The micro-environment of chronic infections and its influence in biofilm persistence

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Understanding biofilms
The problem
With and without foreign body

- 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).

The *in vivo* Biofilm

- *Wound*
- *CF lung*
- *Implant mouse model*

Bjarnsholt et al. Trends in Microbiology Trends Microbiol. 2013 Sep;21(9):466-74
Where?

1) The implant
2) Biopsies
3) Fluid

McConoughey et al. 2014
Penetration of antibiotics to infected bone tissue

Antibiotics

Left tibia

Right tibia

Day 5 – 1 g vancomycin or 1.5 g cefuroxime IV


Study design

- Day 5 – 1 g vancomycin or 1.5 g cefuroxime IV

- Antibiotic-measurements with microdialysis obtained for 8 hours in:
  - Implant cavity
  - Infected cancellous bone
  - Infected subcutaneous tissue
  - Cancellous bone
  - Subcutaneous tissue
  - Blood samples
Acute osteomyelitis model – porcine study

Cefuroxime vs. Vancomycin

- Cefuroxime concentration over time (mg/L)
- Vancomycin concentration over time (μg/ml)

- Healthy cancellous bone
- Healthy SCT
- Infected cancellous bone
- Infected subcutaneous tissue
- Implant cavity
- Free plasma

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Local antibiotics

How much Gentamicin is needed in the inoculum to prevent infection in the porcine model

-the inoculum: 10 µl with $10^4$ CFU of *S. aureus*
  + 16 x MIC of Gentamicin n=3
  + 160 x MIC of Gentamicin n=3
  + 1600 x MIC of Gentamicin n=3
  + 16000 MIC of Gentamicin n=4

The MIC of Gentamicin towards the used *S. aureus* strain was, based on an in-vitro study, estimated to be 0.25 µg/ml

CFU on implants after sonication

Saline
10^4 CFU SA
10^4 CFU SA + Gentamicin 16xMIC
10^4 CFU SA + Gentamicin 160xMIC
10^4 CFU SA + Gentamicin 1600xMIC

Y = Log2(y) transformed data
Summary

• The effect of antibiotics does not only rely on the sensitivity towards the pathogen but also on tissue penetration.

• The penetration is associated with the size of the pathological changes.

• Just 3 mm of pathology results in extreme reduced antibiotic penetration.

• Single extremely high local antibiotic concentrations is needed to prevent biofilm formation.
Biofilms in chronic wounds

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

S. aureus

P. aeruginosa


S. aureus biofilm
P. aeruginosa biofilm
The second matrix

Gradients

Folsom et al. BMC Microbiology 2010, 10:294

Fazli et al. JCM 2009

S. aureus biofilm
P. aeruginosa biofilm
The microenvironment

Schematic drawing by Kasper Kragh
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
Biofilm aggregates at wound edges

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
Pseudomonas aeruginosa transcriptome during infection

In vivo transcriptome groups separately than in vitro growth experiments!

15 human samples (cystic fibrosis, chronic wounds, acute burns)

87 in vitro samples

(1,707 shared genes)
In agreement with other current research!
Summary

In vitro vs. in vivo

Gradients of oxygen, nutrients and antibiotics

Model vs. Chronic infection
Biofilm clinical vs. in vitro

Forget about this if you would like to understand the clinical biofilm
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