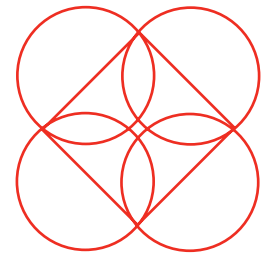


Association for the Advancement
of Wound Care



AAWC

Advancing Your Practice: Understanding Wound Infection and the Role of Biofilms

Wound microbiology

The complex issue of wound infection

Critical colonization

Biofilms and delayed wound healing



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Association for the Advancement of Wound Care

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Editor:

Keith F Cutting Principal Lecturer, Buckinghamshire New University, Chalfont St. Giles, UK.

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Randall Wolcott Director, Southwest Regional Wound Care Center, Lubbock, Texas, USA.

Contributors:

Jose Contreras Ruiz Professor, Interdisciplinary Wound and Ostomy Care Centre, "Dr. Manuel Gea Gonzalez" General Hospital, Mexico City, Mexico.

Keith F Cutting Principal Lecturer, Buckinghamshire New University, Chalfont St. Giles, UK.

David Leaper Visiting Professor, Department of Wound Healing, Cardiff University, Cardiff, UK.

Robert J. Snyder Medical Director, Wound Healing and Hyperbaric Center, University Hospital, Tamarac, Florida, USA.

John G. Thomas Professor, Department of Pathology, West Virginia University School of Medicine, Morgantown, USA. Cardiff University, School of Dentistry, Cardiff, UK.

Randall Wolcott Director, Southwest Regional Wound Care Center, Lubbock, Texas, USA.

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USA

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Foreword

The multidisciplinary science of microbiology is relatively young. Anton van Leeuwenhoek (1632-1723) was the first of the “Microbe Hunters”, using finely ground lenses to document the initial observations of bacteria. Medical interest in the role of bacteria may have been initiated through Ignaz Semmelweise (1818-1865). By insisting upon hand washing, this obstetrician from Vienna, Austria was able to reduce the incidence of child bed fever from 25% to 1.3%. However, microbiology did not gain a foothold in contemporary medicine until the late 1800s with the introduction of germ theory through the work of Louis Pasteur (1822-1895) and Robert Koch (1843-1910).

In 1867, Joseph Lister, a Scottish surgeon, published two short but revolutionary papers, which pioneered the principles of antiseptic surgery. Lister, in acknowledging the writings of Pasteur, signified the association between airborne bacteria and surgical sepsis. He then introduced the use of carbolic acid solution into his regular surgical procedure. By 1870, he claimed that mortality from amputations had dropped from over 40% to 15%. It was not until 1928 that Sir Alexander Fleming on discovering the inhibitory effects of a mold (*Penicillium notatum*) in an uncovered culture of staphylococci –and the publishing his findings in 1929– that the role of antibiotics in bacterial infections had begun!

The bacteriology of wound healing continues in a faltering evolutionary style. Classification, quantification and documentation continue to generate much controversy. The terms aerobic and anaerobic now appear almost simplistic with bacterial bioburden being too ethereal a description when applied equally to wounds of varied etiology. “Best practice” guidelines for the treatment of wound infections are inconsistent. The role of systemic vs. topical antimicrobials is now evolving and just when clinicians think they understand wound bacteriology, the literature calls attention to “critical colonization” and “biofilms”.

It is with this historical perspective in mind, that the Association for the Advancement of Wound Care in cooperation with ConvaTec, is very pleased to present *Advancing Your Practice: Understanding Wound Infection and the Role of Biofilms*. It is our belief that the outstanding articles presented in this position paper will serve as a thoroughly researched foundation for contemporary, clinical excellence in the microbiology of wound healing.

At least for the next few years!

John M Macdonald MD, FACS

President

The Association for the Advancement
of Wound Care



WOUND MICROBIOLOGY

John G Thomas

Professor, Department of Pathology, West Virginia University School of Medicine, Morgantown, West Virginia, USA

Visiting Professor, Cardiff University, School of Dentistry, Cardiff, UK

Introduction

Wound microbiology may be considered a complex and sometimes misunderstood area in clinical medicine, not least because a wound provides an environment in which the microbial ecosystem is very dynamic and unstable.

The human body contains an estimated 10^{14} microbial cells¹ and these outnumber mammalian cells 10-fold. These microbiota are necessary for health but have the potential for causing disease given the opportunity. Infections occur when microorganisms overcome the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provoke a series of local and systemic host responses.

Wound microbiology

The majority of dermal wounds are colonized with aerobic and anaerobic microorganisms, often referred to as the “indigenous” or normal microbiota that originate predominantly from mucosal surfaces such as those of the oral cavity and gut.

These microbiota play an important role in preventing colonization by pathogens of significant virulence (colonization resistance). The role and significance of microorganisms in wound healing have been debated for many years. Some consider the microbial density to be critical in predicting wound

healing and infection, while others consider the types of microorganisms to be of greater importance². However, these and other factors such as microbial synergy, the host immune response and the quality of tissue must be considered collectively in assessing the probability of infection. Whatever the outcome of these processes, wound microbiota are considered to be polymicrobial. The polymicrobial ecosystem of the wound is composed of a vast array of microorganisms which can be classified according to their nutritional and environmental requirements. One fundamental factor significant to wounds is the availability of oxygen which dictates which types of microbes can proliferate (Table 1).

With acute and chronic wound infections, mixed populations of both aerobic and anaerobic microorganisms are commonly found^{2,3}. When anaerobes are evident, this is indicative of a more complex micro-environment in the wound. The existence of anaerobic bacteria in wounds may be significant but their presence is often overlooked as many standard laboratories do not routinely screen for them. Examples of common bacteria that have been isolated from chronic wounds may be seen in Table 2. However, the mere presence of these bacteria does not constitute an infected wound⁴.

Table 1

Classification of microorganisms according to their oxygen requirements

Type	Requirements
Obligate aerobe	Must have access to oxygen
Obligate anaerobe	Will only grow in the absence of oxygen
Facultative aerobe	An anaerobic organism will grow in the presence of oxygen
Facultative anaerobe	An organism that can grow in the presence and absence of oxygen



Definition of terms

biofilm

A surface-associated microbial community that is composed of various phenotypes and commonly various genotypes, which encases itself in a 3-dimensional matrix of extracellular polymeric substances (EPS) (e.g. polysaccharides, nucleic acids and proteins) and demonstrates increased resistance to cellular and chemical attack.

colonization

Bacteria that have adhered to superficial tissue, have begun to form colonies without generating a host immune response and are not considered to be associated with a delay in healing.

The age of a wound influences microbial composition and diversity, and the development of the microbial ecosystem can be divided into 3 phases.

Phase I is predominately described as an aerobic process and the organisms most representative are classified as Gram-positive obligate aerobic or facultative anaerobic. This is an acute process.

Phase II is transitional, occurring as the levels of oxygen are reduced by obligate aerobes, e.g. in poorly perfused tissue. This environment will encourage growth of anaerobic microbes, specifically obligate anaerobes.

If such an environment persists, phase III may develop, reflected by a change in the predominant microbiota to a mixed microbial community favouring organisms that persist over time with less standard pathogenicity; key pathogenic features include enzymes and toxin production.

Historically, most cultures isolated from chronic wounds are based on the traditional culture methodology, either aerobic or anaerobic and have relied upon traditional methods of sampling and laboratory detection. Advanced technology now utilizes molecular techniques that allow for the identification of viable but non-culturable (VBNC) bacteria, that otherwise would remain undetected by traditional methods. This is a

significant advance in wound microbiology. The significance of these VBNC organisms requires clarification specifically related to the area of bacterial synergy, which is known to be important in bacterial pathogenicity and in biofilm formation.

Wound infection

The list of microbes associated with skin and soft tissue infections is growing. This list (Table 2) while not exhaustive, illustrates the complexity of the microbiology involved in wound management.

Bacteria, specifically staphylococci, almost never appear as a single isolate in infected wounds as they are most often found in synergistic relationships with other bacteria. In many wounds, when using culture techniques, the number of aerobic isolates recovered range from 1-8 with an average of 2.7 organisms per wound⁵. However, when molecular techniques are used, significantly more bacteria are found to be present⁶.

Infected chronic wounds are biochemically and microbiologically complex with many deep wounds frequently hypoxic as a consequence of poor blood perfusion. This creates an ideal growth environment for microbes, including fastidious anaerobes that will proliferate as residual oxygen is consumed by obligate, facultative aerobic and anaerobic bacteria.

Table 2

Bacteria commonly isolated from chronic wounds

Aerobes	Anaerobes
<i>Acinetobacter baumannii</i>	<i>Bacteroides</i> spp
Coliforms	<i>Fusobacterium</i> spp
<i>Enterococcus faecalis</i>	<i>Peptostreptococcus</i> spp
MRSA	<i>Porphyromonas</i> spp
<i>Pseudomonas aeruginosa</i>	<i>Prevotella</i> spp
<i>Staphylococcus aureus</i>	<i>Veilonella</i> spp
<i>Staphylococcus epidermidis</i>	
<i>Streptococcus pyogenes</i>	



Gram stain

A method of staining bacteria in the laboratory that distinguishes between different types of bacteria.

microbiota

Microorganisms that are normally associated with a particular tissue or organ.

planktonic

Free floating bacteria, not attached to a surface.

sessile

Attached bacteria not free floating.

synergy

Bacterial synergy is created when bacteria work in concert to create an outcome (pathogenesis) that is greater than individual inputs.

Bacterial species rarely exist in pure culture in wounds and as such, within a wound, the microbiology exists within a community structure. The complexes that occur within wounds are not clearly understood. A better understanding and knowledge base regarding bacterial interactions will be important in managing polymicrobial infected wounds. An example of a polymicrobial infected wound is considered to be a biofilm community⁷.

Biofilms which are considered by some to be associated with delayed wound healing⁸ are by definition sessile, and this stationary mode of growth will reduce the hazards which bacteria are accustomed to within the free floating or planktonic state. Biofilms and their potential to delay healing are discussed in more detail in this publication by Cutting (pages 10-11) and also by Wolcott *et al.* (pages 12-16).

Sampling infected wounds

It is important to remember that the quality of the laboratory report is dependent on the quality of the specimen and that simple cultures provide limited information. Additionally, if unrepresentative samples are

obtained, unrepresentative reports will be generated. If a swab is taken, the specimen must be accompanied with significant clinical information, including specific anatomic site, classification of wound and prior or ongoing antibiotic therapy, and transported in appropriate media and processed within the recommended time frame.

Recovery of true wound bacteria when bordered by skin flora is difficult as these are often classed as contaminants. Consequently, assessing the true microbiology of a wound infection does not have the same clarity as a sample recovered from sterile fluid such as blood or cerebrospinal fluid. Ideally, wound microbiology should only be interpreted in combination with the clinical diagnosis.

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THE COMPLEX ISSUE OF WOUND INFECTION

David Leaper

Visiting Professor,
Department of Wound
Healing,
Cardiff University,
Cardiff, UK

Robert J. Snyder

Medical Director,
Wound Healing and
Hyperbaric Center,
University Hospital,
Tamarac, Florida, USA

A wound interrupts the integrity of the skin and by removing its protective function at that point facilitates the ingress of micro-organisms. Wound infection is a global cause of morbidity and mortality across all wound types and data related to the associated prevalence/incidence of wound infection therefore demands our attention.

Infection in acute and surgical wounds

Health care associated infections (HAIs) affect 15-20% of patients in health care², with an incidence between 7.0-7.8%^{3,4}. They are broadly divided into four categories: respiratory, including hospital and ventilator associated pneumonias; urinary; bacteremia; and surgical site infections (SSIs). Surgical site infection (SSI) can be categorized as:

- superficial, involving skin and subcutaneous fat;
- deep, involving deeper fascial and muscle layers;
- and space or organ infection⁵

The development of a SSI depends on the pathogenicity and number of bacteria present in a wound following a surgical procedure, balanced against the host response⁶. Most SSIs are related to patients' endogenous organisms, present in skin or from an opened viscus (endogenous infection). Exogenous infection follows contamination of a traumatic wound, inadequate theater sterility during

surgery or introduction of organisms during inadequate postoperative wound care.

Staphylococcus aureus is the most commonly cultured organism from SSIs but after prosthetic surgery *Staphylococcus epidermidis* (coagulase negative staphylococcus [CNS]) is more likely⁷. When the large bowel is opened, tissues are contaminated by a range of organisms, including enterobacteriaceae and anaerobes which may act in synergy. These bacteria may present as resistant forms relating to antibiotic misuse and inadequate "search and destroy" policies. These organisms include meticillin-resistant *Staphylococcus aureus* (MRSA) and multiple resistant CNS.

Most SSIs take between 5-10 days to present, although a streptococcal SSI may present earlier as cellulitis. Some SSIs may present many months postoperatively, particularly after joint surgery. This is why the Center for Disease Control and Prevention (CDC) definition involves a 30-day surveillance for wounds in general and a year after prosthetic surgery⁸. Most SSIs respond to removal of sutures with drainage of pus, if present, but occasionally require debridement and open wound care with topical antimicrobials⁹. Spreading infection requires systemic antibiotics. However, in primary care it is likely that over 15% of postoperative wounds are treated with antibiotics¹⁰. Wound complications are often

Note

These figures do not include those wounds resulting from trauma or those of neoplastic origin and make no reference to the additional infection incidence/prevalence burden.

Twelve years ago, George¹ estimated the worldwide burden of wounds to be:

Surgical wounds	40-50 million
Leg ulcers	8-10 million
Burns	7-10 million
Pressure ulcers	7-8 million



Definition of terms

health care associated infections (HAIs)

Infections acquired within a health care setting during the course of treatment for other conditions.

meticillin

'Meticillin' replaces the more familiar 'methicillin' in accordance with the WHO International Pharmacopeia guidelines 2005.

erroneously diagnosed as infections when they present with exudate from a gaping wound edge or with a superficial separation which can be closed using secondary suture or skin closure strips. In larger open wounds, granulation tissue must be healthy with a low bioburden to allow secondary suture.

The impact of SSIs can be severe and life-threatening, they may be associated with other HAIs, leading to sepsis and multiple organ failure¹⁴. Over a third of postoperative deaths are related, at least in part, to SSIs¹¹ which also contribute to appreciable postoperative morbidity and mortality^{3,4}. In Europe, there have been several prevalence studies^{7,12-15} but they have not matched those of the National Nosocomial Infections Surveillance of the United States¹⁶. Other clinical outcomes of SSIs include poor scars which are cosmetically unacceptable; persistent pain and itching; restriction of movement; and a significant impact on emotional well-being¹⁷. Deep SSIs may be responsible for delayed healing in abdominal wall incisions, leading to incisional hernia¹⁸.

Current rates of SSIs vary from 1.4% to over 15% in clean wound surgery alone¹⁹⁻²² depending on definitions and surveillance. These SSIs have been estimated to cost United States health care \$10b annually, ranging from \$44 for a superficial SSI to more than \$30k for a sternal or joint infection²³. A European perspective put the annual cost of SSIs between €1.47b-19.1b to the European health care system². Patients who develop an SSI have a lower health-related quality of life than those who do not²⁴.

The definition and surveillance of SSI requires guidelines and resources if reporting is mandatory and used as a performance

indicator. The definitions and methodology should be consistent with other systems to enable international comparisons, for example the Hospitals in Europe Link for Infection Control through Surveillance (HELICS)²⁵.

Infection in chronic wounds

Chronic wounds include venous leg ulcers, diabetic foot ulcers, pressure ulcers and ischemic ulcers as well as atypical lesions. Although these chronic wounds have different etiologies, they share many inflammatory and immune processes; biochemical activity; microcirculatory changes; and a microbial bioburden which may progress to invasive infection; all of which may delay healing.

The prevalence of leg ulceration is between 1.5 and 3.0 per 1,000 people. The majority of these are secondary to venous disease. The annual cost to the National Health Service in the UK is estimated at around £300 million²⁶. Foot ulcers are the most common cause of hospitalization for patients with diabetes. In diabetic foot ulceration, peripheral arterial disease was found in 49% of subjects with infection present in 58% of the study population²⁷. Fifty-nine percent of diabetic amputations are preceded by infection²⁸. A 9.1% incidence of wound infection has been found in diabetic patients, mostly involving soft tissue with 19.9% of these having culture-positive osteomyelitis²⁹.

In pressure ulceration, the mortality rate is higher than 50% when there is associated bacteremia³⁰ with additional financial costs when a pressure ulcer becomes infected³¹. This can rise as high as £1,920 for a cellulitic episode in a patient with a grade 3 or 4 pressure ulcer. Should osteomyelitis occur, £16,500 can be added to "normal" daily



health care costs. These 2004 costs do not include the special precautions that are required when MRSA is present and are conservative estimates³¹.

All chronic wounds contain a bacterial bioburden which is different from that found in acute, potentially contaminated, surgical and traumatic wounds³². Bacteria compete for available oxygen and nutrients and may produce enzymes which destroy growth factors and stimulate excessive production of matrix metalloproteases (MMPs) further delaying healing³³.

Immunocompromised patients, particularly those with Type 1 diabetes, often lack the systemic signs of fever, elevated white blood cell count, despite the presence of serious infection³⁴. Unfortunately, there is no simple universal method to identify all bacterial species in one step and complex, time-consuming protocols have been developed for this purpose³⁵.

As the framework of “best practice” continues to evolve, clinical definitions of wound infection remain inconsistent, leading to variations in clinical impressions. Clinical assessment based upon touch, color and smell remains subjective. This invariably fosters the use of systemic broad-spectrum antibiotics with the associated risks of selection for resistance. It has been shown that systemic antibiotics fail to reach adequate tissue levels in chronic granulation tissue and may therefore be of limited value³⁶. This suggests a paradigm shift favoring the use of topical antiseptics in conjunction with systemic therapy, where appropriate.

This is particularly relevant in patients with diabetes who have neuropathic ulcers and in patients who have pressure ulcers.

The classical signs of infection have been modified to include signs specific to chronic wounds including exudate with persistent inflammation, delayed healing, discolored or friable granulation tissue that bleeds easily and pocketing of the base of the wound and malodor³⁷. Although clinical judgment is considered subjective, producing results less meaningful than comparisons made against the alleged gold standard of quantitative analysis using tissue biopsy³⁸, accurate clinical diagnoses of wound infection have been achieved³⁹.

Experience plays a vital role in evaluating infection. Many clinicians have not received appropriate guidance in clinical diagnosis of wound infection and consequently lack specific knowledge of the subtle signs specific to chronic wound appraisal.

Accuracy in diagnosis of wound infection is complicated by recent insight into the role of biofilms⁴⁰. More evidence elucidating the role of biofilms in chronic wounds is needed.

Wound infection can be over- or under-diagnosed, even when positive wound cultures have been obtained⁴¹.

Recent clinical criteria, relevant to six wound types (acute and chronic), to assist in diagnosis of wound infection, have been suggested⁴² but these require validation:

- Acute/surgical
- Arterial ulcers
- Burns (partial and full thickness)
- Diabetic foot ulcers
- Pressure ulcers
- Venous leg ulcers

The need for universally accepted definitions of infection is clearly apparent.



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CRITICAL COLONIZATION



Keith F Cutting

Principal Lecturer,
Buckinghamshire New
University, Chalfont St.
Giles, UK

The term “critical colonization”, coined over 10 years ago¹, has continued to attract significant attention. The existence of such a state has not received universal acceptance as skepticism appears to surround the reality of such a prodromal phase of infection, coupled with a lack of consensus on definition². Davis defined critical colonization as “multiplication of organisms without invasion but interfering with wound healing” but did not pursue development of this concept to make it more meaningful in either microbiological or clinical terms. The development of the wound infection continuum model popularized the concept and placed the emphasis on progression to infection being dependent on an increase in microbial load³. This continuum commenced with sterility, a state that is not a feature or a therapeutic goal in chronic wounds. To avoid the inference that critical colonization is no more than a transitional state from surface colonization to potential invasion of bacteria into viable tissue, the following definition has been suggested.

It is hypothesized that where critical colonization is thought to be present, there is an alteration in the bioburden and that this is associated with delayed healing. Infection (diagnosis) may only be evident retrospectively. Evidence suggests that delayed healing in a chronic wound that has no signs of clinical infection (critical colonization) is directly related to the microbial bioburden⁵. The absence of a ‘traditional’ host response is pivotal to understanding the concept of critical colonization and is probably better understood from a microbiological rather than a clinical perspective. It is unclear whether causes other than infection result in delayed healing, but as colonization is typical, it would appear that a microbial cause is most likely. Relying on an increasing number of bacteria

(quantity) as cause of progression to infection does not take account of the diversity and richness of the bioburden or the degree of bacterial virulence, pathogenicity or the fact that not all viable cells are culturable.

Microorganisms may be regarded as existing in at least two distinct phenotypes – planktonic (free floating) and sessile (attached) states. A community of microorganisms that are attached on a surface are referred to as biofilms.

Biofilm formation in a wound is a potential cause of chronic wound infections^{6,7}. Although the prevalence of biofilms in wounds has yet to be established, empirical evidence suggests that a strong association exists with chronic wounds and this is explained in more detail in the following paper by Wolcott *et al.* Inaccuracies in diagnosis of wound infection will inevitably transpire if the relationship of biofilms to wound infection is not carefully explored.

Non-healing wounds can be associated with an impaired inflammatory response as a result of compromised host immunity. Bacteria are thus able to persist within the wound and establish a bacterial community (biofilm) that not only can evade the host’s natural defenses but which is resistant to antibiotic therapy and neutrophil attack. Thus, a chronic inflammatory state is sustained in the wound unless successful strategies are employed that assist in managing the biofilm infection.

The distinction between acute and chronic wound infections and the role of biofilms also need to be considered. Chronic, biofilm infections often involve a variety of genotypes, including skin commensals that cannot be eradicated by the host’s immune system because the host’s immune functions



A recalcitrant
(critically colonized)
wound.



Definition of terms

critical colonization

The inability of the wound to maintain a balance between altered bioburden and an effective immune system, denoted by an unexplained delay in healing but not necessarily deterioration in the wound or other overt signs of clinical infection⁴.

Adapted from White, Cutting and Kingsley 2006.

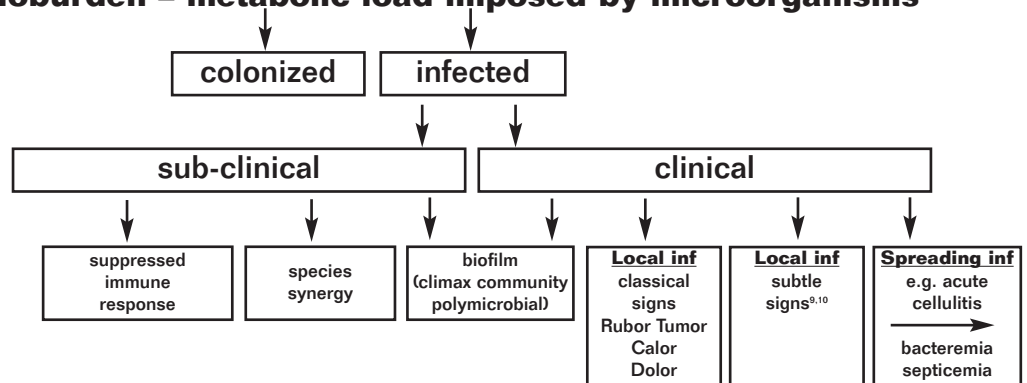
are compromised or the biofilm of bacteria is too tenacious⁸. One hypothesis to explore is that acute infections that promptly respond to antibiotic therapy may be caused by bacterial cells more characteristic of the planktonic phenotype than to biofilms, although to date studies are lacking in this area. More complicated are events of acute infection arising in the midst of a chronic infection. Could these flare-ups be caused by rapidly-growing, less protected bacteria? This and the therapeutic options to effectively manage pathogenic bacterial communities merit further exploration.

To help position biofilms and their relationship to recalcitrant (critically colonized) infected wounds and to illustrate their immuno-evasive capability, a model of infection has been developed (Figure 1). The development of

such a model, despite inherent limitations, may help to visualize how the absence of an overt host response is common to critical colonization and to wound biofilm.

It may now be reasonable to consider that sub-clinical infection could be synonymous with both critical colonization and biofilm infection, and rationalizing critical colonization in this manner may help convince skeptics that it is indeed a reality. Whether the term survives is irrelevant but what is important is that our understanding in respect of the different guises of wound infection is enhanced, otherwise management will be sub-optimal.

Bioburden – metabolic load imposed by microorganisms



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BIOFILMS AND DELAYED WOUND HEALING



Randall Wolcott

Director, Southwest Regional Wound Care Clinic, Lubbock, Texas, USA

Keith F Cutting

Principal Lecturer, Buckinghamshire New University, Chalfont St. Giles, UK

Jose Contreras Ruiz

Professor, Interdisciplinary Wound and Ostomy Care Centre, "Dr. Manuel Gea Gonzalez" General Hospital, Mexico City, Mexico

Biofilm overview

Biofilms are found widely in nature and have been rigorously studied for many years. However, the study of biofilms in relation to health and in particular wounds is a relatively recent development. The National Institutes of Health (NIH) suggest that 80% of human infectious disease is caused by biofilm, usually manifesting as chronic infection^{1,2}. These chronic infections often viewed as benign are in fact insidious and progressive in nature and produce death tolls each year rivaling that of heart disease or cancer, yet clinicians appear to have developed an extremely passive relationship with biofilm disease including those implicated in wound infection.

Most clinicians are familiar with planktonic bacteria as they are routinely cultured in the laboratory, challenged by antibiotics with sensitivity or resistance recorded and a treatment recommendation made. The problem with this approach is that chronic wound bacteria are quite different from their laboratory planktonic counterparts!

The life cycle of a biofilm community can be seen in Figure 1.

A biofilm is a complex community comprising a mixed population of different micro-organisms. It is typified by the secretion of extracellular polymeric substance (EPS), a glue that protects the bacteria and holds the community together. The EPS matrix protects the individual bacteria from environmental stresses, scavenges nutrients from the environment and provides shelter for the unique heterogeneous micro-niches inside the biofilm. A micrograph demonstrating some key components of the host-biofilm interface may be seen in Figure 2.

The biofilm microcolony achieves a critical density of bacteria (a quorum) through the release of signaling molecules and permits differentiation into a true biofilm society³. This complex system of quorum-sensing molecules is tightly controlled and suggests that biofilm is most appropriately thought of as an organism composed of billions of individual cells and specialized structures. Reproduction is carried out by the biofilm breaking down portions of itself and releasing fragments which contain cells incased in matrix material⁴. These detachment fragments have the ability to attach to a suitable surface, become metabolically active, and reform a biofilm community.

The biofilm community also forms secondary structures, including mushroom-type projections off the surface, water channels and extensions. These structures allow nutrient inflow and waste outflow throughout the biofilm.

Biofilm's defenses (*resistance*)

The survivability of biofilm is a result of adaptation strategies developed over millions of years. These strategies together with brief explanations of their mechanisms may be found in Table 1.



Definition of terms

heterogeneity

The condition or state of being different, dissimilar, not comparable or possessing different forms.

quorum sensing

Bacterial pathway regulated by small communication molecules (pheromones). When there is a critical number (quorum), these molecules stimulate biofilm formation and other community activities.

phenotype

The proteins and other cell components expressed in the bacterial cell.

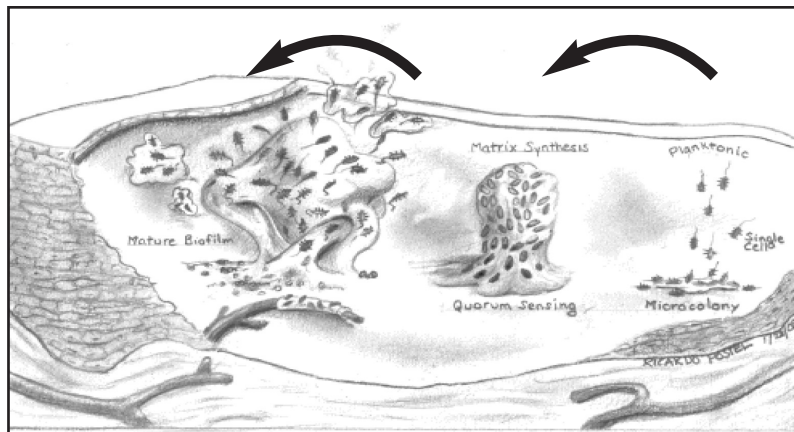


Figure 1 Life cycle of a biofilm community

Reading from right to left, mobile planktonic reproductive bacterium released from a biofilm community and dispersed to find an environment conducive for new colony growth.

Planktonic bacterium finds a suitable surface, attaches and within a few minutes the planktonic bacteria change from their nomadic single-cell state into a biofilm phenotype.

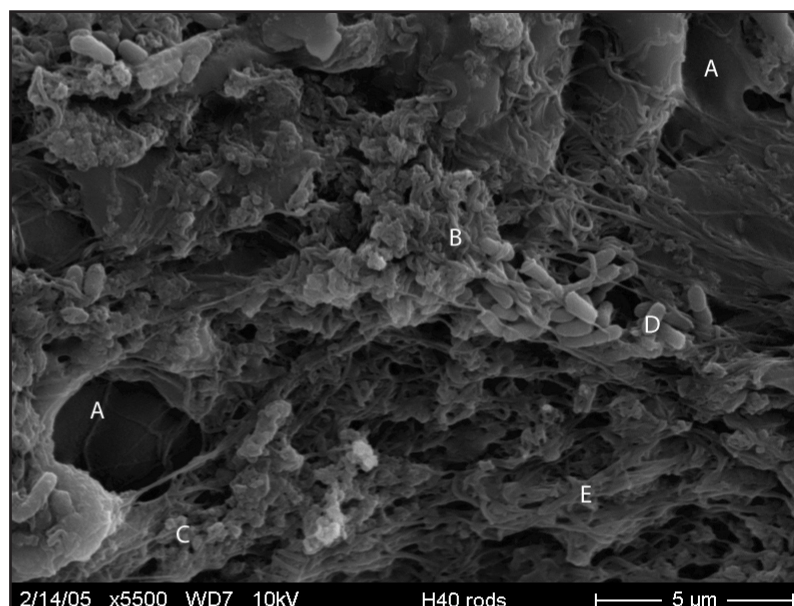


Figure 2 Scanning electron micrograph of a biofilm

- A Host extracellular matrix
- B Rod-shaped bacteria encased in extracellular polymeric substance
- C Spherical-shaped bacteria in extracellular matrix
- D Exposed rod-shaped bacteria
- E Extracellular polymeric substance (matrix) binding the bacterial community to the surface of the host



Table 1

Mechanisms that promote the fitness of biofilms

Strategies	Mechanisms
Extracellular polymeric substance (EPS)	Constructed by the bacteria of the biofilm to protect the community from desiccation, predators, immune cells, and toxins. The components of the EPS can include pathogen and host polysaccharides, proteins, and nucleic acids. The chemical structure of the EPS may also work to prevent some antimicrobials from entering the biofilm.
Enzymatic Protection	Metabolically active cells are able to produce enzymes such as catalase or beta lactamase that can neutralize biocides and antibiotics and shield the inner members of the community ^{5,6} .
Altered microenvironments	By-products of the biofilm create acidic and hypoxic areas which produce slow growth and diversify the ecology of the biofilm.
Plastic phenotype	Biofilms have a dramatically different expression of proteins. Up to 50% of the outer membrane proteins are different from their planktonic counterparts, which demonstrates the phenotypic heterogeneity that can be found within a species.
Heterogeneity	When combined with slower growth, heterogeneity makes most antibiotics less effective.
Quorum sensing	Where groups of bacteria are present, cell-to-cell signaling takes place. The bacterial pheromones facilitate cooperation or result in competitive antagonism, which work together to yield a climax biofilm community that is best suited for the stresses and nutrients of the wound environment.
Evasion of Host Defenses	Most chronic infections are firmly entrenched within the host. Complement pathways, antibodies and even white blood cells have been found to be very ineffective against biofilm.



Imaging studies, including light and electron microscopy of samples from 50 wounds, demonstrated that 60% of chronic wounds possess biofilm, whereas 16 acute wounds failed to show significant biofilm⁷. The chronic wounds healed in over 3 months (a delayed wound healing trajectory), whereas all the acute wounds healed within 3 weeks. This suggests that not only is biofilm present but it may impair healing.

A biofilm model may explain many of the clinical challenges that can make wound care so intricate and complex. It has been established that chronic wounds become “stuck” in a chronic inflammatory state⁸. This chronic inflammation is defined at a molecular level by increases in macrophage-derived MMPs 2 and 9 and neutrophil-derived MMP 8 and elastase⁸. At the cellular level, excessive neutrophils predominate within the wound bed. The presence of biofilm on the surface of the wound (Figure 2) can explain the molecular and cellular findings in chronic wounds.

Differences in opinion of the value of antibiotics in acute and chronic wound care may be found. When antibiotics are used as a single agent, they fail to “heal” a chronic wound the vast majority of times. Clinically, what is often seen following antibiotic administration is a short-term improvement in the wound, that is followed by a subsequent deterioration or recalcitrance. This is possibly due to failure of the antibiotic to reduce the bioburden to a level at which the host defenses can prevail, resulting in reconstruction of the biofilm and enhanced resistance. Clinical support for biofilm’s role in impaired healing is demonstrated by a retrospective study which showed that wounds treated with anti-biofilm strategies were more likely to heal when compared to

those treated by standard care methods⁹. The results provide good working explanations for what is seen clinically in wound care.

Biofilm-based wound management

Suppressing wound biofilm while managing the other known barriers to wound healing (pressure, poor perfusion, poor nutrition, etc.) holds the potential to radically advance wound healing.

Chronic wounds are often managed using a single strategy (e.g. enzyme, topical antiseptic, or a specialty dressing) at a time. Early progress may be observed but often healing is stalled and another strategy is applied. Sequential strategies often result in failure to close the wound.

Using a biofilm model to explain the organization of wound bacteria, it becomes clear that a single strategy is unlikely to succeed. Biofilms are polymicrobial with important interspecies synergies along with the ability to control their environment through modifications of their protective matrix. This has led dentistry and many other industries to adopt a multiple concurrent strategy in managing biofilms.

Dentistry has managed biofilm (dental plaque) successfully over several decades. This has resulted in the well-known daily regimen of: debridement (brushing) at the same time applying an anti-biofilm substance, namely toothpaste. These anti-biofilm agents block reattachment, impair EPS formation, or are biocidal, killing the community members of the plaque. For more recalcitrant plaques, harsher biocides are applied through oral rinses and aggressive debridement can be carried out through flossing, ultrasonic debridement, or professional cleaning. This



process of suppression, which will continue throughout our lifetime, does not aim to *eradicate* the biofilm but to *suppress* it below a level that would cause periodontal disease. The same principles seem reasonable when applied to managing wound biofilm. It is important to note that as biofilm reconstitutes itself and before it has formed a stable climax community, it is much more susceptible to antimicrobials.

Frequent debridement sets the stage for treating agents to be more effective. Debridement provides a cornerstone in the management of chronic wounds and evidence demonstrates that frequent debridement improves wound healing^{10,11}. However, in most wounds, when slough or biofilm is removed from the surface, it rapidly reconstitutes itself on the surface within 24 hours⁴. Clinically, what is seen is a clean-bleeding wound bed post-debridement one day but the next day the slough that was removed the day before debridement is seen on the wound bed. In the laboratory, it takes biofilm about 24 hours to re-establish the biomass of the community.

Topical antiseptics, such as silver^{12,13} and honey^{14,15}, provide some evidence of their value in managing biofilm. Empirically, the authors have noted that iodine preparations, particularly cadexomer, also possess the capability to manage biofilm infection. The goal is not eradication but to get multiple different strategies producing significant stress to the biofilm at the same time.

It is recognized that biofilm demonstrates increased resistance to antibiotics^{16,17}, biocides¹⁸ and host defenses¹⁹. However, when used concomitantly with frequent debridement and other topical agents that impair biofilm defenses, antibiotics can be

more successful. Clinical medicine has found that for biofilm diseases such as osteomyelitis and endocarditis, higher doses of antibiotics for longer periods of time are more successful. In a chronic wound, use of antibiotics as a single agent struggles to suppress biofilm, but when used in conjunction with the other strategies indicated above, does show significant impact in healing wounds.

Because wound biofilms are resistant to antibiotics and host defenses, clinicians struggle to manage successfully many chronic wounds. Aggressively targeting wound biofilm suppresses the bioburden over a period of time to a level at which the host immune response will prevail and resolve the chronic wound.



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