Biofilm Understanding
Dr. Matthew Regulski DPM
Director, Wound Institute of Ocean County NJ

Don’t Stop Your Curiosity

Human Microbiome
“Good Bacteria” in a wound?

Commensals are considered microbes that provide benefits to the host organism. Notably, these interactions require redundant, complex host/microbe interactions that involve various host systems, including dendritic cells, keratinocytes, and antimicrobial peptides (defensins, alarmins, phenol soluble modulins, lipopeptides), which do not exist in the wound bed.

Answer – NO Good bacteria in a wound.

Medical Biofilms

Medical Biofilms

Medical Biofilm US Incidence Annual Cost

Diabetic foot ulcers (P) 3 M 50,000 deaths, 30% of hospital cost for diabetics
Venous leg ulcers (P) 2.5 M
Decubitus ulcers (P) 3-5 M
Surgical site infections 500,000 $5-10 B, 5,000 deaths
Burn wounds 1.1 M 15,000 deaths
Chronic meningitis 1,400-2,800 140-390 deaths
Bacterial prostatitis (P) 162,800
All odontogenic infections
Chronic tonsillitis 11,000 $121.5 M
Gallstones 430,000
Pneumonia (non-VAP) 1.2 M $14-$25 B, 54,000 deaths
Vascular graft infection 16,000 $640 M
Cardiac pacemakers 4,000-20,000
Peritoneal dialysis peritonitis ~20-25,000 on CPD
Ventilator acquired pneumonia 135,000 $1.5 B, 61,000 deaths
Endotracheal tubes 100s of thousands * $5 B
Nosocomial Central venous catheters 250,000 $296 M-$2.3 B, 30-62.5 K deaths
Total 20 Million $100 B, >500k deaths
Medical Biofilms
Context

<table>
<thead>
<tr>
<th>Disease</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>Cardiovascular Disease</td>
<td>2.28 M per year</td>
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<tr>
<td>Cancer</td>
<td>1.5 M per year</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.5 M per year</td>
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<tr>
<td>Medical Biofilm</td>
<td>&gt; 10M per year</td>
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For Comparison

Medical Biofilm > 10M per year > $200 B, > 500,000 deaths

First Medical Device
Biofilm

PCR - Panel of Microbes 8-20

NGS - Next Generation Sequencing

Barrier to Technology
Cost and Time to Deliver

Microbial DNA

Cost and Time to Deliver

25,000 Species
All pyrosequencing procedures were performed at the Research and Testing Laboratory (Lubbock, TX) in accordance with well-established protocols [62].
The microbiome of diabetic foot osteomyelitis

No pathogens were identified in 8 out of the 34 bone samples (23.5 %) with conventional culture techniques. Two out of those eight negative samples did not sequence either.

Our results show that, by using a 16S rRNA sequencing technique, anaerobes were detected in 86.9 % of the positive bone samples (vs. 23.1 % with conventional techniques).

Biofilm Infection

- (a) Bacteria adhered to surface
- (b) Direct visualization of biofilm morphology
- (a) Confined to a particular location
- (b) Resistant to appropriate antibiotics
- (b) Resistant to biocides
- (b) Large number with high diversity in a host lesion
- (b) Infections that wax and wane with exacerbations
- (b) Secondary signs of infection
- (a) Surface select (but is not necessary) for biofilm formation
- (a) Bacterial community in biofilm
- (a) Biofilm seems to limit its size (quorum sensing)
- (a) Elevated proinflammatory cytokines
- (a) Elevated proteinase activity – MMPs
- (a) Diminished activity of growth factors
- (a) Degraded receptor sites (degradation blocked by the addition of MMP inhibitors)

Biochemical Impairment of Chronic Wounds

- Elevated proinflammatory cytokines
- Elevated proteinase activity – MMPs
- Diminished activity of growth factors
- Degraded receptor sites (degradation blocked by the addition of MMP inhibitors)
Biofilm Development

Host Defenses

Molecular Mechanisms

- Staphylococcus Heme Sensing System

... resulting in the tempering of virulence to avoid excessive host tissue damage.

Torres  A Staphylococcus aureus Regulatory System that Responds to Host Heme and Modulates Virulence Cell Host and Microbe Vol 1, 2007
Neutrophils


Biofilms and Chronic Wound Inflammation. JWC Vol 17, 2008

Diegelmann RF. Wound Repair Regen Vol 11 2003

Senescence

Putting Enterohemorrhagic E. coli on a Pedestal (Mar 2009)

Molecular Mechanisms of Senescence

"...new paradigm of bacterial pathogenesis..."

Kim Cell Host and Microbe Vol 8(1), July 2010
**Planktonic Bacteria**
- Single Cell
- Cause of Acute Infection
- Will grow in traditional Culture

**Bio Film**
- "Community of Bacteria"
- Cause of 80% of Infections
- Cause of Chronic Wounds
- Will not grow in traditional culture
- Biofilm cells express a radically different phenotype than planktonic bacteria
- Only Diagnostic tool is Molecular

**Molecular Diagnostics of Nail Samples With Clinical Signs of Infection**
- 50% of Nail Samples had fungal Species Identified
- 50% of Nail samples had no fungal species but did have Bacteria species identified
- Nails with Fungal species identified also had Bacteria
- 10,000 Nail Samples

**Economic Impact in Diabetic Foot Wounds**
- Treatment in 2005
  - 189 patients
  - Culture and Systemic Antibiotics
  - Total Payments for related codes: $11,444
- Treatment in 2013
  - 215 Patients
  - Molecular and Topical Antibiotics
  - Total Payments for related codes: $3,060

Reduction in total charges of 68%
DNA Analysis vs. Traditional Cultures
51 Chronic wounds - Parallel Samples

16S DNA Sequencing
- 46/51 Staph Identified
- 32/51 Pseud Identified

Traditional Culture
- 28/51 Staph Identified
- 8/51 Pseud Identified

DNA Sequencing Outperformed Traditional Cultures
DNA Sequencing identified 145 Genera – Cultures 14!

Attachment

Extracellular Polymeric Substance
SEM of P. aeruginosa Biofilm
Control
Clarithromycin
Biofilm Development

Biofilm 3-D Structure

Biofilm 3-D Structure
Biofilm Detachment

Detached cell cluster from Staphylococcus aureus biofilm

Detachment Rate

Cumulative detached area (μm²)
Eradicating Detachment
Fragments
- Red is dead
- Reconstitute

Quorum Sensing
Stephlococcus aureus

Biofilm Defenses
Mechanisms of Biofilm Tolerance
Antimicrobial Depletion
- Slow Penetration
- Stress Response
- Altered Microenvironment
- Persisters
Slow Penetration

Glutaraldehyde (50 mg/L) Against *Ps. aeruginosa* Biofilm

- *Biofilm*
- *Planktonic*
- Log Reduction
- Time (min)

Biocides vs. Biofilm

- *Vanc vs Staph biofilm >1000x*
- *Tobra vs Pseudomonas biofilm*

Antibiotic Resistance

Biofilm

- *Vanc vs Staph biofilm >1000x*
- *Tobra vs Pseudomonas biofilm*
"The impact of proper specimen management on patient care is enormous"

"Specimen selection and collection are the responsibility of the medical staff and not the laboratory"
Nonviable Bacteria DNA

Bacterial Biofilms and Chronic Rhinosinusitis
Kilty and Desrosiers

“Most convincing was the demonstration in a chinchilla model that live bacteria, although nonculturable, could persist in OME for weeks, whereas DNA strands and DNA from intact but nonviable bacteria could not exist for more than a day [21].”

“Given the extremely short half-life of mRNA, its presence was evidence that viable metabolically active bacterial organisms were likely present in OME.”

“I need my Sensitivity for antibiotics”

“Biofilm-growing microorganisms are significantly more tolerant to antibiotics [6] and corresponding breakpoints have not been established [34]. The S-I-R results can therefore not be used to predict therapeutic success in the case of biofilm infections and offer no guide to clinicians for treating such infections.”

ESCMID Guidelines for the diagnosis and treatment of biofilm infections
Corynebacterium tuberculostearicum: a Potentially Misidentified and Multiresistant Corynebacterium Species Isolated from Clinical Specimens

Nevertheless, partial 16S rRNA gene sequencing still represents the gold standard for the identification of this species. Due to the challenging identification of C. tuberculostearicum, we presume that this organism is often misidentified and its clinical relevance is underestimated.
COST AND COVERAGE OF MOLECULAR DIAGNOSTICS

TRADITIONAL CULTURE
MEDICARE PAYS $250

DNA SEQUENCING
MEDICARE PAYS $285

CUSTOMIZED FOR EACH PATIENT
TOPICAL RX
LIPOGEL BASED
DOSED 3X WEEK
CONTAINS ANTIBIOTICS
ANTIFUNGALS

Benefits DNA Molecular Testing

- Fast Identification of Biofilms and their composition in patients referred to you—**Accuracy and Speed.**
- **GOLD STANDARD** of Microbial Diagnostics
- Medicare Covers the Test and pay $190
- Less Expensive than a Culture

**MOLECULAR DIAGNOSTICS AND PERSONALIZED MEDICINE IN WOUND CARE / ASSESSMENT OF OUTCOMES**

<table>
<thead>
<tr>
<th>Standard of Care Group</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional Culture with Systemic Antibiotics</td>
<td>Molecular Diagnostics with Systemic Antibiotics</td>
<td>Molecular Diagnostics with Customized Topical Antibiotics</td>
</tr>
<tr>
<td>% of Patient Healed</td>
<td>% of Patients Healed</td>
<td>% of Patients Healed</td>
</tr>
<tr>
<td>48.5%</td>
<td>62.4%</td>
<td>90.4%</td>
</tr>
</tbody>
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**Median Number of Days to Heal by Wound Type**

<table>
<thead>
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<th>Wound Type</th>
<th>Standard of Care</th>
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<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Ulcer</td>
<td>N/A</td>
<td>107</td>
<td>28</td>
</tr>
<tr>
<td>Diabetic Foot Ulcer</td>
<td>168</td>
<td>84</td>
<td>32</td>
</tr>
<tr>
<td>Non-Healing Surgical Wound</td>
<td>176</td>
<td>75</td>
<td>44</td>
</tr>
<tr>
<td>Traumatic Abscess</td>
<td>39</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>Venous Leg Ulcer</td>
<td>177</td>
<td>98</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>177</td>
<td>77 (p&lt;0.001)</td>
<td>28 (p&lt;0.001)</td>
</tr>
</tbody>
</table>
God always takes the simplest way.
Albert Einstein

Always listen to good advice...