What is a Biofilm?

Biofilms are mostly made up of exopolymers and microorganisms (mixed strains of bacteria, fungi, yeasts, algae, microbes, protozoa, and other cellular debris), and they have been around long enough to show up in fossil records. In the United States, it has been reported that approximately 16 million new biofilm-based infections are diagnosed every year. Biofilm-associated cutaneous diseases include burns, pressure injuries (ulcers), surgical site infections, and diabetic foot ulcers. Their annual incidence is 1.96 million cases in the United States, where they cause an estimated 268,000 deaths and incur an estimated annual direct cost of $18 billion.1,2

Bacteria live in several forms, including planktonic (free-floating) forms and biofilms. Biofilms form when a planktonic bacterium attaches to a surface that is exposed, such as a wound. Biofilm formation can occur in any setting, whether clinical or natural. Depending on environmental conditions, different types of biofilm can form over the surface. A microcolony of primary biofilm colonizers quickly forms once attachment has been made. A key process in biofilm formation and expression is called quorum sensing. Quorum sensing molecules help bacterial colonies mature by stimulating change in specific genes.2,3 Once the colonies have developed into a mature biofilm, it will then release planktonic cells that migrate into the bulk fluid phase to seek a new surface to colonize. Staphylococcus aureus and Pseudomonas aeruginosa are the most commonly associated quorum sensing bacterial species studied.4
The Stages of Biofilm Development

1. **Attachment** – Planktonic (free-floating) bacteria adhere to the biomaterial surface.

2. **Growth** – Cells aggregate, form microcolonies, and excrete extracellular polymeric substances (EPS). The attachment of these cells is irreversible once the microcolonies are bonded.

3. **Maturation** – A biofilm is formed and matures, and the cells form multilayered clusters. Three-dimensional growth and further maturation of the biofilm provide protection against host defense mechanisms and antibiotics.

4. **Detachment** – The biofilm reaches a critical mass and disperses planktonic bacteria, ready to colonize other surfaces.2,3
How to Identify a Biofilm in Wounds

A biofilm is an adherent population of bacteria in a particular area. Many times, biofilms are not seen and are microscopic. There are no signs and symptoms of infection with biofilms. When wound progress becomes stagnant for approximately 2 weeks, one should suspect a biofilm colony. This is a wound that fails to show signs of healing despite an optimal standard of care. Slough may also indicate that a biofilm is involved.2,4 Clinicians should become familiar with identifying bacterial balance, by noting changes in wound tissue and the amount of exudate. Chronic wound infections contribute to prolonged hospitalization and limb loss, and they pose a potential risk of sepsis to patients. As health care providers, we want to prevent and eliminate infection.

Every wound has the potential for infection, but it is important to differentiate between infection and colonization. Most chronic wounds are colonized by polymicrobial aerobic-anaerobic microflora. Typically, chronic wounds are not cultured unless there are active signs and symptoms of infection. Wound care specialists have become somewhat skilled at visually detecting biofilms, but there is no text edition that provides color photographs of every wound with bioburden. Infection may be present if any of the following are present in the wound area, thereby warranting a culture4,5:

- Erythema
- Induration
- Increased pain
- Increased exudate
- Friable, red granulation tissue
- Odor after wound cleansing

Most Common Pathogens Found in Wounds

| • Aeromonas          | • Fusobacterium       |
| • Bacteroides       | • Klebsiella          |
| • Candida           | • Peptostreptococcus  |
| • Clostridium       | • Proteus             |
| • Enterobacter      | • Pseudomonas         |
| • Enterococci       | • Staphylococcus      |
| • Escherichia       | • Streptococcus       |
Biofilm Identification Techniques

**Swab culture:** A swab culture is the most common technique used for identifying biofilms because it is non-invasive and cost-effective. This type of culture will usually identify the bacterial species of the infection and help steer antibiotic therapy. Surface swabs will unveil only the colonizing organism, and they may not reflect deeper tissue infection. An acceptable alternative to quantitative tissue culture is the Levine quantitative swab technique.5

**The Levine Quantitative Swab Technique:**

1. Cleanse the wound with normal saline.

2. Pat the dry wound bed with sterile gauze.

3. Culture the healthiest looking tissue, excluding exudate, and purulent or devitalized tissue.

4. Spin the end of the sterile applicator over a 1cm×1cm area for at least 5 seconds.

5. Apply sufficient pressure to swab, thereby causing tissue fluid to be expressed.

**Deep-tissue biopsy:** A deep-tissue or punch biopsy for a quantitative culture is the gold standard for identifying wound bioburden and diagnosing infection. Biopsies can be invasive, painful, expensive, and are not always available in all settings. Biopsies must be performed by qualified and trained providers, who are not always available. Providers most frequently use a disposable special circular blade punch tool to remove a plug of deeper layers of skin for testing. Depending on the size, sutures may be necessary to close the wound.5

**Needle culture:** Needle aspiration is a less invasive technique to use in wounds such as puncture injuries. This method includes inserting a small 22-gauge needle into the wound. To obtain a sample of the fluid for biopsy, the clinician pulls back on the plunger and then changes the angle of the needle two or three times to remove fluid from different areas of the wound.6

Careful assessment and documentation of the patient and the wound will help clinicians determine when to perform a wound culture for pathogens and the best technique for gaining the culture sample. Protocol may vary depending on health care setting, so be sure to follow your facility guidelines.7
Why Biofilms Can Be a Challenge

Antibiotics are designed to attack bacteria, and they may only partially eliminate the bacteria contained within a biofilm.\(^2,8\) The dense EPS paralyzes large antibodies and neutralizes microbicides. A biofilm can promote anaerobic bacteria growth and synergism among different bacteria, generate MRSA-resistant proteins, produce negative charges of polysaccharides, and DNA-bind cationic molecules such as silver, antibiotics, and polyhexamethylene biguanide. Because biofilms are so difficult to disrupt, clinical studies show that 60%-90% of chronic wounds contain a biofilm, although only 6% of acute wounds contain a biofilm. The wound appears to be healing and then becomes stagnant again.\(^2,8\)

### Stages of Bacterial Growth in Wounds\(^9\)

<table>
<thead>
<tr>
<th>INTERVENTION WARRANTED</th>
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<tbody>
<tr>
<td>Contamination</td>
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<tr>
<td>Planktonic Growth</td>
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*Planktonic bacteria, staphylococci, streptococci, Pseudomonas and Escherichia coli.

Differentiating Common Terms

**BIOFILM** is any group of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of EPS.

**BIOBURDEN** is normally defined as the number of bacteria living on a surface that has not been sterilized. The term is most often used in the context of bioburden testing, also known as microbial limit testing, which is performed on pharmaceutical products and medical products for quality control purposes.

**SLOUGH** is defined as yellow devitalized tissue that can be stringy or thick and adherent to the tissue bed.

**NON-VIABLE TISSUE** may be black (necrotic) or yellow (slough), and if left in the wound, it creates the ideal conditions for bacterial growth and infection.

**DEVITALIZED TISSUE** may include necrotic tissues, foreign debris, and bacteria, which are removed from the wound area. Tissue that is transitioning from viable to devitalized tissue is called slough. Tissue that is completely non-viable is called necrotic tissue.
Ways to Manage a Biofilm

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. Therefore, clinicians must utilize multiple and concurrent strategies to tackle biofilm colonies, as follows2,10,11:

- **Sequential sharp debridement of wounds** can disrupt 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. Surgical instruments such as curettes, scalpels, and/or ultrasound energy can be used.10

- **Avoid application of skin grafts and impregnated dressings** on wounds until the biofilm has been eradicated. Biofilms use foreign materials as a food source.

- **Treating wounds with an antimicrobial or bacteriostatic dressing** (such as those containing silver, cadexomer iodine, or methylene blue) in an alginate or polymeric foam form will help prevent reformation of biofilms.12

- **Systemic antibiotics** are used to destroy biofilm microbes and prevent reseeding of bacteria on the wound surface. However, exposing bacterial biofilms to the wrong antibiotics may cause thicker biofilms. Antibiotic dosing should be adjusted and monitored if another antibiotic has been taken for systemic biofilm-based diseases.10,13

- **Maggot debridement therapy** studies have shown that the excretions and sections of sterile larvae or maggots contain many bioactive compounds that could prevent, remove, and reduce biofilm formation.14

Conclusions

Treatment and management of biofilms are ongoing challenges for clinicians, health care providers, and microbiologists. Further research is needed to clarify risk factors, identify formation, and treat biofilm to support, guide, and optimize wound care clinical practice in infected or non-healing wounds.

Click here to take our quick quiz to test your knowledge on biofilm management in wound care.
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