Understanding Biofilms – Diagnostics

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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.

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

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

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 ariumcosum

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

Dean Tweed - POSTMEDIA NEWS / IMAGE: Fotolia

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The problem
• 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


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Biofilms and antibiotics

Traditional Antibiotic treatment

Biofilm effect

Planktonic effect

Time after administration

Antibiotic concentration
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.

Biofilm clinical vs. in vitro

Be careful with too much extrapolation
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?
Where?

Non-Tunneled Central Venous Access Device
Sampling from a surface

• Improved Detection of Biofilm-formative Bacteria by Vortexing and Sonication: A Pilot Study
  – Kobayashi, H. et al; Clin Orthop Relat Res; 2009 May;467(5),

• Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro.

• Brief ultrasonication improves detection of biofilm-formative bacteria around a metal implant.
  – Kobayashi, N. et al; Clin Orthop Relat Res. 2007 Apr;457:210-3

• Is the bacteria on the implant or in the tissue?
Surface or not to surface that is not the question

## Diagnosis

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

**Bjarnsholt T.**
The role of bacterial biofilms in chronic infections.  
Fischer’s exact test  
(p=0.001)
Summing up:

• Growth might not be the golden standard
  – Biofilm bacteria might be hidden
  – Biofilm bacteria might be attached
• However, it is not always because bacteria in biofilm cannot be cultured
• Combination of growth and different molecular detection methods
• But you still need to sample right!
What if we cannot see anything?

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

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


So will the biofilm concept change the diagnostic approach for CLABSI pathogen detection?
Prevention?

- Bacteria primarily found in the stratum corneum
  - (Example of bacteria in hair follicle)

Literature:
- 85% in stratum corneum
- 25% in hair follicle
  - Lange-Asschenfeldt et al. 2011

Anti-infective lock therapy!!!
Next step!

- To look again
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