Wound Bed Management:

March 2, 2018

William Tettelbach, MD, FACP, FIDSA, FUHM

Medical Director of Wound & Hyperbaric Medicine Services

Dealing With Infections & Beyond

Intermountain Healthcare
Disclosures:

- National PI DFU Trial, MiMedx
- Advisory Board, Acelity
Objectives

• Appreciate appropriate wound debridement.
• Understand microbiology associated with diabetic foot ulcer (DFU) infections.
• Understand time course of colonization.
• Review bioburden/biofilm and wound healing.
• Review protease activity and protease modulating dressings.
March 30, 2017

- Diabetic male fell while biking
- Left calf ulcer worsening over 1 month
- Referred by PCP
After Sharp Excisional Debridement

March 30, 2017

- Diabetic male fell while biking
- Left calf ulcer worsening over 1 month
- Referred by PCP
February 27, 2017

• Diabetic male with peripheral neuropathy
• Left heel ulcer worsening over 3 months
• Referred by orthopedist
• ABI with evidence of mildly abnormal digital pulsation seen in all left toes. Left ankle and mid foot pulsatility normal.
• MRI suggestive of osteomyelitis.
March 3, 2017

- Sharp excisional debridement
- Obtained deep cultures post debridement
- Prescribed empiric oral antibiotics
  - Minocycline
  - Ciprofloxacin
- Immediately initiated NPWT
- Offloading via knee scooter

After Sharp Excisional Debridement
Mechanisms of Action

1. Draws wound edges together
2. Removes Infectious material
3. Removes exudate
4. Stimulates angiogenesis
March 7, 2017

- After 4 days of NPWT.
- 3/2/2017 wound culture positive for:
  - Enterobacter cloacae
  - Beta-hemolytic Streptococcus spp.
October 10, 2016
Diabetes Foot Ulcers (DFU)

- DFUs are one of the most common complications of diabetes\(^1\)
- The annual incidence of DFUs in diabetic Medicare population is estimated at 6\%.\(^2\)
- The lifetime incidence of DFUs in patients with diabetes may be as high as 34\%\(^3\)

Diabetes Foot Ulcers (DFU)

• In 2010, 73,000 non-traumatic lower-limb amputations performed in adults with diabetes.¹

• More than 60% of non-traumatic amputations occur in people with diabetes.¹

• A foot ulcer precedes 85% of lower-limb amputations in patients with diabetes.

• Contralateral leg amputation follows for 56% of patients within 3-5 years.

• 5-year mortality rate for diabetic patients who have had a single-leg amputation exceeds 70%.

Diabetes Foot Ulcers (DFU)

- 5-year mortality rate for diabetic patients who have had a single-leg amputation is > 50%.

- Higher than overall 5-year mortality rate of
  - Breast cancer (10%)
  - Bladder cancer (19%)
  - Colorectal cancer (33%)
  - All cancers combined (32%)

Diabetic Foot Ulcers (DFUs)

Risk factors predictive of ulcers and amputation\textsuperscript{1-4}

- Previous foot ulceration
- Neuropathy (loss of protective sensation)
- Foot deformity
- Vascular disease

Diabetic Foot Ulcers: A High Infection Risk

- Sustaining a lower extremity wound most common precipitating event for a foot infection¹

If develop a DFU infection:
- 55 times more likely to be hospitalized
- 155 times more likely to have an amputation

Variables achieving independent statistical significance as risk factors for foot infection. Data from a 2-year longitudinal outcomes study of 1,666 patients enrolled in a managed care-based outpatient clinic.

Diabetic Foot Infection: Microbiology

- Staphylococcus aureus

- Streptococcal species
  - Especially Group B
  - Occasionally Groups C or G
  - Less commonly group A

- 89% of DFUs cultured grew two or fewer organisms.¹

- Anaerobic species were isolated in only 5% of all cultures. ¹

Diabetic Foot Infection: Microbiology

- Chronic or more severely infected DFUs tend to be more *polymicrobial*

- Common Gram Negative Pathogens
  - *E. coli*
  - *Klebsiella* spp.
  - *Proteus* spp.

- *Pseudomonas aeruginosa*
  - Associated with water exposure
  - e.g., Puncture wound thru bottom of a shoe
Diabetic Foot Osteomyelitis: Microbiology

Gram Positive Aerobes
- Staphylococcus aureus
  - most common pathogen cultured from bone
- Streptococcus species
- Staphylococcus epidermidis

Gram Negative Aerobes
- Escherichia coli
- Klebsiella pneumoniae
- Proteus species
- Pseudomonas aeruginosa

Anaerobes
- Peptostreptococcus spp.
- Peptococcus spp.
- Finegoldia magna
- B. fragilis

Diabetic Foot Infection: Osteomyelitis

- Probing to bone in infected pedal ulcers is a clinical sign of underlying osteomyelitis in diabetic patients\(^1\)
  - sensitivity of 66%
  - specificity of 85%
  - positive predictive value of 89%
  - negative predictive value of 56%

- If you can palpate small bones of the feet in diabetics with a chronic ulcer, consider it osteomyelitis until proven otherwise

\(^1\) Grayson ML, JAMA 1995;273:721-723
Diabetic Foot Infection: Osteomyelitis

- Plain film rarely useful (unless late in course)
  - good for foreign bodies

- Bone scan - nonspecific
  - Especially in patients with neuropathic osteoarthropathy (Charcot joint)
  - Can be useful when MRI is not an option

- MRI (gold standard for radiological diagnosis)
  - T1 weighted image (low signal intensity)
  - T2 fat-saturated image (hyperintense signal)
  - T1 fat-saturated image post-gadolinium (enhancement)
Diabetic Foot Infections: Treatment

- Vascular Evaluation
- Nutrition Optimization
- Address Comorbidities
- Debridement
- Culture Wounds
- Advanced Wound Dressings
- Offloading
- Appropriate Use of Antimicrobials
Diabetic Foot Infections: Treatment

Inpatient vs. Outpatient

- Critical Ischemic Limb
- Systemic Toxicity
- Metabolic Instability
- Necrotizing Soft Tissue Infection
- Substantial Necrosis / Gangrene
- Need for Urgent Diagnostic / Therapeutic Intervention
- Unable To Care For Themselves
Diabetic Foot Infections: Treatment
Oral antibiotic agents for empiric therapy of mild diabetic foot infections.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Renal Dosing Required?</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicloxacillin</td>
<td>No</td>
<td>Penicillin</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanate†</td>
<td>Yes</td>
<td>β-lactam/β-lactamase inhibitor</td>
</tr>
<tr>
<td>Cephalexin†</td>
<td>Yes</td>
<td>Cephalosporin</td>
</tr>
<tr>
<td>Cefdinir</td>
<td>Yes</td>
<td>Cephalosporin</td>
</tr>
<tr>
<td>Levofloxacin†</td>
<td>Yes</td>
<td>Fluoroquinolone</td>
</tr>
<tr>
<td>Clindamycin†‡</td>
<td>No</td>
<td>Lincosamide</td>
</tr>
<tr>
<td>TMP/SMX§</td>
<td>Yes</td>
<td>Sulfonamide</td>
</tr>
<tr>
<td>Minocycline§</td>
<td>Yes</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Doxycycline§</td>
<td>No</td>
<td>Tetracycline</td>
</tr>
</tbody>
</table>

†Drugs that have been used in published trials of treatment of diabetic foot infections.
‡Suspect inducible clindamycin resistance if staphylococcal isolate is susceptible to clindamycin but resistant to erythromycin. Confirm with D-test.
§Active against community-associated methicillin-resistant *Staphylococcus aureus*. TMP/SMX: Trimethoprim/sulfamethoxazole
### Parenteral or oral antibiotics for empiric therapy of moderate-to-severe DFU infections

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Activity against MRSA?</th>
<th>Activity against B. fragilis?</th>
<th>Renal Dosing Required?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin/Sulbactam</td>
<td>β-lactam/β-lactamase inhibitor</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>β-lactam/β-lactamase inhibitor</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Cephalosporin</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cefepime or Ceftazidime</td>
<td>Cephalosporin</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>Cephalosporin</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>Carbapenem</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Imipenem/cilastatin</td>
<td>Carbapenem</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>Quinolone</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Clindamycin with Ciprofloxacin</td>
<td>Lincosamide / Quinolone</td>
<td>Some</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>Glycylcycline</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Glycopeptide</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Oxazolidinone</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>Cyclic lipopeptide</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
## Diabetic Foot Infections: Treatment

### Antibiotic Selection Overview: Questions a Clinician Should Consider.

<table>
<thead>
<tr>
<th>Question</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is there clinical evidence of infection or critical colonization?</td>
<td>Do not treat clinically uninfected wounds with antibiotics.</td>
</tr>
<tr>
<td>Is there high risk of MRSA?</td>
<td>Include anti-MRSA therapy in empiric regimen if the risk is high or the infection is severe.</td>
</tr>
<tr>
<td>Has patient received antibiotics in the past month?</td>
<td>If so, include agents active against gram-negative bacilli in regimen. If not, agents targeted against just aerobic gram-positive cocci may be sufficient.</td>
</tr>
<tr>
<td>Are there risk factors for Pseudomonas infection?</td>
<td>If so, consider empiric antipseudomonal agent. If not, empiric antipseudomonal treatment is rarely needed.</td>
</tr>
</tbody>
</table>

*a* Such as high local prevalence of Pseudomonas infection, warm climate, frequent exposure of the foot to water.
# Diabetic Foot Infections: Treatment

Duration and route of antibiotic therapy for the treatment of diabetic foot osteomyelitis.

<table>
<thead>
<tr>
<th>Clinical situation</th>
<th>Route of therapy</th>
<th>Duration of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No residual infected tissue (e.g., postamputation)</td>
<td>Parenteral or oral</td>
<td>2–5 days</td>
</tr>
<tr>
<td>Residual infected soft tissue (but not bone)</td>
<td>Parenteral or oral</td>
<td>2–4 weeks</td>
</tr>
<tr>
<td>Residual infected (but viable) bone</td>
<td>Initial parenteral, then consider oral switch</td>
<td>4–6 weeks</td>
</tr>
<tr>
<td>No surgery, or residual dead bone postoperatively</td>
<td>Initial parenteral, then consider oral switch</td>
<td>&gt;3 months</td>
</tr>
</tbody>
</table>

Diabetic Foot Infections: Treatment

Algorithm for the use of HBO₂

Wagner Grading System:

A. Grade 1: Superficial Diabetic Ulcer
B. Grade 2: Ulcer with deep structures involved:
   - ligament, tendon, joint capsule or fascia
   - no active infection (abscess or osteomyelitis)
C. Grade 3: Ulcer with deep structures involved:
   - ligament, tendon, joint capsule or fascia
   - + evidence of infection (abscess or osteomyelitis)
D. Grade 4: Gangrene to portion of forefoot
E. Grade 5: Extensive gangrene of foot

Diabetic Foot Infections: Treatment

• HBO$_2$ as adjunctive therapy
  • Treat at 2.0 to 2.4 ATA once or twice daily
  • Oxygen administered 90 to 120 minutes per session
  • Treatment range: 30 to 40
    • may require up to 60 treatments to achieve sustained therapeutic benefit
    • be aware that the proposed Noridian HBO$_2$ LCD will limit sessions to 40 treatment per year for the diagnosis of osteomyelitis (Overall total allowed will be 60/year per pt)
Diabetic Foot Infections: Treatment

“Appropriate therapy” includes:

- **Antibiotics**
  - at least 42 days for osteomyelitis
  - antibiotics should be culture-directed
  - PICC line for outpatient management

- **Aggressive surgical debridement**
  - remove infected/dead bone, as well as involved hardware if possible

- **Educate & optimize dietary needs**
  - e.g., malnutrition (protein), vitamin D, vitamin C, vitamin A, zinc

- **Address comorbidities**
  - e.g., diabetes, venous stasis, smoking cessation, renal/liver failure

- **Vascular evaluation/intervention if indicated**

- **Adjunct HBO₂ therapy** (not an approved indication for acute osteomyelitis)

- **Offloading**
  - total contact cast
  - walker boot
HBO₂ Benefits Wound Healing Through the Following Mechanism(s)

A. Tissue oxygen tension restored to > 30 mmHg
B. Augments transport of certain antibiotics across bacterial cell walls
C. Promotes capillary angiogenesis
D. Prevents polymorphonuclear leukocytes from adhering to damaged blood vessel linings
E. All of the above
HBO₂ Benefits Wound Healing Through the Following Mechanism(s)

A. Tissue oxygen tension restored to > 30 mmHg
B. Augments transport of certain antibiotics across bacterial cell walls
C. Promotes capillary angiogenesis
D. Prevents polymorphonuclear leukocytes from adhering to damaged blood vessel linings

E. All of the above
Benefits of HBO$_2$

Infected bone is hypoxic*

- **Normal Oxygen Tension** (21% O$_2$ at sea level)
  - Healthy Bone = 45 mmHg
  - Infected Bone = 21 mmHg

- **Hyperbaric Oxygen Tension** (100% O$_2$ at 2 ATA)
  - Healthy Bone = 321 mmHg
  - Infected Bone = 104 mmHg

* Rabbit animal model
HBO$_2$ & Antibiotics with Osteomyelitis in Rats

Mendel et al. Undersea Hyperb Med 26:169, 1999
Burn Wounds

- Estimated over 2 million burn injuries in United States per year \(^1\)
- Result in approximately 14,000 deaths
- Approximately 20,000 admissions to a specialized burn unit
- About 75,000 require hospitalization annually
  - 25,000 remain hospitalized for > 2 months
- Most Common areas involved: upper extremity, head and neck
- Most Common Mechanism of burn injury: flame and scalding

---

Stages of Burn Wounds

➤ First-degree (superficial) burns
First-degree burns affect only the epidermis, or outer layer of skin. The burn site is red, painful, dry, and with no blisters. Mild sunburn is an example. Long-term tissue damage is rare and usually consists of an increase or decrease in the skin color.

➤ Second-degree - (partial thickness) burns
Second-degree burns involve the epidermis and part of the dermis layer of skin. The burn site appears red, blistered, and may be swollen and painful.

➤ Third-degree (full thickness) burns
Third-degree burns destroy the epidermis and dermis. Extends into the subcutaneous tissue. The burn site appears white or charred. There is no sensation in the area since the nerve endings are destroyed.

➤ Fourth-degree burns
Full thickness that extends into muscle and bone
Types of Burns

➤ **Thermal burns**: Burns due to external heat sources which raise the temperature of the skin and tissues and cause tissue cell death or charring. Hot metals, scalding liquids, steam, and flames, when coming in contact with the skin, can cause thermal burns.

➤ **Radiation burns**: Burns due to prolonged exposure to ultraviolet rays of the sun, or to other sources of radiation such as x-ray.

➤ **Chemical burns**: Burns due to strong acids, alkalis, detergents, or solvents coming into contact with the skin and/or eyes

➤ **Electrical burns**: Burns from electrical current, either alternating current (AC) or direct current (DC)
Despite various current topical treatment regimens designed to eradicate the bacterial load within the burn wound, sepsis remains the leading cause of death in burn units around the world. ¹

Advances in resuscitation, surgical management, control of infection, control of the hypermetabolic response and rehabilitation have resulted in dramatic improvements in burn mortality and morbidity in the last 60 years. ²

Relentless increase in microbial resistance to antibiotics and other antimicrobials has led to a renewed search for alternative approaches to prevent and combat burn infections.

Burn Complications

➤ Burns greater than 10% in children or 15% in adults
   - potentially life-threatening injuries related to hypovolemic shock

➤ A local response to burning includes:
   - direct tissue coagulation
   - burn tissue conversion, where the damaged cells progress to cell death, extending the depth and severity of the original injury

➤ Skin barrier replaced by *eschar*:
   - encourages microbial growth.
   - impedes the immune response.
   - restricts distribution of systemic antibiotics due to its avascularity
Colonization Time Course

- **48 hours within initial thermal insult heavy colonization begins**
  - Gram-positive bacteria, such as staphylococci located deep within sweat glands and hair follicles

- **5-7 days burns are colonized with Gram-negative bacteria derived from:**
  - host’s normal gastrointestinal tract
  - upper respiratory flora
  - hospital environment

- Yeasts and fungi tend to be the latest colonizers.

Diagnosis of Burn Wound Infection: American Burn Association Guidelines

<table>
<thead>
<tr>
<th>SYNDROME</th>
<th>CLINICAL AND PATHOLOGIC CRITERIA: BURN SEPSIS GUIDELINES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound colonization</td>
<td>Bacteria on wound surface at low concentration (&lt;10^2 bacteria/g tissue); no invasive infection</td>
</tr>
<tr>
<td>Wound infection</td>
<td>Bacteria in the wound and wound eschar at high concentration (&gt;10^3 bacteria/g tissue); no invasive infection</td>
</tr>
<tr>
<td>Invasive infection</td>
<td>Pathogens in burn wound at a sufficient concentration (&gt;10^5 bacteria/g tissue [frequently]), depth, and surface area to cause suppurative separation of eschar or graft loss, invasion of adjacent unburned tissue, or sepsis syndrome (see Table 319-1)</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>Bacteria present in wound and/or wound eschar at high concentrations (&gt;10^5 bacteria/g tissue), with surrounding tissue revealing erythema (but this alone does not require therapy), induration, warmth, and/or tenderness</td>
</tr>
<tr>
<td>Necrotizing infection, fasciitis</td>
<td>Aggressive, invasive infection with involvement below the skin resulting in tissue necrosis</td>
</tr>
</tbody>
</table>

Notes

1. Quantitative biopsy can assist in identifying pathogen and antimicrobial resistance profiles, but its ability to confirm a diagnosis is limited.
2. Quantitative swabs are unreliable but might assist in pathogen identification and antimicrobial resistance profiles.
3. Tissue histology can be used, but limited expertise exists.
4. Clinical parameters of wound infection include pain, erythema, color change, unexpected changes in wound appearance and depth, systematic changes, and premature separation of burn eschar.

Hyperbaric Oxygen Therapy Infection

Recommended:

• > 20% total body Surface Area
  - Hands
  - Face
  - Feet
  - Perineum

• Not indicated:
  - Superficial burns
  - Not expected to survive
Hyperbaric Oxygen Therapy Infection

Treatments:

➤ Attempted 3 times first 24 hours
  - Twice daily thereafter
  - > 40% surface area 10 to 14 days
  - Treatment > 20-30 sessions typically used to optimize graft take
  - Rare to exceed 40-50 sessions

➤ 2.0 to 2.4 atm abs
  - 90 minute bottom time
  - Maintain comfortable ambient temperature
Chemical Burn “Draino”
76 y/o Male

5.0 cm x 4.4 cm x 0.1 cm
22 sq cm

4-25-2016
Escharotomy - applied Medihoney
Chemical Burn “Draino”
76 y/o Male

4.5 cm x 4.0 cm x 0.1 cm
18 sq cm

4-28-2016
Further Escharotomy - applied mechanical NPWT 125 mm Hg
Chemical Burn “Draino”
76 y/o Male

7-28–2016
RESOLVED
Topical Antimicrobial Therapies

- Antimicrobial peptides
- **Topical photodynamic therapy**
- Chitosan preparations
- Topical iodine delivery formulations
- Phage therapy
- Medical honey
- Essential oils
- Silver Preparations
- Electrochemically activated solutions
- Sorbact
Topical Antimicrobial Therapies

- Concept of antimicrobial photodynamic therapy
  - The photosensitizer is excited to its triplet state by light of the correct wavelength and then transfers its energy to molecular oxygen forming reactive oxygen species that are able to kill all classes of pathogenic micro-organisms.
Topical Antimicrobial Therapies

The three states only differ in the occupancy and spin states of electrons in the two degenerate $\pi$ antibonding orbitals.
Topical Antimicrobial Therapies

- Concept of antimicrobial photodynamic therapy
  - The photosensitizer is excited to its triplet state by light of the correct wavelength and then transfers its energy to molecular oxygen forming reactive oxygen species that are able to kill all classes of pathogenic micro-organisms.
  - These photosensitizer molecules possess either constitutive cationic charges or basic amino groups.
Topical Antimicrobial Therapies

- Antimicrobial peptides
- Topical photodynamic therapy
- Chitosan preparations
- Topical iodine delivery formulations
- Phage therapy
- **Medical honey**
- Essential oils
- Silver Preparations
- Electrochemically activated solutions
- Sorbact
Topical Antimicrobial Therapies

Medical Honey:

- Antibacterial properties are due to
  - high osmolarity
  - production of hydrogen peroxide formed by the enzyme glucose oxidase present in honey.

- glucose oxidase becomes active only when honey is diluted by wound fluid.

- Non-peroxide antimicrobial activity is due to methylglyoxal and an unidentified synergistic component.
Topical Antimicrobial Therapies

- Antimicrobial peptides
- Topical photodynamic therapy
- **Chitosan preparations**
- Topical iodine delivery formulations
- Phage therapy
- Medical honey
- Essential oils
- Silver Preparations
- Electrochemically activated solutions
- Sorbact
Topical Antimicrobial Therapies

**Chitosan preparations:**

- Exhibit bactericidal activity against pathogens
  - e.g., MRSA
- Made from shrimp and crabshell chitin
- A polycationic polymer
  - Stimulates hemostasis
Topical Antimicrobial Therapies

- Antimicrobial peptides
- Topical photodynamic therapy
- Chitosan preparations
- Topical iodine delivery formulations
- Phage therapy
- Medical honey
- Essential oils
- Silver Preparations
- Electrochemically activated solutions
- Sorbact
Sorbact

Mechanism Of Action

• Hydrophobic interaction binds and inactivates pathogens
• Pathogens are permanently bound and become
• Microorganisms removed with each dressing change
Effective against:

Bacteria:
- Staphylococcus aureus – (MRSA)
- Enterococcus – (VRE)
- Clostridium difficile
- E. coli
- Enterobacter
- H. influenzae
- Serratia
- Vibrio
- Group A Streptococci
- Pseudomonas aeruginosa
- Klebsiella

Fungi:
- Candida albicans
- Aspergillus brasiliensis

Sorbact
A biofilm is an aggregate of microorganisms in which cells are stuck to each other and/or to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS, which is also referred to as "slime," is a polymeric jumble of DNA, proteins and polysaccharides.
Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface.

These first colonists adhere to the surface initially through weak, reversible van der Waals forces.

If not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.
Exhibits high levels of antibiotic, host, pH and disinfecting resistance.

1000x greater antibiotic resistance than planktonic cells.
Hypoxic environment inhibits:

- Oxidative burst
- ATP dependent reactions
- Collagen deposition
# Bioburden - Biofilm

## Table 1. Partial list of human infections involving biofilms.

<table>
<thead>
<tr>
<th>Infection or disease</th>
<th>Common biofilm bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental caries</td>
<td>Acidogenic Gram-positive cocci (e.g., <em>Streptococcus</em>)</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Gram-negative anaerobic oral bacteria</td>
</tr>
<tr>
<td>Otitis media</td>
<td>Nontypable strains of <em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>Musculoskeletal infections</td>
<td>Gram-positive cocci (e.g., <em>staphylococci</em>)</td>
</tr>
<tr>
<td>Necrotizing fascitis</td>
<td>Group A streptococci</td>
</tr>
<tr>
<td>Biliary tract infection</td>
<td>Enteric bacteria (e.g., <em>Escherichia coli</em>)</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>Various bacterial and fungal species—often mixed</td>
</tr>
<tr>
<td>Bacterial prostatitis</td>
<td><em>E. coli</em> and other Gram-negative bacteria</td>
</tr>
<tr>
<td>Native valve endocarditis</td>
<td>Viridans group streptococci</td>
</tr>
<tr>
<td>Cystic fibrosis pneumonia</td>
<td><em>P. aeruginosa</em> and <em>Burkholderia cepacia</em></td>
</tr>
<tr>
<td>Melioidosis</td>
<td><em>Pseudomonas pseudomallei</em></td>
</tr>
<tr>
<td>Nosocomial infections</td>
<td>Gram-negative rods</td>
</tr>
<tr>
<td>ICU pneumonia</td>
<td><em>Staphylococcus epidermidis</em> and <em>S. aureus</em></td>
</tr>
<tr>
<td>Sutures</td>
<td><em>S. epidermidis</em> and <em>S. aureus</em></td>
</tr>
<tr>
<td>Exit sites</td>
<td>Gram-positive cocci</td>
</tr>
<tr>
<td>Arteriovenous shunts</td>
<td><em>P. aeruginosa</em> and Gram-positive cocci</td>
</tr>
<tr>
<td>Schieral buckles</td>
<td><em>E. coli</em> and other Gram-negative rods</td>
</tr>
<tr>
<td>Contact lens</td>
<td>A variety of bacteria and fungi</td>
</tr>
<tr>
<td>Urinary catheter cystitis</td>
<td><em>Actinomyces israelii</em> and many others</td>
</tr>
<tr>
<td>Peritoneal dialysis (CAPD) peritonitis</td>
<td>A variety of bacteria and fungi</td>
</tr>
<tr>
<td>IUDs</td>
<td><em>S. epidermidis</em> and <em>C. albicans</em></td>
</tr>
<tr>
<td>Endotracheal tubes</td>
<td><em>S. epidermidis</em> and others</td>
</tr>
<tr>
<td>Hickman catheters</td>
<td><em>S. aureus</em> and <em>S. epidermidis</em></td>
</tr>
<tr>
<td>Central venous catheters</td>
<td>Gram-positive cocci</td>
</tr>
<tr>
<td>Mechanical heart valves</td>
<td>A variety of enteric bacteria and fungi</td>
</tr>
<tr>
<td>Vascular grafts</td>
<td><em>S. aureus</em> and <em>S. epidermidis</em></td>
</tr>
<tr>
<td>Biliary stent blockage</td>
<td><em>S. aureus</em> and <em>S. epidermidis</em></td>
</tr>
</tbody>
</table>
Solution with Least Cytotoxicity & Active Bactericidal Activity

A. Sterile saline solution
B. Puracyn or Vashe solution (hypochlorous acid)
C. Hibiclens skin cleanser (chlorhexidine gluconate)
D. Betadine solution (povidine-iodone)
E. All of the above
Solution with Least Cytotoxicity & Active Bactericidal Activity

A. Sterile saline solution
B. **Puracyn or Vashe solution (hypochlorous acid)**
C. Hibiclens skin cleanser (chlorhexidine gluconate)
D. Betadine solution (povidine-iodine)
E. All of the above
Cell proliferation assays performed by culturing fibroblasts in the presence of commonly used antiseptics.

- H$_2$O$_2$ and povidone-iodine reduced both migration and proliferation of fibroblasts in a dose-dependent fashion.

- Silver-containing antiseptics and chlorhexidine exhibited reductions in proliferation at high concentrations, but enhanced growth at lower doses.
## Cytotoxicity & Bacterial Activity of Commonly Used Antiseptics

<table>
<thead>
<tr>
<th>Agent Tested</th>
<th>Use</th>
<th>Manufacturer</th>
<th>Nontoxic Dilution</th>
<th>Toxicity Index</th>
<th>Time to 4 Log₁₀ Kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0.9%)</td>
<td>Wound</td>
<td></td>
<td>$10^0$</td>
<td>1</td>
<td>&gt;24 hours</td>
</tr>
<tr>
<td>Shur-Clen's</td>
<td>Wound</td>
<td>ConvaTec</td>
<td>$10^0$</td>
<td>1</td>
<td>&gt;24 hours</td>
</tr>
<tr>
<td>Puracyn, OTC</td>
<td>Wound</td>
<td>Innovacyn</td>
<td>$10^{-1}$</td>
<td>10</td>
<td>10 min</td>
</tr>
<tr>
<td>Dermagran Wound Clenser</td>
<td>Wound</td>
<td>Derma Sciences</td>
<td>$10^{-1}$</td>
<td>10</td>
<td>&gt;24 hours</td>
</tr>
<tr>
<td>Prontosan Wound Irrigation Solution</td>
<td>Wound</td>
<td>B. Braun Medical</td>
<td>$10^{-2}$</td>
<td>100</td>
<td>&gt;24 hours</td>
</tr>
<tr>
<td>Dermal Wound Clenser</td>
<td>Wound/Skin</td>
<td>Molniycke</td>
<td>$10^{-3}$</td>
<td>1,000</td>
<td>&gt;24 hours</td>
</tr>
<tr>
<td>Hibiclens (Chlorhexidine gluconate solution 4.0% w/v)</td>
<td>Skin</td>
<td>Hollister Woundcare</td>
<td>$10^{-4}$</td>
<td>10,000</td>
<td>&gt;24 hours</td>
</tr>
</tbody>
</table>

Russell Hoon, Suriani Abdul Rani, Ramin Najafi, Lu Wang, Dmitri Debabov. Antimicrobial Activity Comparison of Pure Hypochlorous Acid (0.01%) with Other Wound and Skin Cleansers at Non-Toxic Concentrations. SAWC Spring and WHS 2013, Abstract GP-55
Hypochlorous Acid:

An Ideal Wound Care Agent With Powerful Microbicidal, Antibiofilm, and Wound Healing Potency
Reduce Bioburden

Topical Therapy

- Bactericidal
- Noncytotoxic
- Avoid pressures for emergence of antibiotic resistance, e.g.,
  - Hypochlorous acid
  - Sodium Hypochlorite
  - Surfactants / PHMB
  - Silver
Evaluation of the antimicrobial activity of a super-oxidized water, Sterilox, for the disinfection of endoscopes
### Table V  Effect of Sterilox against other micro-organisms at a ratio of 10:1 disinfectant:organism

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Calf serum</th>
<th>Mean log&lt;sub&gt;10&lt;/sub&gt; pre-disinfection count</th>
<th>Mean† log&lt;sub&gt;10&lt;/sub&gt; reduction after exposure for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 min</td>
<td>1 min</td>
</tr>
<tr>
<td>E. coli NCTC 9001</td>
<td>Absent</td>
<td>8.7</td>
<td>&gt;6.7*</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>8.7</td>
<td>–</td>
</tr>
<tr>
<td>E. coli NCTC 12900</td>
<td>Absent</td>
<td>9.0</td>
<td>&gt;7.0*</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>9.0</td>
<td>–</td>
</tr>
<tr>
<td>E. coli 0157 clinical isolate</td>
<td>Absent</td>
<td>8.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>8.8</td>
<td>&lt;1.8</td>
</tr>
<tr>
<td>MRSA clinical isolate</td>
<td>Absent</td>
<td>8.7</td>
<td>&gt;6.7*</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>8.7</td>
<td>&lt;1.6</td>
</tr>
<tr>
<td>C. albicans isolate</td>
<td>Absent</td>
<td>7.2</td>
<td>&gt;5.2*</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>7.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Detection limits of test
† Tests carried out in triplicate

MRSA methicillin resistant S. aureus
S. aureus biofilms were produced by circulating nutrient broth through Tygon® tubing for 4-12 hours.

5-10 ml of S. aureus culture (10^8 colony-forming unit [CFU]/ml) was circulated through the tubing.

Biofilms were treated with hypochlorous acid for 1, 3, 5, 7, and 10 minutes.

After each treatment, 2 cm^2 pieces of tube were cut and neutralized, and bacterial numbers, residual protein, and carbohydrate content measured.
Bioburden - Biofilm
Debriding Device Consisting of Beveled Polyester Fibers

- Effectively removes P. aeruginosa biofilms from wounds
- Less invasive method of wound debridement than traditional sharps debridement
  - Less painful

Beyond Bioburden

Systemic Antimicrobial Therapy

- Active infection
  - e.g., periwound cellulitis

- Critical Colonization / Wound Bed Infection
  - Clinical correlation required
  - Wound stalled or increasing in size
Proteases:

- Enzymes that break down proteins into peptides and amino acids.

- Major proteases
  - matrix metalloproteinases (MMPs), e.g., collagenease
  - serine proteases, e.g., elastase.

- In general, different wound-related proteases act on extracellular matrix (ECM) and connective tissue proteins such as collagen, gelatin, proteoglycans and elastin.
Elevated Protease Activity

Compromise of the microvasculature:

- Non-vascularized tissue.
- Proteolysis and lipolysis is up-regulated in burns \(^1\)

Increases in matrix metalloproteinases (MMPs) leads to breakdown of proteins

- Inhibits wound healing
- provides more nutrients for microbes
- facilitates microbial penetration into the tissue

## Roles of Protease in Normal Wound Healing

<table>
<thead>
<tr>
<th>Main phase of healing</th>
<th>Role of proteases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>• Removal of damaged ECM (aids autolytic debridement)</td>
</tr>
<tr>
<td>Proliferation</td>
<td>• Degradation of capillary basement membrane for angiogenesis</td>
</tr>
<tr>
<td></td>
<td>• Aiding detachment and migration of cells</td>
</tr>
<tr>
<td>Remodelling</td>
<td>• Contraction of scar ECM</td>
</tr>
<tr>
<td></td>
<td>• Remodelling of scar ECM</td>
</tr>
</tbody>
</table>
Protease Activity in Wounds

- There is a burst of protease activity at the start of acute wound healing.
- Protease activity typically peaks in 3 days and declines to low levels in 1 week.
The four phases of acute wound healing

Optimum healing of cutaneous wounds requires a coordinated host response.

Living skin substitutes support the latter stages of tissue repair.

References:
Which wound(s) have elevated protease activity?

A: A 52-year-old non-diabetic male with a long-standing venous leg ulcer. There is minimal exudate after treatment with a topical silver agent. Recently treatment has been changed to a collagenase and compression wrapping. He has minimal pain.

B: A 40-year-old non-diabetic woman with bilateral lateral gutter chronic venous leg ulcers currently being treated with topical alginate and compression. The right leg ulcer (above left) has a clean granulating base. The left leg ulcer has repeatedly developed tenacious necrotic slough requiring curettage.

C: Stage III pressure ulcer treated with ORC-silver/collagen.

D: A non-diabetic patient with an acute wound on the dorsum of her hand after an intravenous line infiltration. The wound is healing.

E: A patient with known vasculitis of the lower extremity.

F: Diabetic plantar neuropathic ulcer treated with topical silver alginate and hyperbaric oxygen.
Clinical observation cannot detect high protease activity.
Protease Activity in Wounds

For point-of-care test to become integral to practice, data will be required that demonstrate the validity of the test in a spectrum of wound types in clinical practice.

Key unanswered questions is how to deal with wounds with:

- high proteases that go on to heal without complication
- low proteases that fail to heal
Protease activity is one of the best available biochemical marker for predicting poor wound healing of both acute and chronic wounds.

Localized bleeding following debridement stimulates influx of alpha-2-macroglobulin (A2M), a chemical agent that acts as a protease inhibitor, thus reducing proteolytic activity.¹, ²

---

Protease-Modulating Dressings

- Collagen based
  - Targets collagenase

- Calcium alginate
  - Binds & inactivates elastases

- Silver containing
  - Displaces zinc ion necessary for MMPs to function\(^1\)
  - Decreases bacterial levels potentially reducing host and bacterial protease production\(^2\)

---


2. Widgerow, AD. Chronic wound fluid—thinking outside the box. Wound Repair Regen 2011; [Epub ahead of print]
It's time to close the book on infectious diseases

U.S. Surgeon General, 1969
The cost of ineffective wound treatment is estimated to be $20-25 billion annually.
Questions?