The Biofilm Battlefield: New Technologies to Win the War

Jeffrey A. Niezgoda, MD
FACHM, MAPWCA, CHWS

WOUND HEALING AND HYPERBARIC SYMPOSIUM
McAllen, TX | September 15, 2018
Disclosure

- Consultant & PI – Next Science
Infection Facts & Figures:

- ~2 M hospital-acquired infections with annual cost of ~$30.5 B
- 721 hospitals had their Medicare reimbursement lowered 1% for having high hospital-acquired infection rates
- $373 M per year due to penalties

2014

Infection Facts & Figures:

18% of Medicare patients were readmitted within one month

~2M readmitted each year

**Wound Infection Facts & Figures:**

>6.5 M  
People suffering from chronic wounds

90% CHRONIC WOUNDS AFFECTED BY BIOFILM

Annual chronic wound costs  
>$25 B

1993-2006  
>80% increase in hospital stays for non-healing pressure ulcers alone  
~$11B dollars per year

>15% OF DIABETIC FOOT ULCERS REQUIRE AMPUTATIONS

70% WOUNDS TAKE MORE THAN 12 WEEKS TO HEAL


Overview

1. Barriers to Wound Healing
2. Bacterial Spectrum in Chronic Wounds
3. What is Biofilm
4. Strength is in the Structure
5. Goal of a Comprehensive Approach
6. Current Trends in Biofilm Treatment
7. A Science Driven Treatment

[https://dx.doi.org/10.3201/eid0809.020063](https://dx.doi.org/10.3201/eid0809.020063) Retrieved August 7, 2018 from CDC website
Barriers to Wound Healing
Normal Wound Healing Physiology

1. Hemostasis
   - Seconds to hours

2. Inflammation
   - Hours to days

3. Proliferation
   - Days to weeks

4. Remodeling
   - Days to months
Normal Wound Healing Physiology - Inflammation

1. Hemostasis
   - Seconds to hours

2. Inflammation
   - Hours to days
   - Reactive Oxygen Species (ROS) and protease “off-target” effects
   - Increased vasodilation and vasopermeability lead to increased exudate
   - Release of cytokines and growth factors
   - Immune cell recruitment
   - Bacterial clearance

3. Proliferation
   - Days to weeks

4. Remodeling
   - Days to months
OXIDATIVE BURST
Neutrophils kill microbes by producing reactive oxygen species, demonstrated here with the dye nitroblue tetrazolium (NBT)
Bacterial Burden

Contamination - Infection Continuum
Many factors affect the progress of microorganisms in a wound from colonization to infection:

- Infection = Dose x Virulence
  Host Resistance
- The number of organisms.
- The virulence factors they produce.
- The resistance of the host to infection.
The Risk of Biofilm to Chronic Wounds

Science has demonstrated that 80-90% of all chronic wounds have biofilm.

10% OF BACTERIA ARE PLANKTONIC/FREE-FLOATING
The periodic release of planktonic bacteria from biofilms has been linked to chronic relapsing infections.

90% OF BACTERIA EXISTS IN BIOFILMS
Bacteria protected by biofilm EPS can be 1000x more resistant to antibiotics than planktonic bacteria.

Pathologic Biofilm Quickly Establishes

1. Colonization by free-floating (planktonic) pathogens

2. Absorption of pathogens into wound bed, permanent attachment

3. Biofilm growth, division

4. Maturation of ‘microcolony’, secretion of 3-dimensional protective layer (EPS)

5. Proliferation of microcolonies

6. Continuous dispersion, formation of new microcolonies

A new understanding of these microbial communities is driving a revolution that may transform the science of microbiology. Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/Formation-of-a-biofilm-is-analogous-to-the-development-of-a-multicellular-organism-with_fig2_253263880 [accessed 13 Aug, 2018]
Biofilm Pathology Takes Over Wound Healing Progression

- When prolonged for $\geq 30$ days, inflammation can cause damage to healthy tissue

2 Inflammation

- Becomes ineffective for the host
- Reactive Oxygen Species (ROS) and protease “off-target” effects
- Biofilm formation releases small molecules which triggers more inflammation
- Increased exudate from inflammation incorporates into EPS and provides nutrition for biofilm

3 Proliferation

4 Remodeling

- Ultimately the ineffective inflammatory response prevents wound healing and makes the body susceptible to other infection


Biofilm provides
- protection,
- nutrition,
- water, and
- a place to for pathogens to flourish

Resists host immune responses
What is Biofilm
Confusion Still Surrounds Biofilm
What is Biofilm?

Keywords: protective, matrix, colonized, armor, infection, polymer, crosslinked, sticky, contamination, interactive, secreted, insoluble, biofilm, driven, structure, persistent, fluid channels, metallic ions, voids, calcium, metallic fibers, polysaccharide, slime, gel, film, filaments, iron, network, colonized, armor, protective.
Biofilm is a community of pathogens enveloped within a complex structure of entangled polymers strengthened with metallic bonds.
Biofilm becomes a three-dimensional problem:

Planktonic pathogens, biofilm encased pathogens and encasing structure

Polymicrobial community of organisms

Slimy tangles of protective polymer fibers linked with metallic bonds

The Strength is in the Structure
Robust bacteria inside a protective powerhouse: the extracellular polymeric substance
What is the Extracellular Polymeric Substance and Why Does it Matter?

**Extracellular**
A non-cellular sticky gel secreted by bacteria to provide a physical barrier of protection while they are encased within the gel.

**Polymeric**
Beginning as simple organic molecules; polymers inside the gel become linked with metallic bonds giving heightened strength to the structure.

**Substance**
An insoluble capsular environment that evolves through a dialogue within itself and with the host for bacterial growth, mutation, and proliferation.
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**Substance**
Once metallic bonds become established, the substance converts to an insoluble capsular environment that interacts with the host for bacterial growth, mutation, and proliferation.
Mature Biofilm Forms in 24-48 hours: a Closer Look at the Structure

- **90%** of the bacteria are enveloped within the structure
- **Free-floating bacteria** are only **10%** of the bacteria in a wound
- **Extracellular Polymeric Substance** showing structural polymers linked with metallic bonds
- **Metallic Bond**
The Structure is Designed by Nature to be Mechanically Resistant

Metallically bonded polymers anchor the structure (EPS) giving it strength and prevent it from being washed off or eradicated by current treatment protocols.
Pathogens Structurally Protected by the EPS

Block

- Large molecules such as large molecule antimicrobials, antibodies and inflammatory cells
- Biofilm matrixes act as diffusion barrier to small molecules like antibiotics

Mutual protection

- Exhibit cooperative protective effects
- Some species can assist other species to attach and incorporate into the biofilm (QUORUM SENSING)

Hibernation (quiescent bacteria)

- Biofilm matrixes have developed a mechanism for a subpopulation to become metabolically quiescent, i.e. to hibernate
The Goal of a Comprehensive Biofilm Treatment Approach
“Historical Approach”
Target the Pathogens

- Kill free-floating (planktonic) pathogens, and
- Kill newly exposed pathogens protected within the EPS without cytotoxicity
Target the Biofilm Structure

Dismantle the Structure with Antibiofilm Strategies

- Physically remove the biofilm structure
  - Local debridement
- Attack and destroy the structure of the biofilm
  - Chemically caustic agents
  - Significant cytotoxicity
Control the Environment

Prevent Reformation without damaging new tissue

- Create physical environment that prevents attachment of planktonic organisms, biofilm reformation, and supports pro-healing processes


Current Trends in Biofilm Treatment
Debridement

- Debridement breaks biofilm into smaller colonies but does not entirely remove it.

- Can spread the biofilm to other wound regions.

- Can amplify biofilm – makes spreading more aggressive and reformation faster than on its own therefore followed by a topical antimicrobial for highest effectiveness.

(Biofilms Made Easy (wounds international) – Phillips ; Skin and Wound Care 2013 – Kevin Wu ; Wound Education Update Feb 2014 – Wounds UK Best Practice Statement)
Traditional Antimicrobial-Antiseptic Therapies

- Non-selective - can impact all wound cells not just pathogens
- Not designed for use in all wound healing phases
- Unable to penetrate into the entire EPS matrix
  - Products may travel through the EPS water channels
- Often cytotoxic at effective strengths
- Targeted antibiotics can lead to resistance

Present Treatment Guidelines
“Wish List” or Ideal Antibacterial Therapy

<table>
<thead>
<tr>
<th><strong>Broad antimicrobial spectrum</strong></th>
<th>Biofilm is often polymicrobial, including gram-positive and gram-negative bacteria and fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No microbial resistance</strong></td>
<td>Has a mechanism of action that does not result in the development of microbial resistance</td>
</tr>
<tr>
<td><strong>High tissue compatibility</strong></td>
<td>Does not negatively impact healthy cells or healing</td>
</tr>
<tr>
<td><strong>Sustained barrier effect</strong></td>
<td>Prevents biofilm re-formation in the wound</td>
</tr>
</tbody>
</table>

Hübner NO, Kramer A. Review on the efficacy, safety and clinical applications of polihexanide, a modern wound antiseptic. Skin Pharmacol Physiol. 2010;23(suppl):17-27
Carpenter, S. D. (2016). Expert recommendations for optimizing outcomes in the management of biofilm to promote healing of chronic wounds. WOUNDS June, 1-19
Present Treatment Guidelines “Wish List” or Ideal Antibacterial Therapy

Limited Effectiveness Without Targeting the EPS STRUCTURE
BEFORE

AFTER

SLIME
HOW TO GET SLIME OUT OF EVERYTHING

Deconstruct & Disrupt EPS
**New Science-based Treatment Directly Targets the Biofilm Structure**

<table>
<thead>
<tr>
<th>Description</th>
<th>Deconstruct EPS</th>
<th>Antimicrobial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolves Biofilm Structure</td>
<td>NA</td>
<td>Broad antimicrobial spectrum</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>High Tissue compatibility (non-toxic)</td>
</tr>
<tr>
<td></td>
<td>✓</td>
<td>No microbial resistance</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>Sustained biofilm reformation barrier effects</td>
</tr>
<tr>
<td>Dry dressing</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Targeted antibiotics</td>
<td>No</td>
<td>✓</td>
</tr>
<tr>
<td>Topical antimicrobials</td>
<td>No</td>
<td>✓</td>
</tr>
<tr>
<td>OP sharp debridement</td>
<td>No</td>
<td>Non-selective</td>
</tr>
<tr>
<td>Biofilm disruption and microbial lysis – new technology</td>
<td>Yes</td>
<td>✓</td>
</tr>
</tbody>
</table>


A Science Driven Treatment
Simultaneous actions: target the structure, the pathogens, control the environment
Power of Xbio™ Technology from the Simultaneous Action of Four Ingredients

1. Citric Acid (Chelating Agent and Buffer)
2. Sodium Citrate (Buffering Agent)
3. Benzalkonium Chloride (Surfactant and Antimicrobial Within the Gel)
4. PEG Gel (Super hydrophilic Agent)
Initiate Deconstruction of the Structure

Citric Acid in the gel binds to the metallic bonds

While the Sodium Citrate buffers the solution to a pH of 4.
The Polymers are Rendered Inactive and the Structure is Dismantled

Sodium molecules split off and cap the free polymer ends, then the remaining Sodium citrate molecule converts to citric acid. This prevents the polymer from re-attaching and replenishes the citric acid sustaining the buffering process.
Destroy Pathogens

*Sodium citrate and Citric acid* in the gel mixture produce an osmotic pressure distending the bacteria cell wall.

The *Benzalkonium chloride* surfactant then attaches to the protein in the cell wall and removes it, aiding in cell lysis.
Defend from Recolonization

- Create a physical environment that prevents attachment of planktonic organisms, or biofilm regrowth and supports pro-healing processes.

Laboratory data on file.
Independent Testing Confirms BlastX Supersedes Current Therapy

*In vitro* studies demonstrate BlastX significantly reduced the number of biofilm bacteria present by 4 to 6 log for two common organisms compared to other topical antimicrobial therapies.

Data on File. Center for Biofilm Engineering at Montana State University. Next Science Report TR-10-12-004
**Persistent Biofilm Disruption Overtime**

Texas Tech *in-vivo* Murine Model of Infection Reduction in 24 hour biofilm growth with LUX-modified bacteria

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>BlastX</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours after</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Initial</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bacterial inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Hours after first</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BlastX Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 Hours after</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BlastX treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BlastX – Broad Spectrum Efficacy

Suspension Time Kill Testing, Planktonic Efficacy, 2 minute application

Log Reduction from Control (CFU)

- S. aureus
- P. aeruginosa
- B. Subtilis
- S. pyogenes
- K. pneumoniae
- A. baumannii
- E. coli
- C. albicans
- A. brasiliensis

Bacteria
Fungus
Biofilm Efficacy Versus Current Technologies

Montana State University - Center for Biofilm Engineering Results
- 72 hour biofilm, drip flow reactor, 24 hour treatment, ~8 log control

*S. aureus*
Biofilm Confocal Imaging Outcomes:
Green Cells = Live,
Red Cells = Dead

![Confocal Imaging Images]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log Reduction from Control (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Next Science BlastX</td>
<td>6.0</td>
</tr>
<tr>
<td>Silvasorb wound gel</td>
<td>1.0</td>
</tr>
<tr>
<td>Microcyn wound gel</td>
<td>1.0</td>
</tr>
</tbody>
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Biofilm Efficacy Versus Current Technologies

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- 72 hour biofilm, drip flow reactor, 24 hour treatment, ~8 log control

*P. aeruginosa*

Biofilm Confocal Imaging Outcomes:
- Green Cells = Live
- Red Cells = Dead

![Control](image)
![Next Science Wound Gel](image)
![Silvasorb Wound Gel](image)
![Microcyn Wound Gel](image)

![Log Reduction from Control (CFU)](chart)

Next Science BlastX
Silvasorb wound gel
Microcyn wound gel
Biofilm Efficacy Versus Current Technologies

Montana State University - Center for Biofilm Engineering Results - MIXED SPECIES
- 72 hour biofilm, drip flow reactor, 24 hour treatment, ~8 log control
Biofilm Efficacy Versus Current Technologies

WuXi AppTec in-vivo Infected Rat Model Testing Results – Chronic Wound Model

Infected Wound Rat Model - Infection Measurements

- Average Log Count (CFU/mL)

Infected Wound Rat Model - Wound Size Measurements

- Wound Area (%)
BlastX Clinical Trials
Two RCT’s Trials on Biofilm that Publish Key Wound Healing Parameters


Mayo Clinic Study Reveals ‘Real World’ Impact of Biofilm Disruption

- **Objective:** study the use of a biofilm-disrupting wound gel designed for wound management (BlastX) to determine if disrupting chronic wound biofilm would be therapeutically efficacious.

- A 12-week, prospective, randomized, open-label clinical trial with 43 patients (22 experimental group using biofilm disrupting gel; 21 patient control group using a broad-spectrum antimicrobial ointment).

- **Treatment failure patients from the Control Group** were allowed to cross over to the experimental group at 4 weeks (n=12)

- Wound Range: **1cm² to 114cm²**  
  Wound age: **4 weeks to more than 20 years** in duration

- Average Wound Size: **11cm²**  
  Average Wound Duration: **21 months**
Wound Area Reduction: $3x$ greater area reduction for BlastX 71% (including the cross over group) vs. 24% for control ($P < 0.001$).
Mayo Clinic Study

Wound Closure: 205%\(^*\) relative increase (RI) in healing versus control (52% BlastX (which includes the crossover patients) vs. 17% control) (\(P<.04\))

*Formula: \(\frac{N-O}{O} = RI\)
Wolcott Study: BlastX vs. SOC in Wound Healing

- Prospective, randomized, clinical trial 45 subjects with chronic wounds > one month duration randomized to either:
  - Mon-Wed-Fri application of BlastX
  - Mon-Wed-Fri application of custom topical antibiotics per culture result (SOC)
  - Mon-Wed-Fri application of BlastX plus custom topical antibiotic
- No differences in demographics (wound size, duration, diagnoses) between groups
- All wounds debrided weekly
- Endpoint:
  - Percent of wounds with 50% wound volume reduction at 4 weeks

Wolcott Study: Results

Demonstrated BlastX was more effective than standard of care/custom antibiotics

\[ P < 0.05 \]

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Clinical Use of BlastX
BlastX: Indications

- Pressure ulcers
- Partial- and full-thickness wounds
- Diabetic foot and leg wounds
- Post-surgical wounds
- First and second degree burns
- Grafted and donor sites
BlastX: Availability

- 1 oz. tube (30ml)
- ¼ oz. tube (7.5ml)
BlastX: How to Use

- Apply to wound in a 3 mm layer – the thickness of a nickel
BlastX: How to Use

- Cover with appropriate dressing, based on amount of exudate:
  - Use exudate absorbers for high exudate with or without contact layer – foams, gauze, pads, hydrofibers
  - Use petrolatum based contact layers for low exudate, or foams with film backing, which are more moisture-retentive

- Can use before applying NPWT sponge

- Apply every 1-3 days, depending on dressing change protocol
BlastX: How to Use

- BlastX should not be used if there is a history of allergy to any of the ingredients.

- Do not utilize alginates with BlastX.

- Do not use with other antimicrobials – not necessary.
Clinical Experience – Case Studies
AZH Wound & Vascular Centers - Milwaukee
CASE STUDY

- 42 year old male
- Multiple Sclerosis
  - wheelchair bound
- Significant venous insufficiency and lymphedema
- Referred for “second opinion” after 2 years of management by Dermatologist for “Pyoderma Gangrenosum”
01/29/2018 BlastX Start
CASE STUDY

- 63 year old male
- PMH: PAD, DM
- Presented with a right foot necrotizing cellulitis and deep abscess
What we learned:

- **Wound Healing Interrupted** – biofilm causes prolonged inflammation and halts wound healing
- **What is Biofilm** – community of pathogens protected by POLYMERIC STRUCTURE
- **Current Trends in Treatment** – combination of debridement / topicals / dressings; not fully effective
- **New Science** – elegant chemistry to dismantle EPS, destroy pathogens, and defend against reformation
Thank You and Questions
To Show My Appreciation…

➢ For allowing me the opportunity to participate in this excellent educational opportunity & conference
➢ For sharing some wonderful South Texas hospitality
➢ For listening to my presentation
➢ For your attentiveness and insightful questions
➢ For you rating my presentation with “all 5’s” on your evaluation forms
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This educational activity is endorsed by the American College of Hyperbaric Medicine & American Professional Wound Care Association.
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