The Biofilm Hypothesis of Chronic Infection

Prof. Philip

A Webber Training Teleclass

Center for Biofilm Engineering

The Biofilm Hypothesis of Chronic Infection

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Hosted by Martin Kiernan

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www.webbertraining.com

The Black Death

Victims... “ate lunch with their friends and dinner with their ancestors in paradise.”

Boccaccio

Cholera

“What frightened people about cholera was the speed at which it struck the victim and brought about a painful death.”

Drinking from the Ganges River during a Hindu ceremony

Staph Biofilm

Biofilm on pacemaker lead

Staphylococci on a pacemaker lead

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Biofilm in CF pneumonia
Genetic defect in chloride ion channel
Lung is never cleared of bacteria despite aggressive chemotherapy
Massive neutrophil invasion contributes to gradual loss of lung function
Evidence of low oxygen/anaerobic bacterial metabolism

Biofilm in periodontitis
Tooth surface poorly defended compared to vascularized tissues
Tetracycline, antiseptic mouthrinses have little efficacy
Host responses, bacterial virulence factors lead to progressive bone loss
Malodor

Biofilm in chronic wounds
Biofilm forms on necrotic tissues
Symptoms wax and wane over weeks to months
Marginal response to topical antimicrobials, systemic antibiotics
Normal healing process arrested
Malodor

Biofilm in chronic osteomyelitis
Biofilm forms on dead bone
Infection persists despite antibiotic therapy
Involucrum of fibrous tissue decreases vascularization of the infection site

Features of biofilm infections
- Form preferentially on foreign bodies, dead or damaged tissue
- Slow to develop, but persistent
- Respond poorly or only temporarily to antibiotics, antiseptics
- Collateral damage to neighboring healthy tissue
- Anoxic niches, anaerobic metabolism

Planktonic vs Biofilm
“Predatory”
“Parasitic”

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Glass capillary biofilm reactor

S. epidermidis biofilm

S. epidermidis biofilm - TEM

Visualizing antimicrobial action

Treatment with 50 mg/l quat

Treatment with 50 mg/l chlorine

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## Treatment with 50 mg/l nisin

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Image 1</th>
<th>Image 2</th>
<th>Image 3</th>
<th>Image 4</th>
<th>Image 5</th>
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<td><img src="image2.png" alt="Image 2" /></td>
<td><img src="image3.png" alt="Image 3" /></td>
<td><img src="image4.png" alt="Image 4" /></td>
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<td><img src="image4.png" alt="Image 4" /></td>
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<tr>
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<td><img src="image4.png" alt="Image 4" /></td>
<td><img src="image5.png" alt="Image 5" /></td>
</tr>
</tbody>
</table>


## Daptomycin

![Daptomycin](image6.png)


## Daptomycin penetration in biofilm

![Daptomycin penetration](image7.png)


## H$_2$O$_2$ fails to penetrate biofilm

![H$_2$O$_2$ penetration](image8.png)


## Antimicrobial penetration

- Antibiotics, peptides penetrate readily
- Reactive oxidants may fail to penetrate

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Colony biofilm

3-day old *S. aureus* colony biofilm

*S. epidermidis* killing by rifampin

*S. epidermidis* colony biofilm

DNA synthetic activity in biofilm

Oxygen profile in colony biofilm

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Physiological heterogeneity
- Growing aerobically
- Growing fermentatively
- Dead
- Dormant

K. pneumoniae biofilm - growth

Protein synthetic activity in biofilm

Pseudomonas aeruginosa biofilms consensus genes - 10 studies

Transcriptomic
- Whiteley 2001
- Hentzer 2005
- Waite 2006
- Mikkelsen 2009
- Folsom 2010

Proteomic
- Sauer 2002
- Seyer 2005
- Southey-Pillig 2005
- Mikkelsen 2007
- Patrauchan 2007

Top 26 genes in Pseudomonas aeruginosa biofilms

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
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<tbody>
<tr>
<td>PA0139</td>
<td>PA1905</td>
<td>PA4236</td>
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<tr>
<td>PA0263</td>
<td>PA2274</td>
<td>PA4352</td>
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<td>PA0515</td>
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<td>PA4610</td>
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<td>PA0588</td>
<td>PA2782</td>
<td>PA5427</td>
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<td>PA0713</td>
<td>PA3126</td>
<td>PA5460</td>
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<td>PA1555</td>
<td>PA3309</td>
<td>PA5475</td>
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<td>PA1556</td>
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<td>PA4067</td>
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<td>PA1746</td>
<td>PA4211</td>
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<td>PA1904</td>
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Pa biofilm consensus genes

<table>
<thead>
<tr>
<th>Activity</th>
<th>P-value</th>
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<tr>
<td>Oxygen downshift</td>
<td>~0</td>
</tr>
<tr>
<td>Oxygen limitation</td>
<td>4x10^-11</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>8x10^-10</td>
</tr>
<tr>
<td>Phenazine biosynthesis</td>
<td>2x10^-6</td>
</tr>
<tr>
<td>Peroxide stress</td>
<td>0.02</td>
</tr>
<tr>
<td>Iron limitation</td>
<td>0.25</td>
</tr>
<tr>
<td>HSL quorum sensing</td>
<td>0.29</td>
</tr>
<tr>
<td>Mg limitation</td>
<td>0.76</td>
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<tr>
<td>Oxidative stress</td>
<td>0.77</td>
</tr>
<tr>
<td>Efflux pumps</td>
<td>0.83</td>
</tr>
<tr>
<td>c-di-GMP</td>
<td>0.84</td>
</tr>
<tr>
<td>Nitrosative stress</td>
<td>0.88</td>
</tr>
</tbody>
</table>

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Table 3. Activities or functions in P. aeruginosa biofilms as indicated by overlap with independently derived gene lists.

<table>
<thead>
<tr>
<th>Unstressed, susceptible</th>
<th>Stress response induced, protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial exposure</td>
<td></td>
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</tbody>
</table>

Table 3. Catalase induction in Pa biofilm

![Catalase induction graph](image)

katB-lacZ


Summary

- Biofilms can cause chronic infections
- Bacteria in biofilms evade killing by host antimicrobials and antibiotics
- Multiple mechanisms of protection in biofilms

Coming Soon

01 March Developing a Sustainable and Effective Approach to Hygiene and Infection Prevention in Home and Everyday Life Settings
Speaker: Dr. Sally Bloomfield, International Scientific Forum on Home Hygiene
Sponsor: World Health Organization First Global Patient Safety Challenge

07 March Achievements in Improving Injection Safety Worldwide
Speaker: Prof. Chuck Gerba, University of Arizona
Sponsor: World Health Organization First Global Patient Safety Challenge

22 March Hand Hygiene: New Frontiers in Messaging and Measurement
Speaker: Dr. Katherine Ellingson, Centers for Disease Control
Sponsor: Diversey Inc.

29 March Water and Infection Control
Speaker: Andrew Streifel, University of Minnesota

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