Microbial Life on surfaces - Understanding Biofilms, their diagnosis and treatment

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Understanding biofilms
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 proper brain development. The Human Microbiome Project is using the genomes of 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 species
  - Candida albicans
  - Streptococcus salivarius

- **1,000 SPECIES** in the skin include:
  - Pityrosporum ovale
  - Staphylococcus epidermidis
  - Corynebacterium jeikeium
  - T. mentagrophytes
  - Staphylococcus hominis

- **500-1,000 SPECIES** in the intestines include:
  - Lactobacillus rhamnosus
  - Lactobacillus reuteri
  - Lactobacillus gasseri
  - Escherichia coli
  - B. longum
  - B. breve
  - B. fragilis
  - B. longum
  - B. infantis

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

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

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

**Image Credit:** Donal Timoty / POSTMEDIA NEWS / IMAGE: Flicka
Human skin Microbiome
Beneficial and nuisance

Normal bacterial flora in Stratum Corneum
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

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).

2013 Oct 1;12(10):791-808
Biofilms in chronic wounds

Meta analysis - 78% chronic non-healing wounds contain biofilm
Due to heterogenous distribution – 100%

The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data.

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

S. aureus  P. aeruginosa

Different locations

Swabs
The *in vitro* biofilm

Janus Haagensen

Crystal Violet
High throughput

Attached biomass NOT viability
Biofilm formation

A general mental model
The *in vivo* Biofilm

- Wound
- CF lung
- Implant mouse model

Bjarnsholt et al. Trends in Microbiology Trends Microbiol. 2013 Sep;21(9):466-74
In vitro vs. In vivo – Transcriptomics (RNAseq)

Biofilm clinical vs. in vitro

Forget about this if you would like to understand the clinical biofilm
Second matrix and inflammation
The second matrix

Folsom et al. BMC Microbiology 2010, 10:294

Gradients

Fazli et al JCM 2009
Inflammatory response

Day 0

Larsen et al. 2015
Larsen et al. 2015
Ågren et al. 2014

Day 4

Normal skin
Wound site
Normal skin

Ågren et al. 2014
Fluorescent slide scan of full-thickness skin biopsy – zoom at wound edge

Bay L, Kragh KN, Eickhardt SR, Poulsen SS, Gjerdrum LMR, Ghathian K, Calum H, Ågren MS, Bjarnsholt T.; Bacterial Aggregates Establish at the Edges of Acute Epidermal Wounds.; Adv Wound Care. 2018 Apr 1;7(4):105-113
Human skin Microbiome
Beneficial and nuisance

Biofilm aggregates at wound edges

Bay L, Kragh KN, Eickhardt SR, Poulsen SS, Gjerdrum LMR, Ghatian K, Calum H, Ågren MS, Bjarnsholt T;
Bacterial Aggregates Establish at the Edges of Acute Epidermal Wounds.; Adv Wound Care. 2018 Apr 1;7(4):105-113
Summary

In vitro vs. in vivo

Model vs. Chronic infection
The microenvironment
Next step!

- To look again
Acknowledgments

University of Copenhagen:
Maria Alhede
Kasper N Kragh
Anne K Nielsen
Stephanie G Crone
Blaine Fritz
Lasse Kvich
Lene Bay
Anne Hesselvig
Elin Jørgensen
Tim Holm Jakobsen
Vibeke A Kristensen

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Michael Givskov, Klaus Qvortrup, Oana Ciofu, Tim Tolker-Nielsen, Søren Sørensen, Michael Kühl, Mette Burmølle
Hans Petter Hougen, Henrik Elvang Jensen
Louise Kruse Johansen

Rigshospitalet:
Peter Østrup Jensen
Mette Kolpen
Niels Høiby
Claus Moser
Kim Thomsen
Michael Tvede
Claus B Andersen
Preben Homøe

Others:
Matt Malone, Rune Nørager
Klaus Kirketerp-Møller, Lise H Christensen
Trine Rolighed Thomsen, Claus Sternberg
Christine R Hansen, Tanja Pressler
Mark Shirtliff, Claus Manniche
Benny Dahl, Anders Odgaard
Søren Orth-Nissen
Marvin Whiteley
Kendra Rumbaugh
Steve Diggle
Funding Sources

- The LEOfoundation
- Human Frontier Science project
- Capital Region Research Foundation for Health Research
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•  www.biofilmcourse.ku.dk
•  www.coursera.org/course/bacteria