What’s in that wound bed? Slough, Eschar, or Biofilm?

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Disclaimers

• Speaker does not endorse any one particular company’s products, is not employed by industry, has no financial interest in the listed commercial companies.

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Participants will describe:

• Key characteristics of chronic non-healing wounds
• Impediments to wound healing
• Characteristics of slough, eschar, and biofilm in open wounds
• Evidence-based approaches to address or remove slough, eschar, and biofilm from open wounds
• Potential antibiofilm treatment strategies
Chronic Wounds vs. Acute Wounds

• All chronic wounds begin as acute wounds
• Common chronic wounds
  – Venous ulcers of the lower extremities
  – Diabetic foot ulcers
  – Pressure ulcers
  – Complex trauma and surgical wounds

Key characteristics of chronic wounds

• Imbalanced at microcellular level
• Stuck in inflammatory phase\(^1\-^4\)
  – High MMPs / Low TIMPs (inverse correlation)
  – High inflammatory cytokines
  – Low growth factors
  – Fibroblast inhibition\(^5\)
• Does not follow expected pathway to healing (less than 50% improvement in 4 weeks)\(^6\)
Intrinsic impediments to wound healing

- **Physiological, potentially modifiable:**
  - Nutrition
  - Blood sugar control
  - Immune compromise (HIV, Sickle Cell)
    - Improved management of certain conditions
  - Pain, psychological stress
  - Edema – e.g. lymphedema
  - Tissue viability, perfusion & oxygenation

- **Physiological, not modifiable:**
  - Advanced age
  - Certain comorbid conditions (CVA, SCI, neurodegenerative, cancer, etc.)
Extrinsic impediments to wound healing - potentially modifiable

- **Medications**
  - chemotherapy, steroids, anticoagulants

- **Persistent or repetitive trauma**
  - immobility - failure to off-load, inappropriate shoes or mobility devices, wet-to-dry dressings

- **Exposure**
  - Smoking/nicotine, alcoholism
  - Environmental, toxic chemicals, hygiene (personal & environmental), parasites, pets, etc.

- **Physical barriers**
  - Rolled wound edges, non-viable tissue (slough, fibrin, eschar)

- **Invasion** – virulent pathogens (biofilm)

T-I-M-E-(s) Principle for WBP

- **T** - remove non-viable Tissue in wound
- **I** - address Infection (prevent, treat, remove problematic organisms/biofilm)
- **M** – manage Moisture
- **E** – address wound Edges
- **S** – address Surrounding Skin
Documenting wound assessments

- Location
- Suspected etiology, contributing factors
- Size (W X L X D in cm)
- Undermining, tunneling (clock method)
- Exudate (color, amount, odor)
- Wound bed tissue (color, amount viable)
- Wound edges and surrounding tissue
- Last treatments used, compliance, wound response, patient/CG education

Describing wound tissue

- Color of wound bed (in percentages)
- Viable (living tissue with good perfusion)
- Non-viable (dead/dying host tissue)
- Boggy (wet spongy consistency)
- Fluctuant (moving in waves, movable & compressible, variable/unstable)
- Friable (bleeds easily with light touch)
- Hypergranulating (overgrowing baseline)
- Pale (anemic looking)
Characteristics of slough in wounds

• **What it is**
  - Non-viable host tissue (or “avascular fat”)
  - *Typically* it is moist, white, yellow, grey, or tan dead tissue; loose or adherent; includes white blood cells, fibrin, and other proteins
  - May have “chicken fat” appearance

• **What it is not**
  - Alive – slough by itself is not living tissue
    - Slough will not “grow” on dressings
  - Biofilm - may have bacteria/biofilm on it
  - ?Blood clot, dried exudate, softened scab?

Fibrin

• **Fibringen** is a glycoprotein in vertebrates that helps in formation of blood clots.

• **Fibrin** is an insoluble, non-globular protein formed from fibrinogen during the clotting of blood. It is formed by the action of the protease thrombin (clotting enzyme) on fibrinogen which causes it to polymerize.

• The polymerized **fibrin**, together with platelets form a hemostatic plug or clot over a wound site.

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Slough

- Best ways to remove

Characteristics of eschar in wounds

- What it is
  - Dry, dead host tissue

- What it is not
  - Scab / crust (dried exudate)
  - Dry Gangrene (condition where tissue dies caused by ischemia due to underlying illness, injury, and/or infection). Fingers, toes, & limbs most often affected.

- When not to remove
  - If providing reliable protective barrier
Eschar

• When to remove
  – Integrity is compromised (no longer acting as body “bandaid”)  
  – Impediment to healing

• Best ways to remove

Biofilm – What is it?

• “Any group of microorganisms in which cells stick to each other and often these cells adhere to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).” Wikipedia

• “Van Leeuwenhoek, using his simple microscopes, first observed microorganisms on tooth surfaces and can be credited with the discovery of microbial biofilms.” Rodney Donlan (2002). Biofilms: Microbial Life on Surfaces. Emerging Infectious Diseases, 8(9), 881-890.
Biofilm – what it is not

• Not the same as surface or “free-floating” (planktonic) bacteria
• Not typically identified using traditional culture swab techniques (identifies mostly planktonic bacteria)
• Not easy to eradicate!
  – Exhibits increased tolerance to antimicrobial, immunological & chemical attack compared to planktonic bacteria

Biofilms in >80% of Biopsies of Chronic Wounds
Versus 6% of Acute Wounds

Very likely more prevalent in chronic wounds than we think!\(^7\)

Characteristics of biofilm in open wounds

- Mostly* unable to see it with naked eye
- Polymicrobial
  - Aerobic + non-aerobic bacteria
  - gram pos + gram neg
  - Fungus + virus
- Hydrophilic polymeric protective coating
- Quorum sensing
- Attached 2mm below wound bed surface
- Grows back in 48 hours


Distribution of aerotolerance of bacterial populations in chronic wounds

Figure 1
Distribution of Bacterial Populations in Chronic Wounds in Relation to Aerotolerance. Diabetic, venous, or pressure ulcer types were analyzed separately using pyrosequencing and the resulting populations grouped into 3 categories based upon their suggested aerotolerance. This figure graphically illustrates the relative distribution of these functional categories among the wound types.

**Why are bacteria in biofilms so difficult to kill?**

1. **Extracellular polymeric substance (EPS) of biofilm**
   - Dense matrix impairs diffusion of large antibodies
   - EPS materials chemically react (neutralize) microbicides
   - Negative charges of polysaccharides and DNA bind cationic molecules like Ag\(^+\), antibiotics, PHMB\(^+\)

2. **Persister bacteria have low metabolic activity**
   - Antibiotics only kill metabolically active bacteria

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

4. **Synergism between different bacteria**
   - MRSA secrete resistance proteins
   - Pseudomonas secrete catalase that destroys H\(_2\)O\(_2\)

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**Biofilm**

- **When to remove**
  - When biofilm presence has negative consequences
  - When interferes with wound healing
  - When especially virulent (β hemolytic streptococci)
  - To prevent re-growth

- **Best ways to remove**
  - DEBRIDEMENT
  - Total kill

Slide material: Courtesy of G. Schultz, PhD
Evidence for debridement methods

- **Sharp**
  - Scalpel, scissors, curette

- **Enzymatic (collagenase)**
  - Pros (gentle) Cons (slow)

- **Autolytic (exudate/MMPs)**
  - Pros (gentle) Cons (slow)

- **Ultrasonic (low and high frequency)**
  - With and without forced water

- **Mechanical (debriding gauze, wet-to-dry)**
  - Surfactants (w/wo mechanical wiping)

- **Larval/biological – medicinal maggots**

**Ultrasonic debridement: PA biofilm**

3 day old PA biofilm 3 day old PA biofilm *after* low frequency non-contact, non-thermal ultrasound
Gauze debridement of biofilm bacteria on pig skin explants

Effect of wiping only: total and biofilm PA bacteria

Yang Q, Larose C, Porta AD, Della Porta AC, Schultz GS, Gibson DJ. A surfactant-based wound dressing can reduce bacterial biofilms in a porcine skin explant model. Int Wound J 2016; Slide material courtesy of G. Schultz, PhD
Yang Q, Larose C, Porta AD, Della Porta AC, Schultz GS, Gibson DJ. A surfactant-based wound dressing can reduce bacterial biofilms in a porcine skin explant model. Int Wound J 2016; Slide material courtesy of G. Schultz, PhD.
### Wiping only time course: A. baumannii

**Viable Bacteria (CFU)**

- Total Bacteria
- Biofilm Bacteria

**Day 1**
- Before Wiping: 1.0E+08
- After Wiping: 1.0E+07

**Day 2**
- Before Wiping: 1.0E+06
- After Wiping: 1.0E+05

**Day 3**
- Before Wiping: 1.0E+04
- After Wiping: 1.0E+03

**Slide material courtesy of G. Schultz, PhD**

### Wiping + surfactant gel: A. baumannii

**Viable Bacteria (CFU)**

- Total Bacteria
- Biofilm Bacteria

**Day 1**
- Before Wiping: 1.0E+08
- After Wiping: 1.0E+07

**Day 2**
- Before Wiping: 1.0E+06
- After Wiping: 1.0E+05

**Day 3**
- Before Wiping: 1.0E+04
- After Wiping: 1.0E+03

- **Not impressive**

**Slide material courtesy of G. Schultz, PhD**
**Total *Acinetobacter baumannii* treated with Surfactant gel + antibiotics made daily**

![Graph showing the effect of different treatments on *Acinetobacter baumannii* colony forming units over time.]

**LDT Evidence?**

- **Gray, M. (January 01, 2008) Systematic Review**¹⁴
  - Is maggot debridement effective for removal of necrotic tissue from chronic wounds?
  - January 1960 to February 2008: 4 studies (pooled n=193);
    - 3 studies <60 subjects
  - Compared MDT (LDT) to autolytic/other debridement
    - pressure ulcers, leg ulcers, burn wounds
  - Concluded: “evidence base for the efficacy of maggot debridement therapy (MDT) in the management of necrotic wounds is sparse.”
  - “Even though clinical evidence supporting the use of MDT for debridement of wounds is lacking, clinical experience strongly suggests that this technique is an effective and safe method of debridement for selected patients.”

- Increased evidence from 2008 to 2017
  - more than 300 studies!
Evidence for larval debridement

- Multiple actions of Lucilia sericata larvae in hard-to-heal wounds: Larval secretions contain molecules that accelerate wound healing, reduce chronic inflammation and inhibit bacterial infection.
- A randomized controlled trial of larval therapy for the debridement of leg ulcers: Results of a multicenter, randomized, controlled, open, observer blind, parallel group study.
- Antimicrobial peptides expressed in medicinal maggots of the blow fly Lucilia sericata show combinatorial activity against bacteria.

LDT – mechanisms of action

- Larval enzymes: protease, collagenase, ammonia, allantoin and urea, lysozymes\textsuperscript{20,22,25,26,28,33}
- Increase in alkalinity - breaks down necrotic tissues\textsuperscript{26-28,31,33}
- Antimicrobial action of LDT secretions: peptides (dipterincins, lucifensin); chymotrypsin disrupts protein adhesion-mediated biofilm formation; crude methanol extract\textsuperscript{21-25, 29-31}
- Improved antibiotic effectiveness (re-susceptibility to antimicrobials observed after LDT)\textsuperscript{29}
- Stimulate fibroblast proliferation and promote fibroblast motility; may improve angiogenesis (amino acid derivatives), vascular perfusion, and tissue oxygenation; may reduce scarring; reduces inflammation\textsuperscript{20, 26-28, 32, 33}
Larval Debridement

PA01 biofilm culture before LDT

PA01 biofilm culture 24 hours after LDT

Potential antibiofilm strategies

- Prevention – reduce risk factors
- Debridement
- Selecting suitable topical products
- Selecting suitable systemic products
- Combined approaches
  - “one-two” punches
  - LDT + advanced therapies / skin grafts
  - Ultrasonic treatments + antimicrobials
  - Surfactant gels + mechanical disruption
Summary

What is in that chronic wound bed? Slough, Eschar, Biofilm?

- Examine – not only with naked eye
- Determine - what is it?
- Address – targeted treatment
- Evaluate treatment effectiveness, wound progress
- Prevent regrowth

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Questions?

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Specific References


Specific References


Specific References


Additional Resources & References

Wound Bed Preparation:
http://www.woundsinternational.com/media/issues/87/files/content_49.pdf

Larval Debridement Therapy:


