Multispecies biofilms in chronic infections: clinical impact and treatment considerations

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Are biofilms always bad?

A. Yes
B. No
Everywhere a liquid flows across a surface...
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.

**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 rhamnusus
- Clostridium difficile

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

**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
In the wrong place in the human body

Voice Prosthesis

Caries

Catheter
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.

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?
- What to treat?
Biofilms in chronic wounds

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

Correlates with the findings by Gjødsbøl et al.

- Wounds in total: 22
- Wounds with bacteria (culturing): 19
- Wounds with aggregates (FISH)*: 13
- Wounds with S. aureus (culturing): 12
- Wounds with S. aureus (FISH): 2
- Wounds with P. aeruginosa (culturing): 5
- Wounds with P. aeruginosa (FISH): 9

p<0.02
p<0.0001

Kirketerp-Møller.... and Bjarnsholt; The distribution, organization and ecology of bacteria in chronic wounds; J Clin Microbiol. 2008 Aug;46(8):2717-22
### Sampling

**Are bacteria in biofilms culture negative?**

<table>
<thead>
<tr>
<th>Wounds</th>
<th>Number</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wounds in total</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Wounds with bacteria (culturing)</td>
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<td>p&lt;0.0001</td>
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Kirketerp-Møller.... and Bjarnsholt; The distribution, organization and ecology of bacteria in chronic wounds; J Clin Microbiol. 2008 Aug;46(8):2717-22
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
Cystisk fibrose

1-3 pathogens per lung
Where?

McConoughey et al. 2014
Distribution of species

S. aureus  P. aeruginosa

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>C</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
The *in vivo* Biofilm

- No mushrooms
- 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
Two ongoing projects

Herniation
Unibac-TXR, Pacnes-FITC, DAPI

Breast implant tissue with anaplastic large cell lymphoma
Unibac-TXR, DAPI
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
Model biofilm for studies of interspecies interactions

1 Pseudomonas lutea
2 Stenotrophomonas rhizophila
3 Xanthomonas retroflexus
4 Ochrobactrum rhizosphaerae
5 Microbacterium oxydans
6 Arthrobacter nitroguajacolicus
7 Paenibacillus amylolyticus

- Single species and combinations of 4 species
- Quantification by use of crystal violet assay (Calgary device)

Biofilm formation of single- and four species

2357 - more than 300% increase in biofilm biomass

Ren et al. 2015 ISME J.
How does a chronic infection initiate?

Infections are not ecosystems!
No synergism
ESCMID GUIDELINES

ESCMID* guideline for the diagnosis and treatment of biofilm infections
2014

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• How to study chronic infections
  – Multispecies infections or multispecies biofilms?
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•  [www.coursera.org/course/bacteria](http://www.coursera.org/course/bacteria)