Understanding Biofilms

Thomas Bjarnsholt
Professor, DMSc & PhD
Understanding biofilms
Are biofilms always bad?

A. Yes
B. No
In and on the human body

**THE HUMAN**
Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it’s believed over 1,000 different species live in and on the body.

**MICROBIOME**

**600+ SPECIES**
in the mouth, pharynx and respiratory system include:
- Streptococcus viridans
- Neisseria sica
- Candida albicans
- Streptococcus salivarius

**25 SPECIES**
in the stomach include:
- Helicobacter pylori
- Streptococcus thermophilus

**500-1,000 SPECIES**
in the intestines include:
- Lactobacillus casei
- Lactobacillus reuteri
- Lactobacillus gasseri
- Escherichia coli
- Bacteroides fragilis
- Bacteroides thetaiotaomicron
- Lactobacillus rhamnosus
- Clostridium difficile

**1,000 SPECIES**
in the skin include:
- Pityrosporum ovale
- Staphylococcus epidermidis
- Corynebacterium jeikeium
- Trichosporon
- Staphylococcus haemolyticus

**60 SPECIES**
in the urogenital tract include:
- Ureaplasma parvum
- Corynebacterium aurimucosum

**SOURCES:** NATIONAL INSTITUTES OF HEALTH, SCIENTIFIC AMERICAN, HUMAN MICROBIOME PROJECT

Dean Tweed - POSTMEDIA NEWS / IMAGE: Fotolia
The problem
Planktonic vs. biofilm

- Study from 1956.
- Injected 7,500,000 CFU *S. aureus* in skin of human volunteers = only 50% infected, all resolved
- < 100 CFU onto an implant in humans = 100% infected, did not resolve
- Implants or dead tissue ↑ virulence over 75,000 fold.

Bacteria adhere to tissue (e.g. Bone, Kidney) or colonize device
Biofilms and antibiotics

Traditional Antibiotic treatment

Biofilm effect

Planktonic effect

Antibiotic concentration

Time after administration
MBEC and MBIC

- MBIC – Minimal Biofilm Inhibitory Concentration
- MBEC – Minimal Biofilm Eradication Concentration

Mice were treated with a single intraperitoneal dose of either colistin (16 mg per kg) or imipenem (64 mg per kg).

Cystic fibrosis – the classical example

CF male, 28 years of chronic PA infection

2 week anti PA treatments
20 years daily colistin/tobramycin inhalations

1 kg tobramycin,
10 kg beta-lactam anti-pseudomonas antibiotics
and 1 kg inhaled colistin

Bjarnsholt et al; Pseudomonas aeruginosa biofilms in the Respiratory Tract of Cystic Fibrosis Patients; Pediatr Pulmonol. 2009 Jun;44(6):547-58
Biofilms in chronic wounds

Bjarnsholt et al; Wound Repair and Regeneration, 2008 Jan-Feb;16(1):2-10.
A present problem:

Adverse reactions to polyacrylamide gel are seen as swellings or nodules, and controversy exists whether these are due to bacterial infection or an autoimmune reaction to the filler.

4 days after steroid

Courtesy of Lise Christensen
<table>
<thead>
<tr>
<th>site</th>
<th>Type of PAAG</th>
<th>Time since inj</th>
<th>Initial treatment</th>
<th>Time with AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheek</td>
<td>Aquamid</td>
<td>2 years</td>
<td>Steroid inj, Later AB inj</td>
<td>7 months</td>
</tr>
<tr>
<td>Lip</td>
<td>Aquamid</td>
<td>½ year</td>
<td>Steroid inj, Later ABs</td>
<td>½ year</td>
</tr>
<tr>
<td>Lip</td>
<td>Aquamid</td>
<td>1 month</td>
<td>Steroid + ABs, Later ABs</td>
<td>2 years</td>
</tr>
<tr>
<td>Breast</td>
<td>Amazing gel</td>
<td>2 years</td>
<td>ABs, liposuction</td>
<td>5 months</td>
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<tr>
<td>Tear-trough</td>
<td>Aquamid</td>
<td>2 years</td>
<td>Steroid inj, Later ABs</td>
<td>½ year</td>
</tr>
<tr>
<td>Naso-labial fold</td>
<td>Aquamid</td>
<td>14 days</td>
<td>Steroid ABs+surgery</td>
<td>1½ year</td>
</tr>
<tr>
<td>Lip</td>
<td>Interfall gel</td>
<td>½ year</td>
<td>Steroid ABs+surgery</td>
<td>2½ years</td>
</tr>
<tr>
<td>Penis</td>
<td>Aquamid</td>
<td>2 years</td>
<td>Steroid AB inj+surgery</td>
<td>1½ years</td>
</tr>
</tbody>
</table>
Soft tissue fillers

What is a biofilm

A coherent cluster of bacterial cells imbedded in a matrix” – which are more tolerant to most antimicrobials and the host defence, than planktonic bacterial cells.

Significance of Biofilm infections

- Chronic long-term infections
- Frequently recalcitrant to antibiotic treatment
- Resistant to host defences, such as phagocytosis and killing
- Difficult to treat, in medical implant infections, the only cure may be removal
- Difficult to diagnose
The clinical biofilm

- What?
- Where?
- How to sample?
- How to diagnose?

For diagnosing biofilms in infections, what is the most difficult?
Biofilms in chronic wounds

Bjarnsholt et al; Wound Repair and Regeneration, 2008 Jan-Feb;16(1):2-10.
Correlates with the findings by Gjødsbøl et al.

Sampling

Correlates with the findings by Gjødsbøl et al.

Are bacteria in biofilms culture negative?

Are bacteria in biofilms unculturable?

A. Yes
B. No
NO, but they have to be “sampled” to enable growth.
Multi-species biofilms vs. Multi-species infections

True for 2 out of 13 wounds
Mono-species biofilms vs. Multi-species infections

True for 11 out of 13 wounds
The opportunity!

- Average 5.4 species per wound  
  Thomsen...Bjarnsholt et al (WRR 2010)
- Average 3 species per CF lung  
  Rudkjøbing...Bjarnsholt et al (JCM 2012)
- Many species together in the environment

Dental biofilm


- Intestine
- Soil
- Submerged surfaces
- Pipelines

Okabe et al; APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1999, p. 5107–5116
Where?

McConoughey et al. 2014
Distribution of species


S. aureus

P. aeruginosa

Number of wounds

Distance to wound surface (µm)

S. aureus biofilm

P. aeruginosa biofilm
Heterogeneous distribution of bacteria- Chronic wounds

qPCR *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Position</th>
<th>Wound 1</th>
<th>Wound 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>510±18%</td>
<td>920±9%</td>
</tr>
<tr>
<td>3</td>
<td>No sample</td>
<td>300±13%</td>
</tr>
<tr>
<td>6</td>
<td>760±7%</td>
<td>8200±8%</td>
</tr>
<tr>
<td>9</td>
<td>47±9%</td>
<td>800±10%</td>
</tr>
<tr>
<td>12</td>
<td>280±3%</td>
<td>15±5%</td>
</tr>
</tbody>
</table>


Picture from homepage of Montana State University
For identification of biofilms, what should we look for?

A. Bacteria on a surface
B. Large biofilms
C. Bacteria and inflammation
The *in vitro* biofilm

Janus Haagensen
The *in vivo* Biofilm

- No mushrooms
- Small microcolonies
- Additional layer of host material
- Host defense/inflammation
- Heterogeneous distribution
- Not surface dependable

Bjarnsholt et al. Trends in Microbiology. Trends Microbiol. 2013 Sep;21(9):466-74
<table>
<thead>
<tr>
<th>Biofilm demonstrated in</th>
<th>Visualization method</th>
<th>Approximately diameter size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung infections</td>
<td>Light microscopy</td>
<td>~4-8 µm</td>
<td>Høiby 1977&lt;sup&gt;5&lt;/sup&gt;,</td>
</tr>
<tr>
<td></td>
<td>Light microscopy</td>
<td>~5-100 µm</td>
<td>Baltimore 1989&lt;sup&gt;3&lt;/sup&gt;,</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>~5-100 µm</td>
<td>Kirketerp-Møller&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>~5-50 µm</td>
<td>Løfmann&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chronic wounds</td>
<td>FISH</td>
<td>~5-200 µm</td>
<td>Bjarnsholt 2008&lt;sup&gt;64&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Light and electron microscopy</td>
<td>~35-55 µm</td>
<td>James 2008&lt;sup&gt;75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soft tissue fillers</td>
<td>FISH</td>
<td>~5-25 µm</td>
<td>Bjarnsholt&lt;sup&gt;65&lt;/sup&gt;</td>
</tr>
<tr>
<td>Otitis media</td>
<td>FISH</td>
<td>~15-25 µm</td>
<td>Hall-Stoodley 2006&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>~10-80 µm</td>
<td>Nistico 2011&lt;sup&gt;77&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>~4-40 µm</td>
<td></td>
<td>Homoe&lt;sup&gt;67&lt;/sup&gt;</td>
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<tr>
<td>Implant etc associated</td>
<td>Electron microscopy</td>
<td>~500 µm</td>
<td>Marrie 1982&lt;sup&gt;78&lt;/sup&gt;,</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>~50 µm</td>
<td>Waar 2005&lt;sup&gt;79&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Electron microscopy</td>
<td>~5-15 µm</td>
<td>Costerton 2010&lt;sup&gt;80&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>~5-30 µm</td>
<td>Veeh 2003&lt;sup&gt;81&lt;/sup&gt;</td>
</tr>
<tr>
<td>Catheter and shunt associated</td>
<td>Electron microscopy</td>
<td>~5-1000 µm</td>
<td>Marrie 1983&lt;sup&gt;82&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Electron microscopy</td>
<td>~20-500 µm</td>
<td>Marrie 1984&lt;sup&gt;83&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Fluorescence microscopy</td>
<td>~20-1200 µm</td>
<td>Stoodley 2010&lt;sup&gt;84&lt;/sup&gt;</td>
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<tr>
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<td>FISH and electron microscopy</td>
<td>&gt;1000 µm</td>
<td>Parsa 2010&lt;sup&gt;85&lt;/sup&gt;</td>
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<tr>
<td>Chronic osteomylistis</td>
<td>Electron microscopy</td>
<td>~25 µm</td>
<td>Gristina 1985&lt;sup&gt;86&lt;/sup&gt;,</td>
</tr>
<tr>
<td></td>
<td>Electron microscopy</td>
<td>~25 µm</td>
<td>Marrie 1985&lt;sup&gt;87&lt;/sup&gt;,</td>
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<tr>
<td></td>
<td>Light and electron microscopy</td>
<td>~5-50 µm</td>
<td>Sedghizadeh 2009&lt;sup&gt;88&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chronic Rhinosinusitis</td>
<td>Electron microscopy</td>
<td>~5-30 µm</td>
<td>Cryer 2004&lt;sup&gt;89&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Fluorescence microscopy</td>
<td>~5-20 µm</td>
<td>Li 2011&lt;sup&gt;90&lt;/sup&gt;</td>
</tr>
<tr>
<td>Contact linses</td>
<td>Electron microscopy</td>
<td>~50-100 µm</td>
<td>Stapleton 1995&lt;sup&gt;91&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Two ongoing projects

Herniation
Unibac-TXR, Pacnes-FITC, DAPI

Breast implant tissue
with anaplastic large cell lymphoma
Unibac-TXR, DAPI
Second matrix and inflammation
The second matrix

Folsom et al. BMC Microbiology 2010, 10:294

Fazli et al JCM 2009
Surface or not to surface that is not the question

Single layer of attached bacteria

By Maria van Gennip (Alhedé)
But size does not matter!
PMNs versus biofilm bacteria

## Diagnosis

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Pitfalls and difficulties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culturing</strong></td>
<td>Bacterial presence is confirmed</td>
<td>Heterogeneous distribution</td>
</tr>
<tr>
<td></td>
<td>Antibiotic susceptibility</td>
<td>Finding the focus</td>
</tr>
<tr>
<td></td>
<td>Direct quantification</td>
<td>Pathogens vs. contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biofilms or planktonic samples can be culture-negative</td>
</tr>
<tr>
<td><strong>PCR etc</strong></td>
<td>Fast results even when culture is negative</td>
<td>Heterogeneous distribution</td>
</tr>
<tr>
<td></td>
<td>Low cut-off (used to be $10^{5-6}$)</td>
<td>Finding the focus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pathogens vs. contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biofilm or planktonic</td>
</tr>
<tr>
<td><strong>Microscopy</strong></td>
<td>Biofilms are confirmed</td>
<td>Heterogeneous distribution</td>
</tr>
<tr>
<td></td>
<td>Interactions with tissues</td>
<td>Finding the focus</td>
</tr>
<tr>
<td></td>
<td>Inflammatory cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Results even when culture-negative</td>
<td></td>
</tr>
</tbody>
</table>
Online Lecture Library

Slide withheld at request of author
What if we cannot see anything?

Medical history of biofilm predisposing condition (implanted medical device, CF, IE, chronic)

Recurrence of the infection (particularly if evidence is provided that the same organism is responsible at multiple time points)

Documented evidence/history of antibiotic failure or persistent infection despite adequate choice of antibiotic agent

Evidence of local or systemic signs and symptoms of infection that resolve with antibiotic therapy, only to recur after therapy has ceased such as:

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Towards Diagnostic Guidelines for Biofilm-Associated Infections;
FEMS Immunol Med Microbiol. 2012 Apr
Slide withheld at request of author
Next step!

- To look again
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