- **form strongly attached microcolonies within 2–4 hours**
- **develop initial EPS and become increasingly tolerant to biocides, eg antibiotics, antiseptics and disinfectants, within 6–12 hours**
- **evolve into fully mature biofilm colonies that are extremely resistant to biocides and shed planktonic bacteria within 2–4 days, depending on the species and growth conditions**
- **rapidly recover from mechanical disruption and reform mature biofilm within 24 hours.**

This suggests that serial wound debridement/disruption could provide only a brief window of opportunity, ie less than 24 hours, in which antimicrobial treatments are more effective in reducing both planktonic and biofilm microorganisms in wounds.

Why have we just found out about biofilms in wounds?

It is only relatively recently that biofilms have been generally accepted as a factor that can contribute to delay in healing in skin wounds[7,8].

Chronic skin wounds often lack overt clinical signs of infection and often have low bacterial burdens as measured by standard clinical microbiology laboratory assays[18]. However, standard clinical microbiology tests are optimised to culture planktonic bacteria, and do not adequately measure biofilm bacteria, which require special cultivation techniques[19,20].

The term critical colonisation was developed in an attempt to acknowledge the concept that bacteria play a critical role in the failure of wounds that do not have obvious infection to heal[21]. In reality, the concept of critical colonisation/localised infection probably describes the presence of a biofilm in a chronic wound.

Can we see biofilms?

Biofilms are microscopic structures. However, in some situations, when allowed to grow undisturbed for an extended period of time, biofilms can become thick enough to be seen with the naked eye. For example, tooth plaque can accumulate and become clearly visible within a day. Some bacteria in biofilm phenotype produce pigments, which may aid visual detection of biofilm. For example, *Pseudomonas aeruginosa* produces the quorum sensing molecule pyocyanin, which is green, when in biofilm phenotype[22]. Even so, green discolouration of a wound is not always indicative of a *Pseudomonas* biofilm.

Can biofilms be distinguished from slough?

Wound slough has been described as a viscous, yellow, and relatively opaque layer on wound beds, while biofilm found in wounds has been suggested to appear more gel-like and shiny[23]. Nevertheless, there may be a link between biofilms and slough. Biofilms stimulate inflammation, which increases vascular permeability and production of wound exudate and the build up of fibrin slough[24]. Therefore, slough may indicate the presence of biofilm in a wound. However, such a link between slough and biofilms in chronic wounds is yet to be fully defined.

Currently, the most reliable method to confirm the presence of microbial biofilm is specialised microscopy, eg confocal laser scanning microscopy.

How do mature biofilms 'protect' bacteria?

Biofilms greatly enhance the tolerance of microorganisms embedded in the matrix to the immune system, antimicrobials and environmental stresses (eg nutritional or oxygen limitation). This tolerance may approach complete resistance to factors that would easily kill these same microbes when growing in an unprotected, planktonic state[9,25].

Blocking

One simple way that EPS protects microbes is by preventing large molecules (eg antibodies) and inflammatory cells from penetrating deeply into the biofilm matrix. Mature biofilm may also act as a diffusion barrier even to small molecules like antimicrobial agents[26].

Mutual protection

Another unique property of polymicrobial biofilms is the cooperative protective effects that different species of bacteria can provide to each other. For example, antibiotic resistant bacteria may secrete protective enzymes or antibiotic binding proteins that can protect neighbouring non-antibiotic resistant bacteria in a biofilm[2], as well as transfer genes to other bacteria that confer antibiotic resistance, even between different species[27]. Studies have also shown that the specific characteristics of the EPS of biofilms established by one species can play a significant role in the ability of other species to attach and incorporate into an existing biofilm[28].

Hibernation (quiescent bacteria)

Another survival strategy that many bacteria in biofilms have developed is for a subpopulation to become metabolically quiescent, ie to hibernate[2,29,30]. Because bacteria need to be metabolically active for antibiotics to act, hibernating bacteria in biofilms are unaffected by antibiotics that would normally kill active bacteria[2,31].

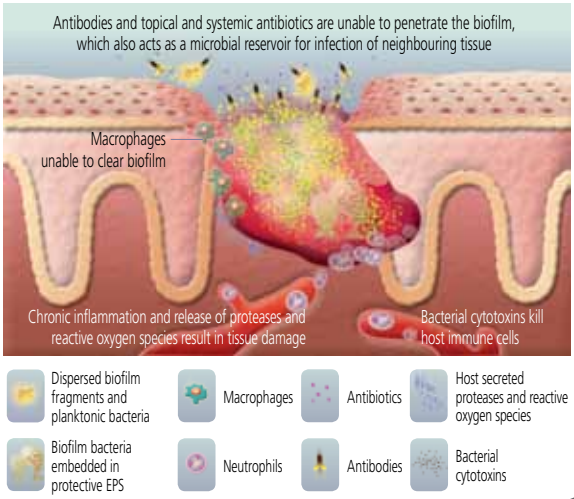
Research has shown that the lowest concentration required to kill or eliminate bacterial biofilm for many antibiotics actually exceeds the maximum prescription levels for the antibiotics[31-34]. Thus, standard oral doses of those antibiotics, which effectively kill the normally susceptible bacteria when grown planktonically in a clinical laboratory, may have little or no antimicrobial effect on the same type of bacteria in biofilm form in the patient.

How do biofilms delay wound healing?

Biofilms stimulate a chronic inflammatory response in an attempt to rid the wound of the biofilm (Figure 2). This response results in abundant neutrophils and macrophages surrounding biofilms. These inflammatory cells secrete high levels of reactive oxygen species (ROS) and proteases (matrix metalloproteinases (MMPs) and elastase). The proteases can help to break down the attachments between biofilms and the tissue, dislodging the biofilms from the wound[35]. However, the ROS and proteases also damage normal and healing tissues, proteins and immune cells and have 'off target' effects that impair healing.

The chronic inflammatory response is not always successful in removing the biofilm and it has been hypothesised that the response is in the interest of the biofilm. By inducing an ineffective inflammatory response, the biofilm protects the microorganisms it contains and increases exudate production, which provides a source of nutrition and helps to perpetuate the biofilm[36].

Figure 2 Delayed wound healing: host factors, microbial factors



Are there conditions that predispose a wound to develop a biofilm?

It is not known whether there are conditions that predispose wounds to developing a biofilm. However, general conditions that impair the immune system or reduce the effectiveness of antibiotic drugs may favour the development of biofilms in wounds. These include tissue ischaemia or necrosis, poor nutrition and comorbidities that impair immune function.

What are the principles of managing biofilms?

Even when a wound is strongly suspected of containing a biofilm, there is no one-step solution for treatment. A proactive approach using a combination strategy based on elements of wound bed preparation[37] may be helpful (Figure 3) and aims to:

- **reduce the biofilm burden**
- **prevent reconstitution of the biofilm.**

This approach is sometimes called 'biofilm-based wound care'.

How can biofilm burden be reduced?

Evidence to date suggests that physical removal, ie debridement or vigorous physical cleansing, are the best methods for reducing biofilm burden[37].

Debridement involves the removal of necrotic and contaminated tissue and matter from a wound so that healing can occur. There are numerous methods of debridement, ranging from sharp debridement to techniques usually thought of as wound cleansing, eg wound irrigation[38,39]. Choice of method of debridement or cleansing by a clinician will be heavily influenced by knowledge, training and competency, and must take into account safety and ethical considerations[40].

Research into the management of wound biofilms has so far used sharp debridement and ultrasonic debridement with the aim of opening all tunnels and removing undermining, all devitalised tissue, slough and discoloured or soft bone[41]. However, because of the difficulties of visualising biofilms, the impact of debridement and the best method of debridement for biofilm management is not yet clear.

Frequency of debridement/ cleansing

No form of debridement or cleansing is likely to remove all of a biofilm, and so any remaining bacteria/biofilm has the potential to regrow and form mature biofilm within a matter of days. As a result it is suggested that debridement in a wound suspected of containing biofilm needs to be performed regularly. As yet, the ideal frequency of debridement is not clear; in a study of patients with critical limb ischaemia, debridement was weekly[41].

Some products are suggested to have additional roles in wound cleansing by aiding removal of bacteria and debris, and disturbing biofilm. A promising technology, for example, lies in the surfactant properties of some polyhexamethylene biguanide (polyhexanide or PHMB) wound cleansing formulations (eg Prontosan®). The surfactant component (betaine) of the cleansing agent reduces surface tension and aids removal of debris and bacteria by irrigation[42,43].

If a wound is still not progressing following regular debridement with one method, it may be necessary to consider a more 'aggressive' form of debridement with specialist referral as appropriate.

How can biofilm reconstitution be prevented?

Biofilm may reform in a wound by:

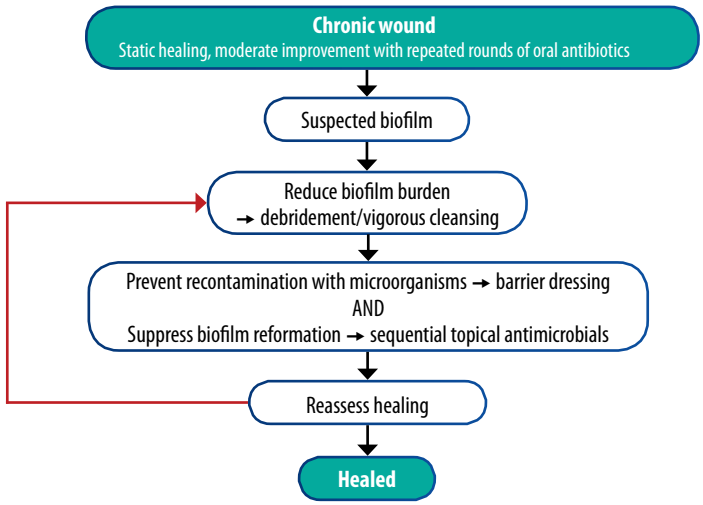
- **growth of fragments left behind following debridement/cleansing**
- **spread of planktonic bacteria released from the remaining biofilm**
- **growth of biofilm by newly introduced microorganisms.**

Principles involved in preventing reconstitution of the biofilm include prevention of further wound contamination (ie the use of dressings), and the use of antimicrobial agents to kill planktonic microorganisms.

The polymicrobial nature of many biofilms indicates that a topical broad spectrum antimicrobial that kills rather than inhibits microorganisms is the most appropriate. Details of the effects of antimicrobials on biofilm reformation are not yet known. However, the broad spectrum microbicidal antimicrobials most widely used in wound care are silver, iodine, honey and PHMB. These are available in a range of formulations.

An emerging principle for the use of topical antimicrobials is changing to a different antimicrobial if there is lack of progress. As yet, there is no evidence to suggest which antimicrobial is preferable first line; choice will be dependent on how the antimicrobial will be used. For example, is it to be left in place for several days? If so, a sustained release formulation will be required to cover the period of use. Patient sensitivities/ allergies must also be taken into account.

Figure 3 Principles of wound biofilm management



How will I know when the biofilm has gone?

The lack of definitive signs and readily available laboratory tests for biofilms means that it is not possible to state categorically when a wound has become biofilm free. The clearest clinical indicator is likely to be healing progression, along with a reduction in the production of exudate and slough.

Until clear guidance becomes available, clinical judgement will be required to decide when and how to modify the management of wounds with suspected biofilm. For example, when healing is progressing well, it may be appropriate to change debridement method or reduce debridement frequency, and/or reconsider whether the use of a topical antimicrobial is necessary.

Additional important concepts include frequent wound reassessment and a holistic approach to patient health to boost the immune system and to promote wound healing.

Future developments

There is a need to develop methods or devices to quickly detect the presence of biofilm before and after selected treatments. Initially, this would help guide researchers and healthcare providers to develop effective wound management strategies. Later, it would aid monitoring of treatment progress.

Both currently available and novel antimicrobial agents and treatment methods are being scrutinised for their efficacy against biofilm, both as biofilm eliminators and biofilm inhibitors. For example, recent studies of the antimicrobial efficacy of various wound dressings against mature *Pseudomonas aeruginosa* biofilm cultured on porcine skin has revealed significant biofilm eliminating properties of cadexomer iodine[44]. However, the complex ever changing polymicrobial nature of biofilm, complicated by biofilm bacterial phenotypic heterogeneity means that antibiofilm efficacy of agents must be verified on a patient by patient basis.

How do we explain biofilms to patients?

Patients can be reassured that biofilms can be effectively treated by a combination of debridement and/or cleansing to remove the biofilms, application of dressings to block new bacteria from reaching the wound, and the use of antimicrobials to kill bacteria left in the wound bed. Patients should be told that treatment needs to be repeated and regular because biofilms can reform within a day and prevent wound healing.

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Glossary	
Biofilm phenotype	Biofilm phenotype microorganisms express genes optimal for developing an attached community embedded in a protective self-secreted 'slime' matrix and surviving environmental stresses.
Commensal	A microorganism that lives on/in a tissue, usually without causing disease.
Extracellular polymeric substance (EPS)	The 'slime' produced by microorganisms in a biofilm comprising polysaccharides, proteins, glycolipids and bacterial DNA; it protects the microorganisms living in the biofilm from the host's immune system and antimicrobial agents.
Phenotype	The characteristics of a microorganism that result from an interaction between the environment and the microorganism's genes.
Planktonic	Refers to free-floating microorganisms that are expressing genes optimal for single, unattached growth.
Quorum sensing	A mechanism used by microorganisms to communicate within and between bacterial species. It is used to detect and respond to changes in the environment (including the presence of other microbes or nutritional limitations). Quorum sensing induces changes in bacterial gene expression that aim to promote survival of the microorganisms.
Sessile	Microorganisms that are firmly attached to a surface by means of receptors and/or proteins called adhesins.

Author details

PL Phillips[1], RD Wolcott[2], J Fletcher[3], GS Schultz[4].

1. Postdoctoral Fellow, Institute for Wound Research, Department of Obstetrics and Gynecology, University of Florida, Gainesville, Florida, USA
2. Medical Director, Southwest Regional Wound Care Center, Lubbock, Texas, USA
3. Principal Lecturer, School of Nursing, Midwifery and Social Work, University of Hertfordshire, Hatfield, UK and Senior Professional Tutor, Department of Dermatology and Wound Healing, School of Medicine, Cardiff University, Cardiff, UK
4. Professor, Institute for Wound Research, Department of Obstetrics and Gynecology, University of Florida, Gainesville, Florida, USA

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Summary

Bacterial biofilms are known to contribute to numerous chronic inflammatory diseases and recent evidence suggests that biofilms also play important roles in impairing healing in chronic wounds. Biofilms have high levels of tolerance to antibodies, antibiotics, disinfectants and phagocytic inflammatory cells. Current understanding of biofilms suggests that management of suspected wound biofilm should involve frequent debridement along with interventions such as dressings and antimicrobials to prevent recontamination of the wound and suppress biofilm reformation.

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