Battling Biofilms
Winning the War in Pressure Injuries

Gregory Schultz, PhD
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The National Pressure Ulcer Advisory Panel (NPUAP) serves as the authoritative voice for improved patient outcomes in pressure injury prevention and treatment through public policy, education and research.

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NPUAP Monograph

Released in November 2012, the 254-page, 24 chapter monograph, Pressure Ulcers: Prevalence, Incidence and Implications for the Future was authored by 27 experts from NPUAP and invited authorities and edited by NPUAP Alumna Dr. Barbara Pieper.

The monograph focuses on pressure ulcer rates from all clinical settings and populations; rates in special populations; a review of pressure ulcer prevention programs; and a discussion of the state of pressure ulcers in America over the last decade.

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DISCLOSURES

• Dr. Schultz has research grant funding during the last year from Acelity, Smith & Nephew, Medline, Biomonde, and CorMedix

• Dr. Schultz has given educational lectures in the last year sponsored by Acelity, Smith & Nephew, Medline, and Organogenesis

• Dr. Schultz is a scientific consultant for Acelity, Smith & Nephew, Medline, Medskin Solutions, QuickMed Technologies, AbbVie

• Dr. Schultz is an inventor of BIOGUARD® dressing, PROFIND® protease detector, Herpezyme® anti-herpes drug, and EXC-ASO anti-scarring drug

• Dr. Schultz has significant financial interests in QuickMed Technologies and Biomonde
Overview of Topics

- Review the four sequential phases of normal wound healing and recognize the BENEFICIAL effects of CONTROLLED INFLAMMATION and PROTEASE ACTIVITIES.
- Understand the link between CHRONIC INFLAMMATION caused by PLANKTONIC and BIOFILM BACTERIA and ELEVATED PROTEASE ACTIVITIES that DESTROY proteins that are essential to healing (extracellular matrix, growth factors, receptors).
- Recognize the high TOLERANCE of BIOFILM bacteria to most antibiotics, antiseptics and disinfectants.
- BIOFILM BASED WOUND CARE emphasizes DEBRIDING BIOFILMS and PREVENTING REFORMATION OF BIOFILMS by using effective antimicrobial treatments.

Sequence of Molecular and Cellular Events in Skin Wound Healing

1. Clotting
2. Vascular Response
3. Inflammation
4. Scar Formation
5. Epithelial Healing
6. Contraction
7. Scar Remodeling
Inflammatory cells kill planktonic bacteria by phagocytosis and reactive oxygen species. They also release proteases (MMPs, elastase) that remove denatured ECM components and permit wound healing to proceed. Inflammatory cells are not effective against bacteria in biofilms.

**Controlled Wound Inflammation Is Beneficial**

**Is There a Common Molecular Pathology Of Chronic Wounds??**

- Diabetic foot ulcer
- Arterial ulcer
- Pressure injury
- Venous ulcer
Basic Background of Bacterial Biofilms

- **Planktonic bacteria** – single, non-attached bacteria
- **Biofilm bacteria** – a structured community of bacteria cells enclosed in a self-produced exopolymeric matrix this is tightly adherent (sessile) to living or inert surface
- **Quorum sensing** – process by which bacteria molecules shift growth from planktonic to biofilm phenotypes
- **Exopolymeric matrix** of biofilms consists of predominately of polysaccharides along with bacterial DNA and proteins that are extremely inflammatory to innate and acquired immune systems
- **Biofilms provide a protected mode of growth** – evolutionary defense against natural predators: bacterial viruses, amoeba, and microbicides. Also protects against phagocytosis (inflammatory cells), antibodies, natural reactive oxygen species (ROS), antibiotics, antiseptics, and disinfectants
- **Persister bacteria** are quiescent (not metabolically active) and are not killed by antibiotics that only kill metabolically active bacteria – provides tolerance to antibiotics
Question: How common are bacterial biofilms in chronic skin wounds?

Answer: Latest data indicate approximately 80% of chronic skin wounds have mature bacterial biofilms.
Biofilms Identified in >80% of Biopsies of Chronic Wounds\textsuperscript{1} but in Only 6% of Acute Wounds\textsuperscript{2}

\textsuperscript{1}M. Malone et al., Prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data, submitted.

Question: Does formation of biofilm colonies in a wound retard healing?

Answer: YES or NO
Biofilm Formation by Staphylococcal Species Delays Healing of Mouse Cutaneous Wounds

Schierle et al., Wound Rep Reg 17:354, 2009

Question: How do biofilms impair healing of skin wounds?

Answer: Biofilms stimulate chronic inflammation that leads to highly increased levels of proteases and reactive oxygen species that degrade proteins that are essential for healing.
Hypothesis Of Chronic Wound Pathophysiology

Repeated Tissue Injury, Ischemia and **Bacteria – Planktonic & Biofilms**

- ↑ TNF-α
- ↑ IL-1β, IL-6

**Prolonged, elevated inflammation**

- ↑ neutrophils
- ↑ macrophages
- ↑ mast cells

**Imbalanced Proteases & Inhibitors, Reactive Oxygen Species**

- ↑ Proteases (MMPs, elastase, plasmin)
- ↓ inhibitors (TIMPs, α1PI)
- ↑ ROS

**Destruction of Essential Proteins (off-target)**

- ↓ growth factors / receptors
- ↓ functional extracellular matrix
- ↓ cell proliferation
- ↓ cell migration

**Acute Wound ⇒ Chronic Non-Healing Wound**


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 tolerant to antibodies, phagocytosis and antibiotics. Neutrophils (Panel C) are attracted to the biofilms, but cannot engulf 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. Costerton, Stewart, Greenberg, Science 284, 1999
High Levels of MMP Activity in Chronic Wounds Decrease as Wounds Heal

Trengove, Stacey, Macauley, Bennett, Gibson, Burslem, Murphy, Schultz. Wound Rep Reg 7:442-452, 1999

Low Protease Activity in Chronic Wound Fluids of Pressure Injuries Predicts the Rate and Extent of Healing

Ladwig, Robson, Liu, Kuhn, Muir, Schultz. Ratios of activated matrix metalloproteinase-9 to tissue inhibitor of matrix metalloproteinase-1 in wound fluids are inversely correlated with healing of pressure ulcers. Wound Repair Reg 10:26-37, 2002
MMP-9 Activity Correlates With Wound Healing Time Course

Patient C05 - MMPs Rise and the Wound Stalled, MMPs Decrease and Wound Begins to Close

D Gibson, G Schultz, unpublished data

Fibronectin is Degraded by Chronic Wound Fluids
Fibronectin is Absent in the Base of Chronic Venous Ulcers
Fibronectin Reappears in Ulcer Base During Healing

Fibronectin profile in plasma shows a single intact band at 250 kDa. In contrast, fibronectin is degraded to lower molecular weight fragments in venous stasis ulcers and in diabetic ulcers.
Wysocki and Grinnell. Lab Invest 63:825, 1990

Fibronectin plays a key role in epidermal cell migration. It is degraded by proteases in chronic ulcers, but is stable when inflammation and protease levels decrease, allowing epithelial cell migration. Herrick, Sloan, McGurk, Freak, McCollum and Ferguson. Am J Pathol 141, 1992.
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⁺
- **Persistor bacteria** have low metabolic activity
  - 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
Hypochlorous Acid Very Slowly Penetrates Biofilm Matrix – Reaction-Diffusion Problem

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

Reaction-Diffusion Problem

Biofilms are Highly Tolerant to Antibiotics

Tobramycin rapidly kills planktonic Pseudomonas aeruginosa (●) very effectively, but is not effective against biofilm (○). Walters et al, Contributions of antibiotic penetration oxygen limitation metabolic activity to antibiotic tolerance of P aeruginosa. Antimicrob Agents Chemother 47:317-323, 2003
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


Biofilm Based Wound Care
Biofilm-Based Wound Care Algorithm for Wounds

Evaluate barriers to healing:
- Prior infection
- Ischemia
- Renal failure
- Repetitive trauma
- Pressure
- Diabetes
- Protein/nutrient deficiency
- Corticosteroids
- Other

Initial evaluation

Presentation of patient with chronic wound

Unity of wounds

All wounds are arranged the same.

Determining wound etiology helps direct infection for better outcomes

Increased drainage, wet, dark slough

Dry, necrotic, non-viable slough

Self-sustaining wound

If the wound has...

Effective Debridement

Aggressive manage surface (sharp debridement)

Remove all necrotic tissue (sharp debridement)

Gently manage surface (ultrasound debridement)

Healed

Effective Antimicrobial Dressings & Topical Treatments

continued drainage and slough

continued necrosis

Black attachment (taurocholic acid, EDTA)

Question: Can you see biofilms on the surface of wound beds?

Answer: YES or NO

Best answer is that some biofilm may be visible on the surface of a wound bed, but much of the biofilm is deeper in the wound bed and is very inflammatory!
What Are These Shiny Substances on Wound Beds?

Question: What is this filmy slough on the wound?

Answer: Mainly fibrin - surrogate biomarker for inflammation

Dr Randy Wolcott
Gauze Debridement of Biofilm Bacteria on Pig Skin Explants

Effect of Wiping Only on Total and Biofilm Bacteria
**Effect of Daily Wiping with a Surfactant Gel on Total and Biofilm Bacteria**

![Graph showing the effect of daily wiping on total and biofilm bacteria](image)

**Question:** What effect does non-contact ultrasonic cleansing have on mature biofilms on pig skin explants

**Answer:** Noncontact ultrasonic cleansing alone has minimal effects on mature biofilms (*P. aeruginosa*)

Combining ultrasonic cleansing with selected microbicidal fluids significantly reduces levels of bacteria in mature biofilms

*(Schultz, unpublished data)*
Effects of Non-Contact Ultrasonic Wound Cleansing on Biofilms

- silver solution
- iodine solution
- bleach solution

Larval Debridement Therapy

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 ug/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 (10⁴ – 10⁵ CFU/5 gm).

**Question:** What effects do microbicidal wound dressings have on mature biofilms grown on pig skin explants?

**Answer:** Most microbicidal wound dressings can reduce mature biofilms by 1-log (90%) to 2-logs (99%) except cadexomer iodine dressing that eliminated 7-logs (99.99999%) of biofilm bacteria.

**Can Dressings Disrupt & Kill Mature Biofilms?**

**24 hr Continuous Exposure of Mature PAO1 Biofilm on Porcine Explants**

Question: What effect does NPWT alone or combined with instillation of antimicrobial solutions have on killing biofilm colonies grown on pig skin explants?

Answer: It depends on the instillation solution used.

![Graph showing the effect of different dressings on CFU/ml over time.](image-url)
NPWT with Instillation Therapy

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

NP port
Instill
Check for leaks, NP pressure, Instillation, Saturation of 'wound' and foam before NPWT and final collection canister volume to verify completion of 6 cycles.

Fix center 8mm biopsies cut from under both NP and instill ports for SEM analysis; Trump fixative

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

*P*-Value <0.005 compared to saline control


Step-Down Treatment Strategy for Chronic Wounds
Summary

What we **KNOW** about Biofilms in chronic wounds:

1. Biofilms are present in a high percentage of chronic wounds and they **impair healing** by stimulating chronic inflammation, leading to elevated levels of **proteases and ROS** that degrade proteins that are essential for healing.

2. Biofilms are communities of bacteria encased in a self-produced matrix of polysaccharides, protein and DNA that provides high levels of **tolerance** to antibodies, antibiotics and antiseptics.

3. Topical dressings can reduce biofilm CFUs ~1 to 2 logs except sustained release **cadexomer iodine** dressings - kills biofilm.

4. **NPWT + Instillation** of some wound cleansing solutions **significantly** reduced **CFUs of P. aeruginosa** biofilms compared to NPWT control.

5. **Biofilm Based Wound Care and Step-down Therapy** are part of **Wound Bed Preparation (TIME)**.

Summary

What we **DON’T KNOW** about Biofilms in chronic wounds:

1. Can skin wounds heal with **some low level** of biofilms?

2. How can we quickly identify biofilms on and below chronic wound beds?

3. What is the **optimal method(s)** to debride biofilms in chronic wounds?

4. What is the **optimal method(s)** to kill biofilms in chronic wounds?

5. Are there very promising **new approaches** to kill biofilms and not kill wound cells?
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