



Biofilm's Role in Chronic Wounds and Healing

The authors take a look at the relationship between microbial species, biofilm-forming capabilities, and their antibiotic sensitivities.

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Van Leeuwenhoek first studied biofilms in the 1650s, but it was not until 1978 when J.W. Costerton presented his research on biofilm bacteria that the medical field expressed interest. Biofilms can be present in all types of wounds. It has been shown that less than 10% of acute wounds carry biofilms, while chronic wounds average or exceed 60%.³

Healthcare costs are currently rising yearly and chronic wounds play a major role in sustaining the expanding budget. The United States healthcare system has over 5.7 million patients being treated annually for chronic wounds, with an estimated cost of 20 billion dollars.

Chronic wounds come in all shapes, sizes, and depths, with an array of etiologies and co-morbidities. Clinical assessment in chronic wounds can be challenging but vital in the process of staging a wound, due to the fact that a wound can linger in one stage versus another for varying periods of time. Classic clinical indications that depict a chronic wound housing a biofilm are that the wound will have excessive moisture with a shiny translucent surface, or opaque, loosely-attached patches with possible secretions of specific pigments.

The base of the wound will contain poor-quality granulation tissue. Signs of local infection in the peri-wound area include redness, heat, swelling, pain, and malodor. The pa-

tient may have a history of antibiotic failure or recurring infection, culture negative results despite signs of bacterial infection, and a non-progressing wound despite all underlying co-morbidities being addressed.⁷ Practitioners have become sound at identifying chronic wounds; however, evaluating the contents of the wound has become one of the challenges.

A growing interest in the healthcare field is the specifics of the microbial components within chronic

wounds. Bacteria promote bacterial growth through the formation of a biofilm.

The vital component of a biofilm is the exopolysaccharide (EPS), predominantly a polysaccharide-base substance forming a matrix not only encasing the bacteria or poly-microbial collaboration, but also fungi, viruses, proteins, extracellular DNA, and other biological by-products. Formation of a biofilm starts with the bacteria attaching to the wound surface, then secreting and creating the EPS.

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wounds. Most commonly discussed topics in wound care are identifying tissue type, controlling infectious pathways, promoting a healthy moisture balance, and promoting epithelialization. Current emphasis is on the environment that the microbes produce within the wound. Research has identified that gram-negative bacteria are more prevalent than gram-positive bacteria. Chronic wounds consist of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas*. Diabetic wounds tend to be poly-microbial when compared to non-diabetic wounds, which are more likely mono-microbial in nature. Researchers have uncovered a sophisticated civilization that pro-

Cell proliferation permeates within the EPS, leading to growth and differentiation. The EPS allows for environmental protection and protection from an array of treatment protocols. EPS promotes ion exchange, maintains moisture balance, regulates enzyme activity, and provides vital nutrients.

Fully developed biofilms function as sophisticated grids, providing channels for passage of nutrients, ions, and enzymes. The cell-to-cell communication provides the advancement of the virulence factor for biofilms with quorum sensing (QS).³ Bacteria are able to produce cellular sequencing with the use of adhesion molecules, communication

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molecules, and effector proteins that establish specific secretory systems for communication, all leading to a stable environment for survival.

QS thrives when the wound is left in a chronic state. The communication increases with cell-to-cell contact channels or proteins. This communication can affect up to 5% of the gene expression by autoinducers (AI). Gene expression is regulated by AI entering the cells and binding to trans-membrane receptors, causing signal transduction and gene expression. Research has identified the activators of gram positive QS systems using small peptides termed auto-inducing peptide pheromones and gram negatives being controlled by two regulator proteins Lux1 and Lux2 (as an autoinducer synthase which produces AIs). This is one glimpse into gene regulation and transduction that occurs within the biofilm environment.³

Cells within the EPS act differently compared to free motile bacteria. Metabolic activity is vastly reduced. This reduction in activity is the key role in the persistence of many chronic wounds. The gold standard for treating an infection is the use of antibiotics that seek actively dividing cells by targeting peptidoglycan produced in the cell wall, protein synthesis, or DNA replication.

Building and maintaining the biofilm affects the bacteria and allows the genetic makeup to create irreversible genotype changes, causing widespread resistance within the wound environment. Maintenance of the biofilm has been shown to be a result of persister cells. If bacteria within the wound environment are not eliminated, persister cells can regenerate the biofilm with a microbial population that resembles the past susceptibility profile.⁷ This key concept is extremely frustrating to the patient as well as to the practitioner treating chronic wounds.³

Persister cells (PC) are protected from the immune system by the biofilm EPS matrix. Antibiotics penetrate the biofilm with varying degrees. Each antibiotic has a different penetration ability, depending upon the dosage

and the specific tissue that is targeted. The revival of the infection comes from the PCs surviving the initial antibiotic bactericidal treatment and then reforming the biofilm. The continuing infection may be a replica of the initial infection, or have altered metabolism and gene expression.

Many antibiotics that are used to treat infections target the metabolically active cell, targeting ribosomes, binding proteins, gyrase, and topoisomerase during DNA replication, while persister cells are relatively

micro-organisms within a biofilm. A DNA diagnostic method such as polymerase chain reaction (PCR) is able to identify and quantify micro-organisms present in chronic wounds. DNA diagnostic testing identifies with great accuracy and creates precisely tailored treatment, reducing unnecessary antibiotic use, and the prevalence of drug-resistant bacteria.

Standard culture techniques have been recently questioned for accuracy. In chronic wounds, all medical professionals have an understanding that

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dormant or highly down-regulated. Current medical treatment is directed to target the active cells. Current and further research on the mechanism of Daptomycin in killing bacteria can shed new light on treatment protocols for chronic wounds. For example, Daptomycin is a lipopeptide that does not need an active cell to conduct eradication. The mechanism of action is through accumulation of the antibiotic in the bacterial membrane, causing rapid depolarization. To date, this process is poorly understood, and further microbiologic research of persister cells needs to be conducted.⁴

Clinical cultures have been the gold standard method to identify organisms present in a wound. Today, clinical cultures have shown to be ineffective in positively identifying all relevant bacteria within a wound. There are two major problems with identifying organisms in a wound. One is a phenomenon known as antimicrobial synergism, which plays a role in the ability to identify multiple organisms in the same wound.

The second problem is that antimicrobial susceptibility examinations test for free-living metabolically active bacteria, but in a biofilm most bacteria are dormant or relatively inactive.³

Polymerase Chain Reaction (PCR)

Advances in technology have created multiple avenues for testing

Staphylococcus and Pseudomonas are prominent organisms. The reported predominance of these organisms could be from ease of culturing and not a true representative sample of the wound. Studies using pyro-sequencing methods have shown profiles of Peptinophilus, Streptococcus, and Clostridium to be significant contributors to chronic wounds with biofilm formation.

Current research has shown an association between Pseudomonas and lactate metabolism and Burkholderia and glycerol metabolism. These carbon sources can be targeted to customize a therapeutic intervention that hinders the opportunistic pathogens.²

This research into amino acid metabolic pathways indicates the complexity of communication within the biofilm environment and can give practitioners potentially accurate and cost-effective means to fight chronic wounds.

Frequent debridement is a pivotal mechanism in healing chronic wounds and improving healing outcomes. Current commonly-used debridement techniques include autolytic, mechanical, sharp, hydro-surgical, larvae therapy and mechanical de-sloughing.⁹ Eradicating the thick layer of EPS that protects the infecting microbial population from the immune system and antimicrobials is the first step in the treatment algorithm.

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Debridement forces disorganization within the EPS and causes an increase in metabolic activity. Stimulating the cellular activity in the wound as the biofilm is trying to reconstitute itself leaves the biofilm vulnerable to standard treatments, which have been developed to target active cellular activity.

Dressing Materials

A major focus in the wound care field is the advancements in dressing material. Dressings have become plentiful and have many different attributes and functions. The attributes range from supporting the wound environment, absorbing exudates, providing optimum moisture balance, preventing maceration of the peri-wound and controlling bacterial colonization. Advances in nanoparticles, specifically silver nanoparticles, have shown the ability to eradicate bacteria within a biofilm.¹⁰

Nanoparticles are three-dimensional structures containing a certain molecule in clusters stabilized by coating ligands with a regulated release pattern.⁶ The mechanism silver nanoparticles use is a process of blocking exopolysaccharide synthesis, resulting in eradicating biofilms.⁵

Further research into the synergistic effects of silver nanoparticles and molecule extracts has shown promising results. Manufacturing of nanofibrous meshes made up of ultra-fine intrinsic properties such as a high-surface area for fluid absorption, nanoporosity allowing cell respiration, and gas permeation preventing dehydration, have shown promising results in recent research. The mesh fibers tend to imitate the fibrous architecture of the natural extracellular matrix, providing a comparable substrate. Electrospun scaffolds have been shown to promote cell migration and proliferation.¹ Electrospun meshes provide essential requirements for wound healing, but the elementary aspects and parameters for optimal wound healing are still poorly understood.

The effects of degrading the biofilm matrix with synergy of cidal treatment agents and the specific properties of wound gels on the

breakdown of the EPS have recently been studied.

Ingredients include a pH buffer system of an acid and conjugate base with a high osmolarity and a surfactant. The base of the gel creates a moist environment for proper autolytic debridement and granulation formation. The high osmolarity of the gel causes osmotic imbalance, resulting in the cell wall becoming more permeable and exposing proteins on the cell wall to the surfactant.

The antibacterial components are

interacting with micro-organisms have shown to cause DNA damage, denaturing of proteins effecting the cell wall, and generation of reactive oxygen species. To further the efficacy of LLLT on biofilms, well-designed, long-term, controlled, randomized, and double-blind clinical trials need to be completed before this technology can become a commonplace treatment for fighting chronic wounds.⁹

Many aspects of biofilms and chronic wounds are still undiscovered. Current culturing techniques

Advances in hydrogels have created another avenue to cure and prevent biofilms.

now exposed to numerous surface molecules to conduct their mechanism of action. Combining the anti-biofilm strategies of wound gel to degrade the matrix, and the use of antimicrobial agents improved wound healing outcomes.¹¹

Advances in hydrogels have created another avenue to cure and prevent biofilms. Fundamental research on the bonding nature of chemicals has given insight on how creating an environment can fuel specific reactions to prevent or have bacteria become vulnerable. Hydrogels have shown increasing effectiveness in fighting biofilms by releasing the chemicals within the matrix in a controlled fashion. Hydrogel and treatment molecules have a synergistic effect within the wound environment to control the biofilm formation.⁸

Studies continue to be conducted on laser technology to fight biofilms. Low-level laser therapy (LLLT) as an antimicrobial agent has shown promising results to date. The exact mechanism of action for LLLT is not well understood, but the most accepted theory is that visible light irradiation provokes the photo-activation of enzymes. Laser therapy uses a specific wavelength of light to stimulate processes at the cellular level by increasing oxidative phosphorylation, reducing inflammation, increasing cellular metabolism, and enhancing growth and proliferation of fibroblasts. LLLT

may be inadequate to fully identify the organisms housed within the wound. Advancements in technology have shown that practitioners may be treating only a fraction of what is represented within the wound bed. Further development is needed to understand the community within the biofilm. Advances in understanding how the EPS, QS, and persister cells function as a unit may reveal alternative treatment mechanisms. The medical field must clearly evaluate the relationship between microbial species, biofilm-forming capabilities, and their antibiotic sensitivities. The economic costs associated with chronic wounds are staggering and exponentially growing in the next decades. Further research emphasis may need to switch from specific cell-based therapy to the mechanisms of action within a biofilm, to prevent dysfunction and promote healthy wound-healing potential. **PM**

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