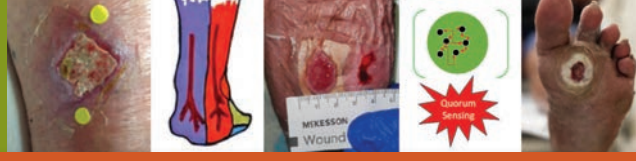

The Role of Biofilm in Non-Healing Wounds in Patients with Diabetes

These cells play a pivotal role in hindering the wound-healing process.

BY J. KARIM EAD, DPM, MS AND ROBERT J. SNYDER, DPM, MBA, MSC

Introduction

According to a recent meta-analysis, approximately 6.3% of patients with diabetes worldwide are affected by diabetic foot ulcers (DFU), translating to approximately 33 million individuals impacted by this condition.[2] DFUs result from a combination of factors related to the underlying pathophysiology of diabetes and associated risk factors. These risk factors include poor glycemic regulation, infection, biomechanical abnormalities, poor nutritional status, peripheral neuropathy, and macro/micro-vascular disease, among others.[2]

DFUs result from a combination of factors related to the underlying pathophysiology of diabetes and associated risk factors.

An emerging area of concern is the role of biofilm on wound surfaces and within the underlying tissues, adding to the list of potential risk factors pertaining to chronic non-healing wounds. The role of bio-

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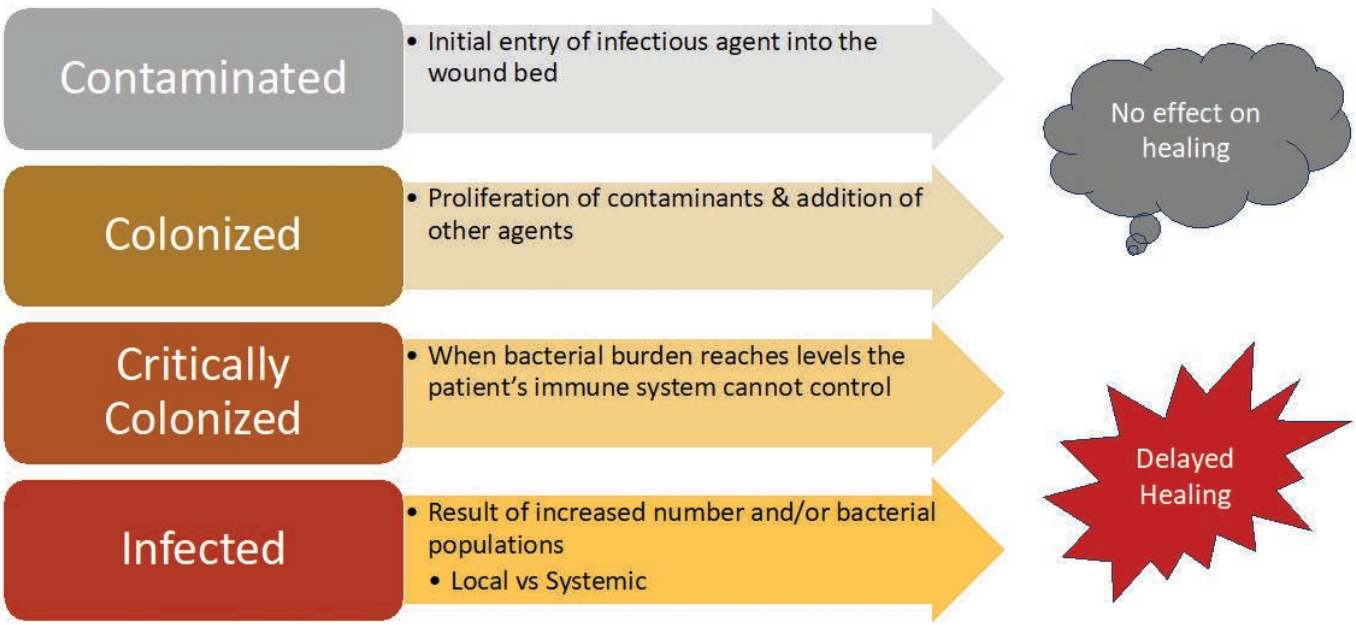
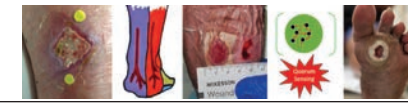


Figure 1: This conceptual framework is used to describe the progression of bacterial colonization in wounds. All chronic wounds have some level of bacterial load. Healing can occur in the presence of normal levels of bacteria that are commonly found on our skin and that can even be helpful in wound healing.[5]



Role of Biofilm *(from page 113)*

film in non-healing wounds among patients with diabetes is a critical factor contributing to the prolonged and challenging nature of these wounds. Biofilms are complex and structured communities of bacteria encased in a protective matrix.[3]

In patients with diabetes, elevated blood glucose levels create a favorable environment for bacterial colonization and biofilm formation.[4] The impaired immune response, compromised circulation, and peripheral neuropathy further exacerbate the problem.[4] When a wound becomes chronic or fails to heal within the expected timeframe, bacteria colonize the wound bed and initiate biofilm formation. Once biofilm is well incorporated, bacteria are protected from the host's immune system and become highly resistant to traditional treatment options (i.e., antimicrobial therapies).[4] Many speculate that biofilms play a pivotal role in hindering the normal wound healing process and exacerbating the already impaired healing environment caused by diabetes-related complications.[4]

Wound infection occurs when the bacterial burden in the wound leads to an inflammatory response, causing damage to the surrounding tissues and a systemic immune response.

The Bacterial Burden Continuum

The "bacterial burden continuum" within the context of a wound framework is a concept that describes the relationship between the severity of a wound and the level of bacterial contamination or infection present within the wound ecosystem.[5] It suggests that wound healing is influenced by the balance between the extent of tissue injury and the microbial load within the wound.[5] It is essential to remember that not all wounds follow a linear path along the continuum, and wound status can change over time.[5] Thus, it is not the presence of the microorganisms, but their interaction and numbers that determine their influence on wound healing for patients.[5]

Critical bacterial colonization and wound infection are two distinct stages in the spectrum of wound bacterial presence, and they are characterized by different clinical features and management approaches.[5] Understanding the difference between these stages is essential for appropriate wound care and infection control (Figure 1). Critical colonization refers to a stage where the wound is heavily colonized by bacteria, but the bacteria do not cause overt signs of infection.[6] The bacterial load in the wound exceeds the body's ability to control their growth without causing systemic symptoms or spreading to surrounding tissues.[6] The wound may show some signs of inflammation and increased exudate (drainage) but lacks the typical signs and symptoms of an active infection.[6] Bacteria at this stage are typically organized within a biofilm ecosystem, which can be more resistant to standard antimicrobial treatments.[6]

Critical colonization refers to a stage where the wound is heavily colonized by bacteria, but the bacteria do not cause overt signs of infection.

Wound Infection

Wound infection occurs when the bacterial burden in the wound leads to an inflammatory response, causing damage to the surrounding tissues and a systemic immune response.[7] Typical signs of local infection include increased redness, warmth, swelling, tenderness, and pus or foul-smelling discharge, among others.[7] Infections can be classified as superficial (limited to the wound surface) or deep (involving underlying tissues, such as cellulitis or abscess formation). Infection complicates the treatment of wounds and impedes the healing process by: damaging tissue, reducing wound tensile strength, and by inducing an undesirable inflammatory response.[8] Thus, controlling or preventing infection is essential in order for the healing process to progress normally. Wound infections can be caused by a variety of bacterial species and may require surgical debridement, systemic antimicrobial therapy (oral vs. intravenous antibiotics), in addition to local wound care.[8]

Systemic signs of infection involve the entire body and indicate that the infection has spread beyond the local wound site. Some of these symptoms include: fevers, chills, tachycardia, and tachypnea, among others.[7] These signs suggest a more severe infection that may require urgent medical attention.[7] Uncontrolled infections can lead to sepsis, a potentially life-threatening condition characterized by systemic inflammatory response and organ dysfunction.[7] Effective wound management involves early detection and appropriate management of both critical colonization and infection, as well as addressing other factors that may impede the wound healing process.[7]

In patients with diabetes, particularly those with poorly controlled blood sugar levels, the immune system can be compromised, making them more susceptible to infections. When a wound occurs, whether due to injury, surgery, or other factors, the risk of infection is higher compared to individuals without diabetes.

Secondary signs of infection in patients with diabetes include the following[9]:

- *Slow or Delayed Healing:* Wounds in individuals with diabetes

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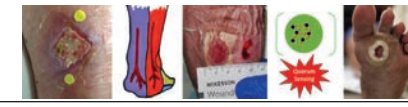

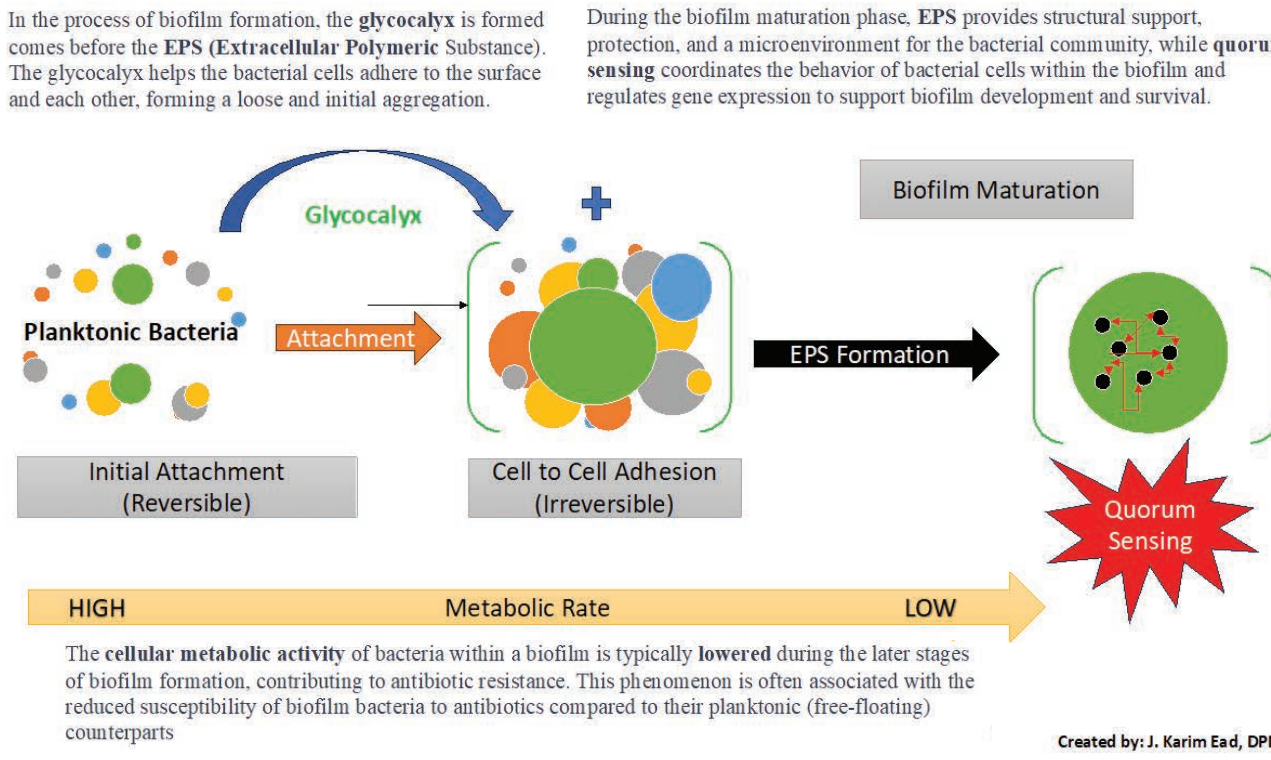

Figure 2: During the biofilm maturation phase, the glycocalyx and Extracellular Polymeric Substance (EPS) and quorum sensing play crucial roles in shaping the structure and behavior of the biofilm community.[25]

Role of Biofilm *(from page 114)*

tend to heal more slowly, making them more susceptible to infections. The impaired blood flow, nerve damage, and high blood sugar levels can hinder the body's natural healing process.

- *Acute Wound Deterioration*

- *Redness and Warmth:* The area around the infected wound may appear red and feel warm to the touch due to the body's immune response and increased blood flow.
- *Foul Odor:* Infected wounds may produce a foul-smelling discharge due to the breakdown of tissues and the presence of bacteria.

To effectively manage non-healing wounds in diabetic patients with biofilms, healthcare professionals need to adopt comprehensive wound care strategies.

- *Increased Pain (in a painless foot):* Infected wounds can become more painful, especially if the infection spreads or becomes more severe. The presence of bacteria and inflammation in the wound can lead to heightened discomfort.
- *Increased Swelling:* Infections can lead to increased localized swelling around the wound site as the body responds to the invading pathogens and inflammation.

- *Discharge or Pus:* The wound may exude a yellowish or greenish fluid known as pus, which contains dead white blood cells and bacteria.

To effectively manage non-healing wounds in diabetic patients with biofilms, healthcare professionals need to adopt comprehensive wound care strategies. This may include regular debridement to remove the biofilm and infected tissue, offloading pressure from the wound to improve blood flow, controlling blood glucose levels, using topical antimicrobial agents or dressings specifically designed to target biofilms, and considering advanced wound therapies when necessary. It's important to note that biofilm-related wound management is a challenging area of research and requires a multidisciplinary approach involving wound care specialists, podiatrists, infectious disease experts, and other healthcare professionals to provide the best possible outcomes for patients with diabetes dealing with chronic/non-healing wounds.

The Biofilm Matrix: Understanding the Pathogenesis and Implications for Effective Treatment

In the process of biofilm formation, the glycocalyx typically comes before the Extracellular Polymeric Substance (EPS). The formation of a biofilm is a complex and dynamic process that involves multiple stages. Here's a general sequence of events[10]:

- *Initial Attachment:* Free-floating (Planktonic) microorganisms encoun-

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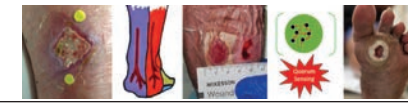

Role of Biofilm *(from page 115)*

ter a surface and attach to it through weak, reversible interactions.[10] During this stage, the bacteria begin producing a thin layer of glycocalyx. The glycocalyx helps the bacterial cells adhere to the surface and each other, forming a loose and initial aggregation.[10]

- *Irreversible Attachment:* Once attached, the microorganisms produce adhesive substances that promote irreversible attachment to the surface, making it more difficult to dislodge.[10]
- *Extracellular Polymeric Substance (EPS) Production:* The attached microorganisms start producing EPS, which consists of polysaccharides, proteins, and nucleic acids. EPS acts as a glue that holds the biofilm together and anchors it to the surface.[10] The EPS also hinders the migration of cells essential for wound healing, such as fibroblasts and keratinocytes. The EPS also create a physical barrier that prevents wound closure and re-epithelialization.[10]
- *Microbial Growth and Colonization:* The microorganisms within the biofilm multiply and grow, creating multiple layers of cells. Different species of microorganisms can co-exist within the biofilm, creating a diverse community.[10]
- *Formation of Water Channels:* As the biofilm grows, it forms channels that allow nutrients and waste products to flow in and out, maintaining the biofilm's viability.[10]
- *Maturation and Three-Dimensional Structure*: Over time, the biofilm ma-

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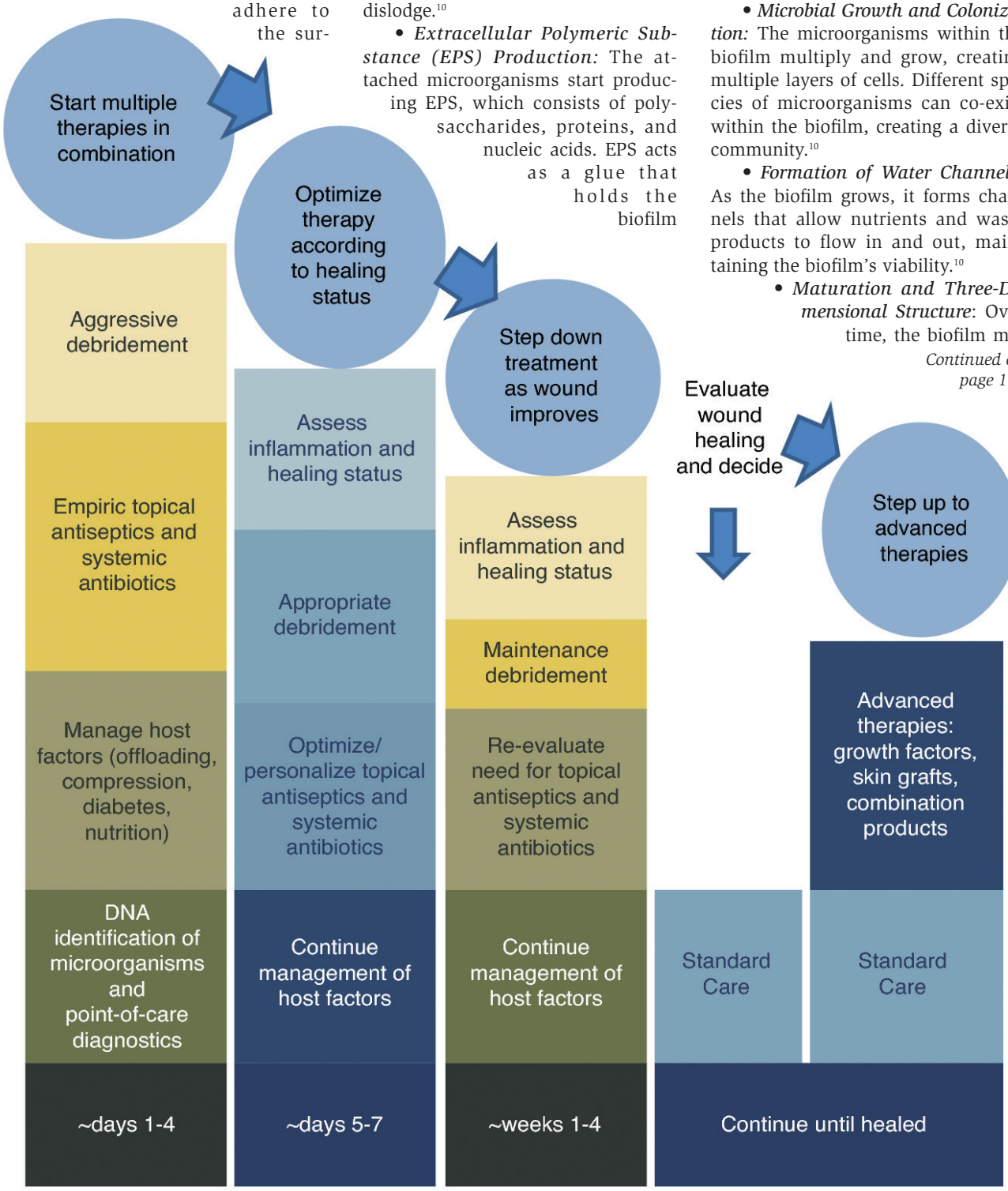

Figure 3: Summary of the step-down/step-up approach to biofilm-based wound care.[24] (Adapted from Schultz, et al.)

Role of Biofilm *(from page 116)*

tures, and its structure becomes more organized and complex. The biofilm can develop into mushroom-like structures, towers, or flat layers, depending on the specific microorganisms and environmental conditions.[10]

- *Spatial Gradients:* Biofilms exhibit spatial gradients, with oxygen and nutrient levels varying throughout the structure. This creates micro-environments where bacteria experience different conditions, leading to variable susceptibility to antimicrobial agents.[11]
- *Detachment and Dispersal:* Within the mature biofilm, some microorganisms detach and disperse to colonize new surfaces, allowing the biofilm to spread and potentially cause infections or other adverse effects.[10]
- *Resistance to Antimicrobial Agents:* The biofilm's EPS matrix provides protection and reduces the effectiveness of antimicrobial agents, making it more challenging to treat biofilm-related infections.[10]

Quorum sensing is a communication system used by many bacteria to coordinate their behavior and regulate gene expression in response to changes in population density.

Quorum Sensing

Quorum sensing is a communication system used by many bacteria to coordinate their behavior and regulate gene expression in response to changes in population density.[12] It allows bacteria to monitor their environment and respond collectively when a certain threshold of bacterial cells is reached.[12] This system plays a crucial role in the pathogenicity of biofilms.[12] The biofilm's EPS hinders the host's immune cells from effectively reaching and eliminating the bacteria.[12] The biofilm structure reduces the ability of immune cells to detect and respond to the bacteria, allowing the biofilm to persist and evade the immune system.[12]

Both the glycocalyx and the EPS (extracellular polymeric substance) play important roles in the formation and maintenance of biofilms in chronic non-healing wounds, but they are distinct components with different functions within the biofilm structure.

Glycocalyx

The glycocalyx is a layer of glycoproteins, glycolipids, and other carbohydrates that covers the surface of many cells, including bacteria. In the context of biofilms, the glycocalyx forms a protective and adhesive matrix around bacterial cells. It is produced by the bacteria themselves and consists of polysaccharides and other molecules. The

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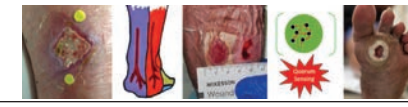

Role of Biofilm *(from page 117)*

glycocalyx serves several key functions in biofilms:

- *Adhesion:* The glycocalyx helps bacteria adhere to surfaces, including the wound bed, allowing them to establish and maintain a stable attachment.
- *Protection:* The glycocalyx provides a physical barrier that protects bacteria within the biofilm from environmental stressors, immune cells, antibiotics, and other threats.
- *Nutrient and Waste Exchange:* The matrix of the glycocalyx allows for the exchange of nutrients and waste products within the biofilm structure.
- *Cooperation:* Bacteria within the biofilm can communicate and coordinate their activities using signaling molecules embedded in the glycocalyx. This communication, known as quorum sensing, enables the bacteria to act collectively and adapt to changing conditions.

EPS (Extracellular Polymeric Substance)[25]:

The EPS is a complex mixture of polymers, including polysaccharides, proteins, nucleic acids, and lipids, produced and secreted by bacteria within a biofilm.[25] It forms the structural framework within the maturated biofilm and plays a crucial role in its development and maintenance. EPS has several important functions in biofilms:

- *Matrix Formation:* The EPS forms a three-dimensional matrix that holds the bacterial cells together in a cohesive structure, promoting stability and protection.
- *Retention of Water and Nutrients:* The EPS matrix retains water, which helps create a micro-environment with different chemical and physical properties compared to the surrounding environment. This micro-environment supports bacterial growth and allows for efficient nutrient and waste exchange.
- *Antibiotic Resistance:* The EPS matrix can create physical barriers that limit the penetration of antibiotics into the biofilm, contributing to antibiotic resistance in chronic wound infections.
- *Mechanical Protection:* EPS adds mechanical strength to the biofilm, helping it resist physical disturbances and shear forces.

The presence of the biofilm triggers a prolonged and exaggerated inflammatory response at the wound site.[12] Chronic inflammation can impair the normal wound-healing process, delaying the formation of granulation tissue and the closure of the wound.[13] Even after successful treatment of an initial infection, residual bacteria within the biofilm can cause recurrent infections. Bacteria within the biofilm ecosystem exhibit higher resistance to antimicrobial agents, including oral and topical antibiotics. Bacteria living in a biofilm can exhibit a 10 to 1,000-fold increase in antibiotic resistance compared to similar bacteria living in a planktonic state.[14]

The presence of the biofilm triggers a prolonged and exaggerated inflammatory response at the wound site.

Biofilms are also resistant to immune killing and clearance. The aforementioned EPS acts as a physical barrier, preventing antibiotics from effectively reaching and killing the bacteria within the biofilm.[15] Some antibiotics can penetrate biofilms, including fluoroquinolones, rifampin, and ampicillin.[16] However, these antibiotics are not able to eradicate 100% of biofilm bacteria.[16]

Biofilm-forming bacteria are encased in a matrix that can exclude antibiotics and the host immune response.[16] Other antibiotics, like tobramycin, ciprofloxacin, and tetracyclines, preferentially kill the metabolically active bacteria located in the outer part of the biofilm.[17] However, the non-growing bacteria in the inner part of the biofilm survive treatment.[17] The lower the antibiotic concentration, the longer it takes to kill 5% of the bacteria in the biofilm.[18] This holds true for several antibiotic subclasses, and it applies solely when the antibiotic concentration surpasses the Minimum Inhibitory Concentration (MIC).[18]

To effectively act on biofilm-forming microorganisms, antimicrobial agents must overcome several key obstacles that are inherent to the biofilm structure and behavior. Some include:

- *Increased Number of Resistant Mutants:* Within the biofilm, there is a higher likelihood of genetic mutations occurring due to the dense bacterial population and exposure to stressors, including antimicrobial agents. This increased mutation rate can lead to the development of resistant bacterial strains, making it more challenging to eradicate the biofilm.[19]
- *High Cell Density:* Biofilms consist of a high concentration of bacterial cells in a confined space. This high cell density can create a physical barrier, limiting the penetration of antimicrobial agents into the deeper layers of the biofilm.[20]
- *Complex Molecular Exchange Pathways:* Bacteria within the biofilm communicate and interact through various signaling molecules. This communication allows the biofilm community to act collectively, leading to increased resistance to antimicrobial agents.[21]
- *Substance Delivery Challenges:* The biofilm matrix hinders the efficient delivery of antimicrobial agents to the bacterial cells. The EPS acts as a protective shield, preventing the antimicrobials from reaching their targets effectively.[20]
- *Efflux Pump Activity:* Biofilm-forming bacteria may up-regulate efflux pumps, which are cellular transporters that actively pump antimicrobial agents out of the bacterial cells. This efflux mechanism reduces the intracellular concentration of antimicrobials, contributing to resistance.[22]

Continued on page 119

Role of Biofilm *(from page 118)*

• *Persister Cells:* Within the biofilm, some bacteria can enter a dormant and highly tolerant state known as persister cells. These cells are not actively dividing and are highly resistant to antimicrobial agents, allowing them to survive treatment and potentially re-initiate the infection once conditions become favorable.[23]

Understanding the microbiology/molecular framework of biofilms in chronic non-healing wounds is of vital importance as it provides critical information about the specific bacterial species present, their antimicrobial resistance profiles, and their potential role in hindering wound healing. However, to date, there are no routine diagnostic modalities currently available to confirm biofilm presence.

Various techniques can provide more information on the bacterial network; such as tissue biopsy, curettage, sonication, or specialized swabs designed to collect biofilm samples more effectively. However, there are significant and potential challenges associated with these processes. Identifying the biofilm-forming bacteria helps in selecting appropriate antimicrobial therapies that specifically target the biofilm.

Biofilm bacteria may exhibit different antibiotic resistance patterns compared to planktonic bacteria, making targeted treatment crucial for successful wound management.[14] Additionally, these complex communities can be highly heterogeneous, with various bacterial species present in different parts of the wound.[14] Standard wound swab cultures may not accurately detect biofilms since they only sample the wound surface and may miss deeper biofilm layers.[24]

Advanced specialized sampling techniques and advanced imaging methods are required for more accurate detection.[24] For instance, advanced imaging techniques like confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) can be employed to visualize and confirm the presence of biofilms in the wound samples.[24] Without bedside diagnostic tests, clinicians should rely on particular signs and symptoms to confirm the presence of biofilms.[24]

The most effective treatment pathway to address biofilm-infested chronic wounds involves a comprehensive and multi-modal approach.[24,25] Since biofilms are highly resistant structures, a combination of strategies is needed to treat them effectively.[24] First, an accurate assessment of the wound is crucial to determine the presence and extent of the biofilm.[24] This includes evaluating wound size, depth, presence of undermining or sinus tracts, signs of infection, and tissue viability. Debridement is an essential step to remove necrotic tissue, debris, and biofilm from the wound bed.[24]

This can be achieved through various methods such as sharp debridement, mechanical debridement, enzymatic debridement, or autolytic debridement. Biofilms have been shown to regain antibiotic resistance 72 hours after debridement, suggesting reduced efficacy of treat-

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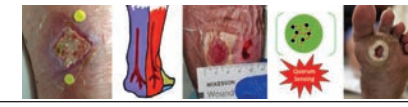

Role of Biofilm *(from page 119)*

ment and delayed wound healing.[26] This crucial window highlights the importance of timely removal/debridement of all non-viable tissue in order to create a healthier wound environment that is more responsive to healing.[25]

Topical antimicrobial agents play a crucial role in targeting the bacteria within the biofilm. These agents can be applied directly to the wound bed after a thorough debridement to disrupt the biofilm and control infection. The application of topical antimicrobial agents within the 72-hour window can potentially suppress biofilm reformation.[25]

In some cases, systemic antibiotics may also be prescribed based on the severity of the infection. Use of advanced wound dressings with antimicrobial properties can aid in reducing biofilm burden and promoting wound healing.[24] Silver, iodine, and other antimicrobial-impregnated dressings have been shown to be effective in managing biofilm-infested wounds.[27] Managing blood glucose levels is critical in promoting wound healing and reducing the risk of infection.[28] Proper glycemic control helps prevent the development of chronic wounds and supports overall wound healing processes.[27]

Patient education on proper wound care protocols, early reporting of any non-healing wounds, and maintaining good foot hygiene are essential for successful wound management and the prevention of biofilm formation. Regular follow-up visits with healthcare professionals are necessary to monitor wound progress, evaluate the effectiveness of the dynamic treatment plan, and make adjustments as needed.

The current understanding of biofilm pathogenesis and its impact on wound healing has led industry leaders to develop innovative approaches to combat biofilm and accomplish their role in the wound-healing process.

Some of these emerging therapies include:

- *Antimicrobial Peptides (AMPs):* Antimicrobial peptides are short chains of amino acids with potent antimicrobial properties. Researchers are investigating the use of specific AMPs that can disrupt biofilm structures and kill bacteria within the biofilm. AMPs offer the advantage of broad-spectrum activity against various microbes while potentially minimizing the risk of antimicrobial resistance.[29]
- *Enzymatic Disruption:* Enzymatic agents are being studied to break down the extracellular matrix of biofilms. Certain enzymes can degrade the biofilm's EPS, making it more susceptible to traditional antimicrobial agents and the body's immune response.[30]

—For example: Bromelain-based debridement is considered a non-invasive method of wound debridement, which can be particularly beneficial for patients who cannot tolerate more aggressive debridement techniques.[31]

—This method involves the use of a concentrate of proteolytic enzymes enriched in bromelain, which is derived from pineapples.[31]

—Bromelain is a mixture of proteolytic enzymes that can break down proteins.[31]

—Bromelain's enzymatic action is generally more selective in targeting non-viable tissue, minimizing damage to healthy tissue.[31]

- *Quorum Sensing Inhibition:* Quorum sensing is a communication mechanism used by bacteria to coordinate their activities, including biofilm formation. Inhibition of quorum sensing disrupts this communication, potentially preventing biofilm development or making existing biofilms more vulnerable to eradication.[32]
- *Bacteriophages:* Bacteriophages are viruses that infect and destroy specific bacteria. Researchers are exploring the use of bacteriophages that target biofilm-forming bacteria. These phages can penetrate the biofilm and lyse the bacterial cells, reducing the biofilm's integrity and viability.[33]
- *Nanoparticles:* Nanoparticles, particularly those with antimicrobial properties, are being studied as a means to disrupt biofilms. Some nanoparticles can penetrate the biofilm matrix and release antimicrobial agents directly at the site of infection.[34]
- *Photodynamic Therapy (PDT):* PDT involves the use of photo-sensitizers that, when exposed to specific wavelengths of light, produce reactive oxygen species.[33] These reactive oxygen species can damage bacterial cells and disrupt biofilm structures.[35]

Early detection, coupled with effective biofilm-targeting strategies, holds the potential to improve outcomes, reduce amputation rates, and enhance the quality of life for patients with diabetic foot ulcers.

While these novel therapies show promise, more research is needed to understand their safety, efficacy, and clinical applicability fully. As our understanding of biofilm pathogenesis continues to evolve, these innovative therapies hold the potential to revolutionize wound management and improve outcomes for patients with chronic wounds, especially those with diabetes.

Conclusion

Non-healing wounds among patients with diabetes continues to pose a significant challenge to both patients and healthcare providers due to their chronicity and resistance to healing. The presence of biofilms in DFUs plays a pivotal role in hindering the wound-healing process and contributing to recurrent infections. Biofilms create a hostile environment that shields bacteria from the immune system and antimicrobial

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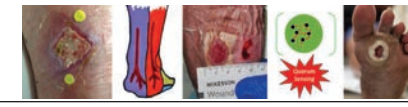

Role of Biofilm *(from page 120)*

treatments, leading to prolonged inflammation and delayed tissue repair.

As researchers delve deeper into the mechanisms of biofilm formation and resistance, promising treatment approaches are emerging, including biofilm-disrupting agents, advanced topical antimicrobials, and innovative combination therapies. Nevertheless, the management of DFUs and biofilms demands a personalized and multidisciplinary approach, focusing on wound debridement, optimizing glycemic control, and implementing preventive measures.

Early detection, coupled with effective biofilm-targeting strategies, holds the potential to improve outcomes, reduce amputation rates, and enhance the quality of life for patients with diabetic foot ulcers. Continued research and collaboration among experts in the field are essential to unraveling the complexities of biofilms and advancing wound care interventions in this challenging clinical scenario. **PM**

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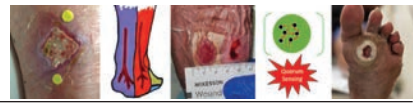

Role of Biofilm *(from page 121)*

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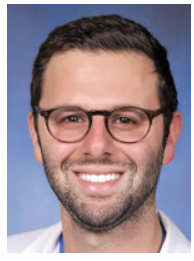
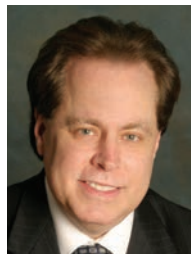


Dr. Ead is a fellowship-trained podiatric surgeon who is a graduate from the University of Southern California's Limb Preservation fellowship program under the tutelage of Drs. Laura Shin and David Armstrong. He received his surgical training from HCA Florida Westside Hospital. In the Fall of 2023, Dr. Ead will be establishing a new Podiatry service at the St. Frances Cabrini—CHRISTUS Health system in Alexandria, Louisiana.



Dr. Snyder is Dean, Professor, and Director of Clinical Research at Barry University School of Podiatric Medicine. He has served as Principal Investigator on more than 60 randomized clinical trials. In 2018, Dr. Snyder was inducted as Faculty Fellow of Podiatric Medicine, Royal College of Physicians and Surgeons (Glasgow).