The wound microbiome: striking a bacterial balance

INTRODUCTION

Clinicians aim for every wound to perform the perfect healing cascade to wound closure. Wound chronicity is a major concern, and clinicians must find the missing pieces of the puzzle for timely wound healing. Implementing the key elements of wound bed preparation assists in removing barriers with each stage of wound healing. The key elements include effective wound cleansing, debridement, bacterial balance, and exudate management. [1] If there are inconsistencies within the wound healing continuum, they will result in impeded healing caused by significant factors such as biofilm formation and infection. Biofilm has a natural ability to rebuild. This process can take 72 hours, and biofilm can be detected within 24 hours after debridement, thus making wound healing a major challenge. To make understanding biofilms even more complex, all biofilms and host factors differ. [2]

Malone et al. determined that the prevalence of biofilms in chronic wounds was 78. 2% (confidence interval, 61. 6–89, P < 0. 002) and only 6% in acute wounds. [3] These species degrade proteins that are a necessary element for the wound healing process. Microorganisms nimbly seek out the optimal environment to thrive and grow. Full-thickness wounds, non-viable tissue types, exudates, dry wounds, and dressings can provide this welcoming microbial surface. Any break in the skin is at risk for possible infection. However, influential factors such as wound type, wound depth, wound location, and level of tissue perfusion are key in the development of a myriad variety of microbiomes. For example, aerobic bacteria live superficially,

whereas anaerobic bacteria live in deeper tissue layers. Predicting healing outcomes can be difficult because we still do not know the depth needed to remove the entire biofilm colony. [4]

Biofilms are made up of a combination of exopolymers and mixed strains of microorganisms. They can include bacteria, fungi, yeasts, algae, microbes, protozoa, and other cellular debris. Bacteria can live in several forms, including planktonic (free-floating) forms and biofilms, in a clinical or natural setting. Chronic inflammation is stimulated by biofilm, increasing levels of proteases and reactive oxygen species while degrading fibronectin and platelet-derived growth factor (PDGF). Quorum sensing molecules help bacterial colonies mature by stimulating change in specific genes. [5, 6] The exopolymeric matrix (EPM) is responsible for making it difficult to kill biofilms.

(insert stages of biofilm development diagram in wounds from 2018 Biofilms?) [7]

WOUND INFECTION CONTINUUM (insert stages of bacterial growth diagram in wounds from 2018 Biofilms?) [7]

Antibiotics are designed to attack bacteria and may only partially eliminate the bacteria contained within a biofilm. Exposing bacterial biofilms to the wrong antibiotics may cause biofilms to become dense. This dense EPM matrix can paralyze large antibodies and neutralize microbicides. A biofilm is capable of promoting anaerobic bacteria growth and synergism among different bacteria, generating methicillin-resistant *Staphylococcus aureus*

(MRSA)-resistant proteins, and producing negative charges of polysaccharides and DNA binding cationic molecules such as Ag+, antibiotics, and PHMB. Biofilms can also reform in as little as three days after sharp debridement. This is when a wound may appear to be healing but then becomes stagnantagain. [8-11] Wounds that remain in this stagnant state for a longer time can manifest complications such as infection and even limb loss.

KEY RISK FACTORS AND CONTROL MEASURES

Early intervention and prevention are critical in reducing the likelihood of biofilm formation and wound infection. Subsequently, development of an infection is influenced by the virulence of the organism and the immunological status of the patient. Using a consistent sequence of wound infection prevention and management strategies will support the reduction of infection and decrease the recurrence of infection. Assessing key risk factors, implementing a plan of care, setting healing goals, and teamwork providing education to the patient, family, and health care team should be part of wound management.

PREVENTING AND MANAGING BIOFILMS

Clinicians must implement principles of wound bed preparation, utilize multiple concurrent strategies, and manage host factors for prevention and treatment of biofilm formation. For example, a diabetic foot ulcer (DFU) has a multitude of host factors compared with a surgical dehiscence wound type. DFU inflammation can be caused by more than one factor, including biofilm, weight-bearing status, perfusion, nutritional status or low pre-albumin, and

recurrence of injury. [12] Necessary components of wound bed preparation include cleansing, debridement, and appropriate choice of topical agents or dressings. [13]

Sequential sharp and surgical debridement methods for wounds with mature biofilm have been proven in national and international guidelines to disrupt biofilm growth and promote faster healing. [3, 11, 14, 15] Preventing biofilm regrowth remains a challenge partly because biofilms spread in and around blood vessels below the wound surface. [16]

Following wound debridement, topical antibiofilm therapies or products are suggested to help prevent biofilm reformation. Wound bed preparation using mechanical debridement methods and multiple effective antibiofilm antiseptics is key. [13] The wound care industry is rapidly growing, with an array of impregnated dressings in a variety of formats (including collagens, foams, alginates, hydrocolloids, hydrogels, and gauzes) containing antibiofilm agents and accompanying benefits. Antimicrobial agents that contain topical disinfectants, antiseptics, and antibiotics are also used widely with solution and gel forms such as cadexomer iodine, iodine, ionic silver, silver, silver sulfadiazine, PHMB, sodium hypochlorite, methylene blue, gentian violet, and mupirocin. [12]

Biofilm Intervention Model

Schultz et al. [13] introduced the Step-Down Then Step-Up Strategy, which contains a multitude of personalized therapies that are monitored along the protocol continuum, to provide the key essentials to successful wound

healing. The Step-Down Then Step Up Strategy utilizes the following framework:

- Days 1–4
    - Initiate multiple therapies in combination – aggressive debridement, topical antiseptics, systemic antibiotics, management of host factors (offloading, nutrition, diabetes, compression), and DNA identification of microorganisms and point of care diagnostics.
- Days 5–7
    - Optimize and personalize therapy according to healing status – assess inflammation and healing status, perform appropriate debridement, and optimize and personalize topical antiseptics and systemic antibiotics.
- 1–4 weeks
    - De-escalate as wound improves – assess inflammation and healing status, perform maintenance debridement, re-evaluate need for topical antiseptics and systemic antibiotics, continue management of host factors.
- Continue until healed
    - Evaluate wound healing and decide.
- Step up to advanced therapies – advanced therapies consisting of growth factors, skin grafts, and combination products.

CONTROLLING INFECTION

Clinically infected wounds usually require systemic antibiotic therapy. However, there is a worldwide dilemma with antibiotic resistance. Antibiotics are overprescribed as a result of treating and managing an array of wound types. Infection-generating bacteria are developing resistance at a rapid rate, but we must still rely on the most current available agents. Prophylactic antibiotics used without confirmed infection have been linked to delayed healing in all wound etiologies. [4] You can help optimize antibiotic therapy for wounds by 1) prescribing antibiotics only for clinically infected wounds, along with the shortest duration needed to treat the infection; (2) selecting an antibiotic based on clinical and laboratory data; and (3) revising and constraining antibiotic therapy based on response and culture and sensitivity results. Typical cultures can fail to identify specific microorganisms in the biofilm phenotype. DNA sequencing technology has become quite popular and validates the number of bacteria, antibiotic-resistant genes, most common species, and any fungal species found, in a comprehensive report. [17]

CONCLUSION

Further research is needed to clarify risk factors and to identify, treat, guide, and optimize wound practice with infected or chronic non-healing wounds. Health care providers, clinicians, and microbiologists have had ongoing challenges determining precise prevention, treatment, and management of biofilms. We need a better understanding of wound microbiomes, how much of a biofilm can exist before it causes delayed healing, and pinpointing potential biomarkers of complex microorganisms. What we do know is

utilizing a multitude of therapies in conjunction with one another will assist in eradicating biofilm growth and promoting faster healing rates.

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Biofilm Virulence

Wound biofilms not only impede healing but also increase the risk of infection. It is essential that biofilms be addressed and treated in a prompt, consistent manner. Biofilms have been an ongoing challenge due to the majority of resistance bacteria. Research in antibiofilm technology continues to grow, and it is essential to keep up on the most recent evidenced based practice literature for improving patients’ outcomes.

Wound chronicity is costing the healthcare system millions of dollars every year. Microbial communities known as biofilms are generally composed of bacteria, fungi, viruses, proteins, extracellular DNA, biogenic factors, and other types of microorganisms. The organisms are microscopic, but as the biofilm matures it attaches the microbial community to a wet or moist surface as a viscous substance. This viscous substance is referred to an exopolymeric material (EPM). The biofilm then protects the microorganisms from the body's natural immune response, and the prevents antibodies from reaching them. The body attempts to fight biofilm through the inflammatory response but is unsuccessful. Healing tissue is damaged and in return, there is a delayed wound healing. [1, 3]

Biofilms polymicrobial nature and dense exopolymeric material (EPM) matrix paralyzes large antibodies and neutralizes microbicides. Bacteria cells that is encased in expolymeric material (EPM) is much different than the free-living, planktonically bacteria. These bacterial cells have reduced motility and activity. This action is referred to as sessile (non-motile) and increases antimicrobial tolerance. A biofilm can promote anaerobic bacteria growth, synergism between different bacteria, generating MRSA-resistant proteins, producing negative charges of polysaccharides and DNA bind cationic molecules like Ag+, antibiotics, and polyhexamethylene biguanide. [2] Antimicrobial tolerance is increased due to many antibiotic classes targeting only peptidoglycan that is produced in the cell wall (β-lactams), protein (aminoglycoside) synthesis, or DNA replication (quinolones). [4] The transfer of antimicrobial resistant genes carries moving genetic elements. This

transfer can occur between bacteria and or cells from the same or different species. The potential for virulent and infection therefore increases. [5]

Wound exudate amount and consistency can be a useful indicator suspecting a biofilm. There has been a correlation between moisture imbalance, translucent or opaque film above the wound bed, recalcitrance, and local wound infection. [7]

Antibiotics can disturb and eradicate bacteria, but once the antibiotic is suspended, the remaining cells can stir up infection once again cause antibiotic resistance. Antibiotic resistance genes carry mobile genetic elements, such as plasmids. This causes irreversible genotype changes in the bacteria, apart from resistance genes harbored on mobile genetic elements. [5]

On the other end of the spectrum of antibiotic resistance, is antimicrobial tolerance. Bacteria cells that survive antibiotics are known as persister cells. Persister cells block synthesis of peptidoglycan or DNA. The cells then remain sensitive to the antibiotic, and regrowth of the biofilm will occur with a similar susceptibility profile as the original biofilm. Persister cells win and are maintained. [7]

The most virtual step in chronic wound care is to remove necrotic tissue and the microbial bioburden by surgical or sharp debridement. [6] Due to the strong attachment of expolymeric materal (EPM), removing all the underlying biofilm is difficult. The remaining cells attached allow the biofilm an opportunity to regrow, and start the biofilm growth cycle over, increasing the risk of wound infection. [5]

Conclusion

More antibiofilm research is needed to better determine antimicrobial susceptibility patterns, test old and new antibiofilm agents, and to improve current available treatments. We know that biofilms delay healing, but by what mechanism remains to be identified. Sharp debridement of wound biofilm is considered “ gold standard” but is not effective in removing and preventing regrowth of biofilm. QS inhibitors and molecular diagnostic techniques are proving to help increase the ability of treating biofilms, but do not differentiate the biofilm type. Innovations in biofilm eradication-type technology are needed to improve effective and inexpensive antibiofilm treatments.

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Understanding the Wound Infection Continuum

Wound infection is a complex process that can be impacted by a variety of factors, some of which inhibit the ability to heal. The first stage of healing, the inflammatory stage, is particularly susceptible to chronicity. Chronicity can be influenced by many factors, with a common contributor being the presence of infection.[i]The wound infection continuum begins withcontamination and, if left unchecked, will progress to systematic infection.

Patient medical conditions can influence the likelihood of developing infection for a given wound, but there are also other signs that a wound is experiencing colonization or infection. As there exists no single test that can diagnose infection, it is generally up to the clinician to recognize the signs during assessment. If infection is suspected, DNA swabbing and analysis can

provide a greater level of detail about the patient’s microbiome and can often assist with identification of pathogenic bacterial and fungal microorganisms.[ii]This can also help to identify the best treatment method available.

Wound infection development depends upon a variety of microbial and host factors, both of which can change status during any stage of the continuum.

Contamination

Initial wound contamination is near-impossible to detect. Since biofilms are present up to 60% of the time, infection is most frequently caused by bacterial colonization originating from either normal flora or bacteria on one’s body.[iii]When possible, prevention of infection is the optimal goal. Prevention is best achieved by wound cleansing, using a non-cytotoxic wound cleanser or normal saline to reduce debris. This provides the optimal environment for healing.[iv]

Colonization

The differentiation between colonization and infection is challenging to decipher. However, colonization is generally defined as the presence of proliferating or replicating bacteria with no host response.[v]Proliferation does not reach a critical level and there are no evident symptoms, such as inflammation. However, gauging the host response is still challenging.

Critical Colonization

Critical colonization is a relatively new concept added to the wound infection continuum in recent decades and is used to describe the condition in which there is “ multiplication of organisms without invasion but interfering with wound healing.”[vi]In this state, wounds often stagnate, rather than improve in condition, and obvious signs of infection such as fever and inflammation tend to be absent. Despite this, other signs, such as the presence of discoloration and odor, may be observed. The bioburden is elevated beyond colonization to a point in which it impacts the healing process. The critical colonization stage is also the tipping point on the wound infection continuum where treatment becomes necessary as a means to stop the progression to infection.[vii]Effective treatment for critical colonization can generally be achieved through the use of topical antiseptics that control the bioburden so that healing can proceed.[viii]A bioburden level of > 105 bacteria per gram of tissue is the threshold for when critical colonization crosses into infection and at > 106, healing becomes impeded.[ix]

Infection

Like critical colonization, infection is the invasion of proliferating bacteria that is present not only on the surface of the wound, but also in healthy tissue on the periphery of the wound. Infection will cause a host response, but this immunological response will not be sufficient to overcome the bacteria alone.[x]During an active infection, the wound condition becomes degenerative, as opposed to its stagnation during a critical colonization. Signs of inflammation, odor, are discoloration will typically be observed and bleeding becomes more common.[xi]Infection will require antibiotic treatment, either topically or orally.

Antimicrobial vs. Antibiotic Treatment

Antibiotic resistance is becoming an increasingly common problem, and antibiotic regimens should be approached cautiously, as it is often possible to treat wounds in the earlier stages of the infection continuum more effectively with another type of treatment. Wound infection treatment must be addressed by the level of bacteria present in the wound. When wounds have a high bioburden topical antibiotics and antiseptics can decrease the bacterial load.[xii]However, if a wound is clinically infected, a wound culture is the best way to determine the optimal type of antibiotic.[xiii]

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Patient demographics and medical conditions can influence the likelihood of developing infection for a given wound, but there are also other signs that a wound is experiencing colonization or infection. As there exists no single test that can diagnose infection, it is generally up to the clinician to recognize the signs during assessment. While swabbing can be done to determine resistance to treatments, culture results can be misleading since they can fail to detect the presence of biofilm.

Wound infection development depends upon a variety of microbial and host factors, both of which can change status during any stage of the continuum.

Contamination

Initial wound contamination is near-impossible to detect. Since biofilms are present up to 60% of the time, infection is most frequently caused by bacterial colonization originating from either normal flora or bacteria on one’s body. When possible, prevention of infection is the optimal outcome.

During the contamination stage, prevention is best achieved by cleaning the wound with a cleanser or saline spray that reduce the bioburden and clean debris. This provides the optimal environment for healing.

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The differentiation between colonization and infection is challenging to decipher. However, colonization is generally defined as the presence of proliferating or replicating bacteria with no host response. Proliferation does not reach a critical level and there are no evident symptoms, such as inflammation. However, gauging the host response is still challenging. Colonization, unlike critical colonization and infection, does not impede healing and can, under the right conditions and in small amounts, facilitate and accelerate healing.

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Critical colonization is a relatively new concept added to the wound infection continuum in recent decades and is used to describe the condition in which there is “ multiplication of organisms without invasion but interfering with wound healing”. In this state, wounds often stagnate, rather than improve in condition, and obvious signs of infection such as fever and inflammation tend to be absent. Despite this, other signs, such as the presence of discoloration and odor may be observed. The bioburden is elevated beyond colonization to a point in which it impacts the healing process. The critical colonization stage is also the tipping point on the wound infection continuum where treatment becomes necessary as a means to stop the progression to infection. Effective treatment for critical colonization can generally be achieved through the use

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Breaking the Biofilm Cycle

Advancements in molecular microbiology, microscopy technology, and techniques for studies of bacteria has increased the ability to identify the existence of biofilms, but there still remains the unknown. [6] Differentiating between planktonic bacteria versus biofilm bacteria just to name a few. Chronic non-healing wounds harbor bacteria across the wound etiology classification. [7, 8, 9]Malone et al [7] determined the prevalence of biofilms in chronic wounds was 78. 2% (confidence interval, 61. 6-89, $P < .002$). The development of biofilms move through a common pattern: attachment, microcolony formation, maturation, and dispersion. The initial attachment is reversible, but the attachment becomes stronger as cells multiply and change their gene expressions. This cell communication process is referred to as quorum sensing allowing cells to survive.

Clinicians evaluating wounds should be thorough and detailed including the clinical history, any signs and symptoms, and a microscopic culture and tissue to help identify causative microorganisms. [1] Conventional culturing methods lack sensitivity, and studies have proven consistent failure of identifying types of organisms present within biofilm. DNA based technology or molecular methods are better suited that conventional culturing methods in identifying biofilm colonies. [11, 12, 13, 14, 15] Using a multidisciplinary approach using good wound cleansing and established principles of wound care will provide better healing outcomes. Research shows that microorganisms rarely invade healthy tissue unless the wound bed is compromised by drying out. [10]

A multitude of strategies and therapies are most effective in suppressing biofilm activity in a wound. The goal is to target only the biofilm and not the defense and healing mechanisms of the body. Aggressive debridement, topical antiseptics, systemic antibiotics, DNA identification of microorganisms, and management of host factor (offloading, compression, diabetes, nutrition) are all components of a biofilm based wound care approach.

Debridement methods used to aid in biofilm eradication are utilized to prepare the wound bed to move towards healing. Keeping the wound bed clear of devitalized tissue and biofilm is imperative in enhancing wound healing progress. If biofilm colonies contaminate the wound bed, the transition to wound closure becomes complex. [3, 4] Combining debridement methods has been found to be an advantage in managing complex wounds and different pathological tissues since 2006. [9] Developed biofilms harbor physical and metabolic defenses. These defenses enable the biofilm to resist antimicrobials that usually alienate planktonic cells and include resistance to host defenses, biocides, antibiotics, and ultraviolet light. Sequential sharp debridement of wounds disrupts biofilm growth and inhibitory factors and can promote faster healing. It is difficult to predict the outcome because we still do not know the depth needed to remove the entire biofilm colony.[5]

- Biological debridement is the use of maggots, Lucilia sericata (green bottle fly). The flies are grown in a sterile environment and serve to digest dead tissue and pathogens. The sterile maggots are applied to the wound bed with a cover dressing used to “ confine” the maggots to

the wound. There are custom and pre-assembled dressings available, as well as the option to create your own. [3]

- Ultrasound debridementis a focused ultrasonic energy using a curette. The curette gently contacts the wound bed, separating and removing unwanted tissue while preserving healthy granulation tissue. [18] Ultrasound debridement used together with conservative sharp debridement has demonstrated effectiveness in reducing biofilms in vitro datauing semisolid agar or relevant pigskin explant model. [16, 17]
- Enzymatic debridement is performed by the application of a prescribed topical agent that chemically liquefies necrotic tissues with enzymes. These enzymes dissolve and engulfdevitalized tissuewithin the wound matrix. Certain antimicrobial agents used in conjunction with collagenase can decrease the effectiveness of enzymatic debridement. This method can be used in conjunction with surgical and sharp debridement. This method can be expensive depending on the insurance payer source; however, discount programs are available. Enzymatic debridement is commonly used in the long-term care setting because there is less pain and nurses can apply it daily.
- Autolytic debridement is the slowest method, and it is most commonly used in the long-term care setting. There is no pain with this method. This method uses the body’s own enzymes and moisture beneath a dressing, and non-viable tissue becomes liquefied. Maintaining a balance in moisture is important. Dressing frequency and absorbency. Dressing types commonly used are hydrocolloids, hydrogels, and transparent films (semi-occlusive and occlusive).

- Mechanical debridement is by irrigation, hydrotherapy, wet-to-dry dressings, and an abraded technique. This technique is cost-effective, but can damage healthy tissue, and cause painful. Wet-to-dry dressings are frowned on in the long-term care setting by state surveyors because of the options available with advanced wound care dressings. This type of dressing is used to remove drainage and dead tissue from wounds. A wet-to-moist dressing is another option accepted in long-term care. This type of dressing is used to promote moist wound healing and is used to remove drainage and dead tissue from wounds. Deep wounds with undermining and tunneling need to be packed loosely. Without packing, the space may close off to form a pocket and not heal leading to infection or abscess. This type of dressing is to be changed daily, compared with the wet-to-dry dressing, which is changed every 4 to 6 hours.
- Surgical sharp and conservative sharp debridement is performed by a skilled practitioner using surgical instruments such as scalpel, curette, scissors, rongeur, and forceps. This debridement type promoteswound healingby removing biofilm and devitalized tissue. The level of debridement is determined by the level of devitalized tissue removal. Surgical debridement is the most aggressive type of debridement and is performed in a surgical operating room. Sharp and conservative debridement can be performed in a clinic or at the bedside with sterile instruments.
- Topical antibiofilm therapies/products. Impregnated dressings containing antibiofilm agents and accompanying benefits. Dressing categories include: collagens, foams, alginates, hydrocolloids,

hydrogels, and gauzes. Antimicrobial agents that contain topical disinfectants, antiseptics, antibiotics are also used widely with solution and gel forms such as: cadexomer iodine, iodine, ionic silver, silver, silver sulfadiazine, polyhexamethylene biguanide (PHMB), sodium hypochlorite, methylene blue, gentian violet, and mupirocin.

Conclusion

Finding the pieces of the puzzle to biofilms has been ongoing. However, we know more now than a decade ago. Biofilms are known for their considerable defense protection from host immunities and utmost tolerance to antimicrobial agents. There are no normal standard signs and symptoms, or precise method to identify biofilms. Key essentials to preventing, disrupting, and suppressing biofilm regrowth are aggressive debridement, topical antibiofilm strategies, and host factor management strategies.

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