Biofilm Management
Myths and Reality

Gregory Schultz, Ph.D.
Professor of Obstetrics/Gynecology
Director, Institute for Wound Research
University of Florida
Learning Objectives

• Review the four phases of normal wound healing and understand the important roles that controlled inflammation play in healing

• Understand where biofilms fit into the “bioburden spectrum” of contamination, colonization, critical colonization, and infection

• Explain how chronic inflammation leads to elevated levels of protease and reactive oxygen species (ROS) that impair healing by destroying proteins essential for healing

• Recognize key differences between planktonic and biofilm bacteria in terms of tolerance to antibiotics and antimicrobials

• Describe different effects of debridement techniques and wound dressings on reducing biofilms

• Understand basic principles of Biofilm-Based Wound Care and how to prepare wounds to heal using the concepts and principles of Step-Down then Step-Up Therapy
Sequence of Molecular and Cellular Events in Normal Skin Wound Healing

Four Phases of Healing
1. Hemostasis
2. Inflammation
3. Repair
4. Remodeling

Controlled Inflammation of Acute Wounds Is Beneficial for Healing

Inflammatory cells engulf and kill microorganisms by generating reactive oxygen species (ROS) and release proteases (MMPs, elastase) that remove denatured ECM components and permit wound healing to proceed.
In the membranes of neutrophils, NADPH oxidase (NOX) generates superoxide ($O_2^-$), which spontaneously dismutates to $H_2O_2$, and is converted to hypochlorous acid (HOCl) by myeloperoxidase (MPO). These reactive oxygen species (ROS), especially HOCl, participate in the killing of bacteria. The right panels show a bacteria being phagocytized and production of ROS (red color) surrounding the yeast cell.
Question: What happens when the respiratory burst is impaired?

Answer: Severe impairment of host resistance to infection occurs. Clinical condition - Chronic Granulomatous Disease is due to mutated NAPDH oxidase. Characterized by predisposition to bacterial and fungal infections.

Decreased levels of:
- hydrogen peroxide ($\text{H}_2\text{O}_2$)
- peroxynitrite anion (ONOO$^-$)
- oxyhalides ($\text{HOCl}$ a.k.a. hypochlorous acid)
Controlled MMPs Are Necessary for Wound Healing
Debridement, Angiogenesis, Contraction, Epithelial Migration, Remodeling

MMPs ARE NECESSARY FOR SEVERAL KEY PROCESS IN WOUND HEALING

1. removing denatured matrix
2. degrading capillary basement membrane for angiogenesis
3. contraction of ECM by myofibroblasts
4. migration of epidermal cells
5. remodeling of scar
Profiles of MMP-9 in mastectomy wound fluids (□) and matched sera (♦) during early wound repair in nine patients. 


Levels of total MMP-9 protein in intraperitoneal drainage fluid from 58 patients undergoing elective colorectal surgery.

Is There a Common Molecular Pathology Of Chronic Wounds??

Diabetic foot ulcer

Arterial ulcer

Pressure ulcer

Venous ulcer
Repeated Tissue Injury, Ischemia and Bioburden – Planktonic & Biofilms

↑ TNF-α

Prolonged, elevated inflammation
↑ neutrophils ↑ macrophages ↑ mast cells

↑ IL-1β, IL-6

Imbalanced Proteases & Inhibitors
↑ Proteases (MMPs, elastase, plasmin), ↓ inhibitors (TIMPs, α1PI), ↑ ROS

Destruction of Essential Proteins (off-target)
↓ growth factors / receptors, ↑ ECM degradation
↓ cell proliferation, ↓ cell migration,

Acute Wound ⇒ Chronic Non-Healing Wound

Hypothesis Of Chronic Wound Pathophysiology

Chronic Infections Causes by Medical Biofilms

- Chronic sinusitis
- CNS shunt infection
- Contact lens associated keratitis
- Chronic otitis media
- Cochlear implant infection
- Burn infection
- Catheter infection
- Prosthetic valve endocarditis
- Pacemaker infection
- Biliary stent infection
- Peritoneal dialysis catheter infection

18 Clinical Pathologies

Biofilms Identified in >80% of Biopsies of Chronic Wounds but in Only 6% of Acute Wounds

How Does the Immunological Response to Biofilms Cause Tissue Damage and Impair Healing?

In panel A, planktonic bacteria can be cleared by antibodies, phagocytosis, and are susceptible to antibiotics. Adherent bacterial cells (panel B) form biofilms preferentially on inert surfaces or devitalized tissue, and these sessile communities are resistant to antibodies, phagocytosis, and antibiotics. Neutrophils (panel C) are attracted to the biofilms, but cannot engulf and kill biofilm. Neutrophils still release proteases and reactive oxygen species. Phagocytic enzymes (panel D) damage tissue around the biofilm, and planktonic bacteria are released from the biofilm, causing dissemination and acute infection in neighboring tissue.

Chronic Venous Ulcers Have High Levels Of IL-1β and TNFα That Decrease With Healing

Trengove, Stacey, Macauley, Bennett, Gibson, Burslem, Murphey, Schultz. Wound Rep Reg 7:442-452, 1999
High Levels of MMP Activity in Chronic Wounds Decrease as Wounds Heal

MMP-9 Activity Correlates With Wound Healing Time Course

For some patients, protease activities are not the only problem for healing. Basic comorbidities are not corrected – glucose control, off loading, ischemia. Debridement not adequate.

D. Gibson and G. Schultz, submitted
PDGF-AA Immunostaining is Low in Chronic Wounds and Increases in Healing Chronic Wounds

A. normal skin

B. healing acute wound

C. chronic wound

D. healing chronic wound

Pierce et al, J Clin Invest 96, 1336-50, 1995
Degradation of Fibronectin in Base of Chronic Venous Ulcers Reverses With Initiation of Healing

Summary: fibronectin is absent (degraded) in base of chronic venous ulcer, but fibronectin reappears (stable) as ulcer heals.

Conclusion: Inflammation in chronic wounds must be reduced to levels that lead to low protease and ROS that allow wounds to heal.

Action: Bacterial levels (both planktonic and biofilm) must be reduced for healing.

--- how to OPTIMALY do that?
Early intervention with multiple mechanical and effective antibiofilm antiseptics is key then step-up to advanced therapies if needed to complete healing.

Initiate multiple therapies in combination

Aggressive debridement
Empirical topical antiseptics and systemic antibiotics
Manage host factors (off-loading, compression, diabetes, nutrition)
DNA identification of micro-organisms and point-of-care diagnostics

~days 1–4

Optimize & personalize therapy according to healing status

Assess inflammation and healing status
Appropriate debridement
Optimize /personalize topical antiseptics and systemic antibiotics
Continue management of host factors

~days 5–7

De-escalate treatment as wound improves

Assess inflammation and healing status
Maintenance debridement
Re-evaluate need for topical antiseptics and systemic antibiotics
Continue management of host factors

~1–4 weeks

Evaluate wound healing and decide

Step up to advanced therapies

Advanced therapies:
- Growth factors
- Skin grafts
- Combination products
- Negative pressure wound therapy

Standard care

Continue until healed

Principles of Biofilm Based Wound Care

1. **Frequent debridement** of wounds to physically remove biofilm communities

2. Use of an **effective bacterial barrier dressing** after debridement to prevent reformation of biofilms

3. **DNA based identification of bacterial species in wounds** – personalized topical antimicrobials

4. **Alter topical & systemic antimicrobial treatments** to prevent emergence of dominant bacteria from polymicrobial populations


Biofilm Based Wound Care

A combination strategy for managing biofilms

1. Biofilm removal
   - Frequent aggressive debridement

2. Prevention of biofilm reconstitution
   - Antibiotics
   - Biocides
   - Antibacterial agents

Question: Why is debridement of chronic wounds so important for “biofilm based wound care?”

Answer: Because a significant population of bacteria in biofilms develop very high tolerance to antibiotics and antiseptics that normally kill planktonic bacteria very effectively

Healing of Diabetic Foot Ulcers Increases with Frequency of Debridement

**Figure 4.** The incidence of complete healing increases with frequency of debridement in patients receiving rhPDGF-BB or placebo gel. When the frequencies of debridement are equal, the incidence of complete healing is approximately 2 to 3 times as high in patients receiving REGRANEX Gel compared with that of patients receiving placebo gel. Adapted from Steed et al.42

Steed et al., J Am Col Surg, 183: 61, 1996c
Question: Why are bacteria in biofilms hard to kill?

Answer:

- **Exopolymeric material (EPM) of the biofilm**
  - Dense matrix impairs diffusion of large antibodies
  - EPM materials chemically react (neutralize) microbicides
  - Negative charges of polysaccharides and DNA bind cationic molecules like Ag⁺, antibiotics, PHMB⁺

- **Metabolically dormant bacteria**
  - Antibiotics only kill metabolically active bacteria

- **Oxygen diffusion to center of biofilm is limited**
  - Promotes growth of anaerobic bacteria

- **Synergism between different bacteria**
  - MRSA secrete resistance proteins
  - Pseudomonas secrete catalase that destroys H₂O₂

After 60 minutes of exposure to dilute bleach (Dakin’s solution), many bacteria in this biofilm were dying (green cells), but many cells in the interior of the biofilm were still alive (orange cells) Costerton, Sci Am, 2001.
Biofilms are Highly Tolerant to Antibiotics

Tobramycin rapidly kills planktonic *Pseudomonas aeruginosa* (●) very effectively, but is not effective against biofilm (○).

Metabolic Activity of Pseudomonas aeruginosa in Mature Biofilms is Limited to the Surface Layers

-- Only fluorescent bacteria are metabolically active
-- Only located in outer layers of the biofilm matrix
-- Antibiotics only kill metabolically active bacteria

Can you see a biofilm in this wound?

Photo provided by Dr Matthew Malone
What Are These Shiny “Sloughy” Substances on Wound Beds?
Distribution of Bacterial Species in Wound Beds


Images from Prof Bjarnsholt with permission
Wound Slough Harbors Bacterial Biofilms

PROVIDED BY
Dr Randy Wolcott
How Can We Visualize Biofilm on a Wound?

- Ruthenium red staining specific to bacterial biofilm component (EPS: mucopolysaccharides).

- Easy to recognize under visible light (No equipment needed)

- Planktonic bacterial inoculated onto skin does not stain

- 1 day growth of Pseudomonas aeruginosa has minimal staining

Flow Of Biofilm Detection In A Clinical Setting

Wound cleansing

Wound blotting: attaching the nitrocellulose membrane for 10 seconds

Ruthenium red staining for 1 minute

Bedside noninvasive and real-time biofilm detection system

Differences in percent reduction in necrotic area between biofilm-negative and positive pressure ulcers after sharp debridement.

Biofilm Bacteria Are Present In Multiple Locations


Question:
What effects do different debridement techniques have on removing and killing biofilms on dermal explants?

- Larval debridement
- Non-contact ultrasonic debridement
- Negative pressure wound therapy with instillation
- Concentrated surfactant gel
Larval Debridement Therapy Effectively Removes Biofilms

Before treatment

After 24hr treatment

Effects of Non-Contact Ultrasonic Wound Cleansing on Biofilms

Schultz et al submitted
NPWT with instillation therapy combines the benefits of vacuum therapy with automated solution instillation and removal which can help:

- **Cleanse** the wound with instillation of topical wound cleansers in a consistent, controlled manner

- **Treat** the wound with the instillation of appropriate topical antimicrobial and antiseptic solutions and the removal of infectious material

- **Heal** the wound and prepare for primary or secondary closure

NPWT with Instillation Therapy

cycle repeats for duration of therapy
Effects of 6-Cycles of NPWT-Instill Treatments Over 24 Hours on *P. aeruginosa* Biofilm Grown on Pig Skin Explants

![Graph showing effects of NPWT-Instill treatments over 24 hours on *P. aeruginosa* biofilm.](image)

* P-Value <0.005 compared to saline control

Non-Ionic Concentrated Surfactant Gel Removes Degraded ECM

Damaged ECM and Proteins

Surfactant

Binds All That it Can

Necrotic Emulsion Carried Away

H₂O  H₂O  H₂O  H₂O  H₂O

Healthy ECM Left Behind
Concentrated Surfactant Gel Eliminated Bacterial Biofilms Grown on Porcine Skin Explants After Daily Treatments for 3 Days

Effect of Daily Wiping + Concentrated Surfactant Gel on PA Bacteria Biofilms

Question: How quickly can planktonic bacteria form protective biofilms in wounds after debridement?

Which answer is true?

1. 7 days
2. 5 days
3. 3 days
4. 1 day
Biofilm Maturity Studies Indicate Sharp Debridement Opens a Time-Dependent Therapeutic Window

Biopsies from three patients with large (>10 cm²) venous ulcer were split into two tubes containing saline (control) or saline with 200 μg/ml gentamicin (treatment), and after 24 hours of incubation, samples were disperse biofilm into microcolonies and CFU/5 gm were measured. Total levels of bacteria at 0, 1, 2, and 3 days after initial debridement remained consistently high. However, in two of the three wounds, all bacterial were “planktonic” at 1 and 2 days after debridement (full kill by exposure to gentamicin), but by 3 days post-debridement, all three wounds had re-established substantial levels of biofilm bacteria (10³ – 10⁵ CFU/5 gm).

Question: Can all antimicrobical wound dressings effectively kill biofilm colonies grown on pig skin explants?

Answer: YES or NO
Can Dressings Disrupt & Kill Mature Biofilms?

24-Hour Continuous Exposure of Mature PAO1 Biofilm on Porcine Explants

Reduction In Biofilm And Planktonic Bacterial Counts in DFUs Compared To Baseline With IODOSORB and Hydrogel

Step-Down Then Step-Up Treatment Strategy for Biofilm-Based Wound Care

Early intervention with multiple mechanical and effective antibiofilm antiseptics is key then step-up to advanced therapies if needed to complete healing.

- **Initiate multiple therapies in combination**
  - Aggressive debridement
  - Empirical topical antiseptics and systemic antibiotics
  - Manage host factors (off-loading, compression, diabetes, nutrition)
  - DNA identification of micro-organisms and point-of-care diagnostics
  - 

- **Assess inflammation and healing status**
  - Appropriate debridement
  - Optimize / personalize topical antiseptics and systemic antibiotics
  - Continue management of host factors
  - ~days 1–4

- **Optimize & personalize therapy according to healing status**
  - 

- **De-escalate treatment as wound improves**
  - Assess inflammation and healing status
  - Maintenance debridement
  - Re-evaluate need for topical antiseptics and systemic antibiotics
  - Continue management of host factors
  - ~days 5–7

- **Evaluate wound healing and decide**
  - Continue management of host factors
  - ~1–4 weeks

- **Step up to advanced therapies**
  - Advanced therapies:
    - Growth factors
    - Skin grafts
    - Combination products
    - Negative pressure wound therapy

- **Continue until healed**
  - Standard care
  - Standard care

Free download from Wounds International

Introduction
This article discusses that MMPs are and the importance of their role in normal and disrupted wound healing. In particular, it discusses the relevance of MMPs to clinical practice, including current and potential interventions aimed at modulating their activity.

What are MMPs?
The matrix metalloproteinase (MMP) pro-dollars in the larger family of metalloproteinases that play an important role in wound healing.

MMPs are a large class of enzymes that act to degrade extracellular matrix (ECM) proteins. They are found in tissues throughout the body and are involved in a wide range of physiological processes, including wound healing. MMPs are produced by a variety of cell types, including neutrophils, macrophages, and fibroblasts. They are also expressed by a variety of other cell types, including epithelial cells, chondrocytes, and osteoblasts.

MMPs are involved in the degradation of ECM proteins, such as collagens, elastin, and proteoglycans. This process is critical for wound healing, as it allows the wound edges to blend together and the ECM to be remodeled. MMPs also play a role in the resolution of inflammation, as they can degrade the matrix that is responsible for maintaining the inflammatory response.

How are MMPs produced?
In normal wound healing, MMPs are produced from
- activated inflammatory cells (neutrophils and macrophages)
- wound cells (epithelial cells, fibroblasts, and vascular endothelial cells).

What is the method that MMPs are in a tissue reaction or

purified form. They are activated by other proteins that

co-activate the latent form of the molecules. This opens up the

active enzyme in the MMP molecule and allows the MMP to

bind to its substrate. Other members of the group are involved

in the production of other factors that modulate the

inflammatory response.

MMPs are classified based on their size and function. Some MMPs are involved in the degradation of collagen and elastin, while others are involved in the degradation of other ECM proteins.

MMPs are also involved in the resolution of inflammation. They can degrade the matrix that is responsible for maintaining the inflammatory response, allowing the wound edges to blend together and the ECM to be remodeled.


D. Gibson, B. Cullen, R. Legerstee, K.G. Harding, G. Schultz. MMPs
Biofilms are present in a high percentage of chronic wounds. Biofilms impair healing by stimulating chronic inflammation, leading to elevated levels of proteases and ROS that degrade proteins that are essential for healing.

Step-down-step-up (SD-SU) therapy is based on starting with the therapies that most effectively reduce biofilms, inflammation, and proteases, (Step-Down)

Then shifting to advanced therapies (Step-UP) that enhance repair of the wound bed (growth factors, collagen dressings, biological membranes, and NPWT).