Biofilms and wound infections.
Biofilms in chronic wounds?

Klaus Kirketerp-Møller
Orthopedic surgeon, Consultant
The chronic wound

- Arises from different acute wounds that requires different initial treatment
Bacterial Biofilm

- Bacterial biofilm is still one of the main reasons for unsuccessful treatment of implant related infections
- Biofilm formation is probably present in all chronic infections
- Treatments that takes biofilm into account are more likely to be successful

Pressure Ulcers

Cause:
Sustained or repetitive pressure in the immobilized patient

Treatment:
Off-loading mattresses, cushions, ambulation
Diabetic Foot Ulcers

Cause:
Sustained or repetitive pressure in the neuro-ischemic foot

Treatment:
Restoration of circulation. Off-loading in therapeutic footwear, cast, orthoses. Surgery
Controversies

• A diabetic foot ulcer:
Venous Leg Ulcers

Cause:
High hydrostatic pressure in veins in lower extremity due to venous valvular defects

Treatment:
Compression bandage or stockings
The Chronic Wound

- A wound that heals slower than expected?
- A wound that has not healed within 3 months?
- A wound that will never heal?
- A wound that will not heal despite effective treatment of cause?
Causes of the chronic wound (not complete)

- Immunological defects in the patient
  - Cancer
  - HIV infection
  - Inborn defects
  - Diabetes
  - Immunosupression
  - Malnutrition

- Defects in wound healing
  - Diabetes
  - Inborn defects
  - Malnutrition

- Infection
Infection definitions

- Contaminated
  - No bacteriological proliferation
- Colonized wound
  - Bacterial proliferation but no inflammation
- Infection
  - More than $10^5$ bacteria per gram tissue?
  - Clinical infection (inflammation)?
Venous Leg Ulcer Bacteriology

• Gødsbøl et al, Copenhagen Wound Healing Center:
  – *Staphylococcus aureus* (93.5%)
  – *Enterococcus faecalis* (71.7%)
  – *Pseudomonas aeruginosa* (52.2%)
  – Coagulase-negative staphylococci (45.7%)
  – *Proteus* species (41.3%)
  – Anaerobic bacteria (39.1%)
Venous Leg Ulcer
Bacteriology II

• Other works:
  – *Staphylococcus aureus* 38-88%
  – *Pseudomonas aeruginosa* 26-51%

• Differences probably due to sampling technique and clinical population
Chronic wound characteristics

- Slow fibroblasts
- Elevated Metalloproteases (MMPs)
- Decrease in Tissue Inhibiting Metalloprotease (TIMP)
- High rate of Polymorph Nuclear Neutrophils (PMN) in the chronic wound
Role of PMN in wound healing

• Enters the acute wound within 6 hours
• Removes debris and bacteria by phagocytosis
• Attracts other immunological components
• Their function is gradually taken over by macrophages
• PMN normally disappears after 48 hours
*Pseudomonas aeruginosa* in the Chronic Wound

A: Planktonic *P. aeruginosa* in a chronic wound
B: Microcolonies of *P. aeruginosa* in the same wound
Chronic wounds with *Pseudomonas aeruginosa*

- Tends to be larger and heals slowly
  - Gødsbøl et al, Int Wound J. 2006 Sep;3(3):225-31

- Have higher levels of metalloproteases, especially in combination with multiple bacteria
  - Lobmann R et al, Oral presentation DFSG 2005
**Pseudomonas and PMN**

- Quorum Sensing producing *P. aeruginosa* eliminates PMN
- Quorum Sensing blocked *P. aeruginosa* do not!
Diabetic foot ulcer
The story continues
Still working on the same case
Eksperimental infection with *Pseudomonas aeruginosa*

- Rabbit keratitis model
  - Elevated MMP-2 and MMP-9
  - Decreased TIMP-1 and TIMP-2

*Pseudomonas aeruginosa* escapes elimination from the chronic wound

By

- Biofilm formation
  - Increases antibiotic tolerance
- PMN elimination
  - Quorum Sensing dependent virulence factor
    - Rhamnolipid
Patient 9: P. aeruginosa and Proteus
Patient 11: *Staph. aureus, Enterococcus faecalis, P. aeruginosa, Klebsiella pneumonia*
Patient 2: *Staph. aureus, Enterococcus faecalis, P. putida, Corynebacterium species*
Patient 1: P.A
Chronic infection of a wound

- Bacteria
- PMNs
- Virulence factors
- Antimicrobial compounds
In conclusion

We propose

- *Pseudomonas aeruginosa* escapes elimination by biofilm formation
- *Pseudomonas aeruginosa* deteriorates wound healing by PMN elimination
- Presence of *Pseudomonas aeruginosa* explains chronic wound characteristics
  - Elevated MMP
  - Decreased TIMP
  - Chronic inflammatory state (PMN influx)
Wound Treadmill

- Skin defect
- Polymicrobial colonization
- Tissue damage
- Necrosis
- Bacterial colonization
- PMN elimination
- Biofilm Quorum sensing

Trauma
Controversies

• Normaly bacterial biofilm is associated with surfaces
  – Implants
  – Katheters
• This has led to the assumption that bacterial biofilm is on the surface of chronic wounds
Biofilm in Chronic wounds?

Bacterial biofilm is a major barrier to wound healing

- Bacteria protected from topical agents
- Low oxygen in biofilm niches
- Impaired migration and proliferation of keratinocytes
- Bacteria protected from systemic antibiotics
- Host defenses unable to clear infection

Here too?

Here?
Procedure: Split Skin Transplant for Chronic Ulcer

- **Excision:**
- **Transplantation:**
## Results

Success Rate of Split-Thickness Skin Grafting of Chronic Venous Leg Ulcers Depends on the Presence of *Pseudomonas aeruginosa*: A Retrospective Study

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ <em>P. aeruginosa</em></td>
<td>33.3%</td>
</tr>
<tr>
<td>(8/24)</td>
<td>P=0.001</td>
</tr>
<tr>
<td>- <em>P. aeruginosa</em></td>
<td>73.1%</td>
</tr>
<tr>
<td>(49/67)</td>
<td>P=0.001</td>
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</tbody>
</table>

Høgsberg et al. (2011) *Plos One*
Biofilm in Chronic Wounds?

Bacterial biofilm is a major barrier to wound healing

- Bacteria protected from topical agents
- Low oxygen in biofilm niches
- Impaired migration and proliferation of keratinocytes
- Bacteria protected from systemic antibiotics
- Host defenses unable to clear infection

Here!

Here too!
Take Home Message

• All chronic wounds contain biofilm
• Not all chronic wounds stay chronic due to biofilm
• Biofilm is not only present on the surface
• Today we do not have the tools to identify harmful biofilm
Swab or Biopsy?
Distance from Surface

Figure 2. The distribution of the distances from the wound surface to the center of mass of *S. aureus* aggregates (□) or *P. aeruginosa* aggregates (■). The distances are average values obtained from analysis of 15 images for each wound sample.

Nonrandom Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in Chronic Wounds
Mustafa Fazli et al. JCM dec. 2009
### Heterogeneity

<table>
<thead>
<tr>
<th>Method</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
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<tbody>
<tr>
<td></td>
<td>Wound E</td>
<td>Wound F</td>
</tr>
<tr>
<td>q-PCR cultivation</td>
<td>89±11%</td>
<td>200±13%</td>
</tr>
<tr>
<td>DGGE</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>q-PCR cultivation</td>
<td>No sample</td>
<td>86±8%</td>
</tr>
<tr>
<td>DGGE</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>q-PCR cultivation</td>
<td>240±10%</td>
<td>290±8%</td>
</tr>
<tr>
<td>DGGE</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>q-PCR cultivation</td>
<td>310±13%</td>
<td>80±5%</td>
</tr>
<tr>
<td>DGGE</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>q-PCR cultivation</td>
<td>180±8%</td>
<td>93±12%</td>
</tr>
<tr>
<td>DGGE</td>
<td>+</td>
<td>+</td>
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Influence of Microorganisms on the Healing of Skin Grafts from Chronic Venous Leg Wounds

Wulf et al. InTetch Book chapter
# Sampling Technique

## Pro´s et Con´s

<table>
<thead>
<tr>
<th></th>
<th>Swab</th>
<th>Biopsy</th>
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</thead>
<tbody>
<tr>
<td><strong>Pro</strong></td>
<td>Large area</td>
<td>More than surface</td>
</tr>
<tr>
<td></td>
<td>Non-invasive</td>
<td>Larger sample size</td>
</tr>
<tr>
<td><strong>Con</strong></td>
<td>Only surface</td>
<td>Small area</td>
</tr>
<tr>
<td></td>
<td>Invasive</td>
<td>Invasive</td>
</tr>
</tbody>
</table>
A Shift of Paradigm?

- Antibiotics and surgery is often not enough to eradicate bacterial biofilm
- We are looking for a substance that readily kills bacteria throughout the biofilm and is tolerated by the tissue
- This substance could be buffered Acetic acid!
  - 0,5% acetic acid eradicates *P. aeruginosa* (gram negative)
  - 1,0 % acetic acid eradicates *S. aureus* (gram positive)
  - pH dependant effect

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How did this get started?

Acetic acid is used to treat middle ear infections at Rigshospitalet, Copenhagen

Initial experiments were too good to be true....
In Vitro – pH dependency

**P. aeruginosa biofilm treated with 0.5% buffered acetic acid and tobramycin**

<table>
<thead>
<tr>
<th>µg/ml tobramycin</th>
<th>pH 3</th>
<th>pH 4</th>
<th>pH 4.33</th>
<th>pH 4.76</th>
<th>pH 5</th>
<th>pH 5.5</th>
<th>pH 6</th>
<th>pH 6.85</th>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
<td>3.125</td>
<td>1.5625</td>
<td>0.78125</td>
</tr>
</tbody>
</table>

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In Vitro - HCl vs. Acetic Acid

Buffered Acetic acid vs HCl on *P. aeruginosa* Biofilm

No growth

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Case 1: Patient with recent heel ulcer

Anamnesis

- A 38-year old male with T2DM associated neuropathy was presented to Copenhagen Wound Healing Clinic. A heel ulcer was obtained during a vacation due to strenuous walking.

Prior history of treatment with no apparent improvement in wound healing (over period of three months)
- Off-loading, therapeutic shoes, and Aircast.
- Wound treatment with silver dressings and compression.
- Several courses of antibiotics.

Treatment of wound with phosphate buffered acetic acid (patient continued antibiotic therapy)
- 6x20 minutes per day, for 10 days (continuous) in combination with NPWT Therapy
Case 1: Patient with recent heel ulcer

Day 0

Day 11

Day 36 (split skin transplant 10 days prior)

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Case 2: Patient with chronic foot ulcer

Anamnesis
- A 54-year old woman with a past history of IV-drug abuse. Difficult to treat venous insufficiency and a long history of chronic leg ulcers. The wound is infected with *P. aeruginosa* and *E. faecalis*. Have been treated for the last 8 years at Copenhagen Wound Healing Clinic.

Prior history of treatment with recurrent leg ulcers
- Compression and various dressings.
- Numerous antibiotic treatments.
- Numerous skin transplants which have been lost.

Treatment of wound with phosphate buffered acetic acid (patient continued antibiotic therapy with meropenem) 6x20 minutes per day, for 10 days (continuous) in combination with NPWT Therapy
Case 2: Patient with chronic foot ulcer

Day 0

Day 39

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Intellectual Property Rights

• A PCT patent has been filed covering the active substance “WO2009/155931 A1”

• Wound dressings incorporating the active substance is also claimed
Let Us Return to the Paradox

- Most diabetic foot ulcers or other chronic wounds heals with proper off-loading, treatment of infection, edema reduction and management of ischemia.
- How can this paradox be explained?
Healing Despite of Biofilm

• Not all biofilms are harmful
• Virulent biofilms can change virulence pattern
  – Change of habitat due to
    • Off-loading
    • Edema reduction
    • Infection control
    • Alterations in nutrition factors
    • No apparent reason
What Needs to be Done in Research

• How to identify the wounds that are non-healing due to biofilm
• How do we measure virulence?
• Does the actual wound contain harmful biofilm?
• How do we conquer biofilms?
  – Jamming the communication
  – Blocking virulence factors
  – Electric field therapy
  – New drugs that kills bacteria throughout the biofilm
Until then …

- Follow the guidelines and..
- The residual ulcers are likely to contain harmful biofilm
- Please refer to EWMA Journal May 2011 for more details
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   Rigshospitalet

AdvanDx
1st Announcement – Call for Abstracts

30th Annual Meeting of the European Bone and Joint Infection Society

EBJIS 2011

Biofilm and Health Economics in Bone and Joint Infections

15-17 September 2011
The Panum Institute,
Copenhagen, Denmark

www.ebjis2011.org

Thank you for your attention