

Spatial organization of *Pseudomonas aeruginosa* and
Staphylococcus aureus in chronic wounds

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Outline

- Acute vs. Chronic wounds
- Previous and current work
- Results
- Summary & conclusion

Acute vs. Chronic

- Acute wounds
 - caused by external damage to intact skin
 - surgical wounds, bites, burns, abrasions
 - expected to heal within predictable time (several weeks)
- Chronic wounds
 - caused by endogenous mechanisms
 - leg ulcers, foot ulcers, and pressure ulcers
 - stuck at inflammatory phase

Chronic wounds

- Enormous problem worldwide:
 - 1 – 2 % suffer chronic wounds
- Cost billions of \$ to health care systems
- Patients experience :
 - suffering
 - lost of employment
 - reduced quality of life

Chronic wound types

Diabetic foot ulcer



Venous leg ulcer



Pressure ulcer

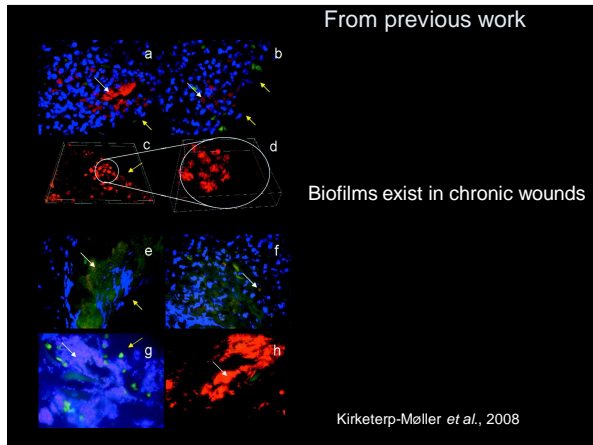
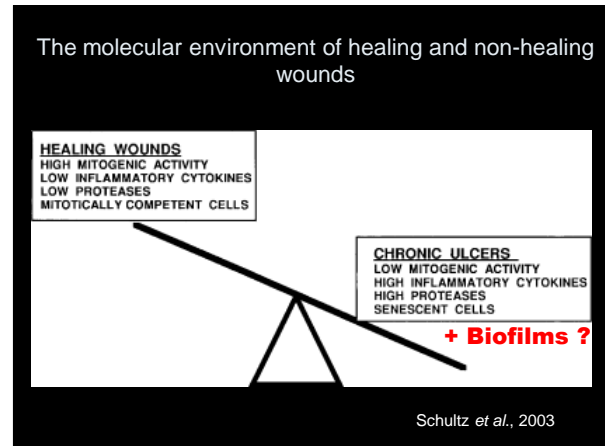
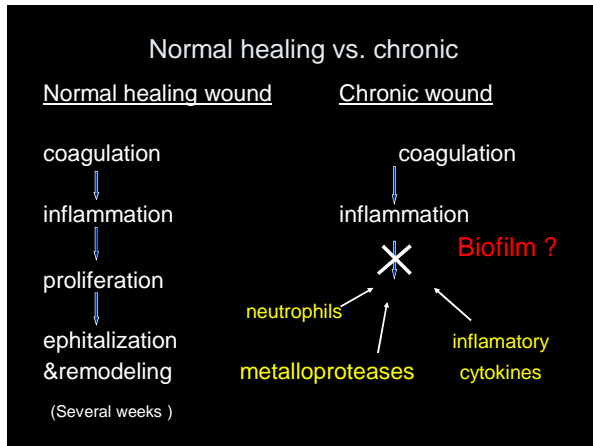


www.erc.montana.edu/biofilmbook/MODULE_07/Mod07_S02_Blue.htm

Venous leg ulcers

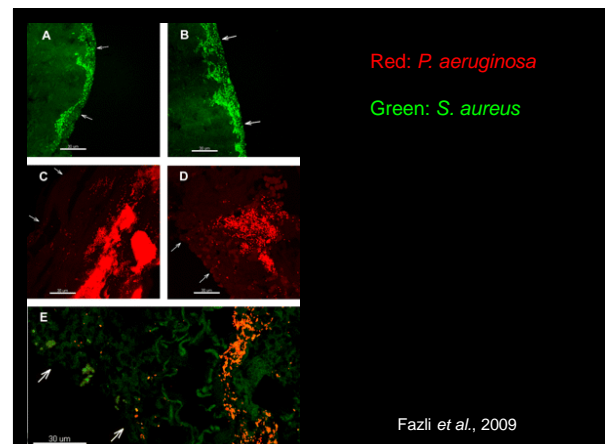
- Failure of valves in the veins of the legs
- This results in:
 - Venous hypertension in veins
 - Increased pressure in capillaries
 - Slow blood flow
 - Edema
 - Inflammation





- ### Our aim
- Characterization of bacterial biofilms in venous leg ulcers
 - *P. aeruginosa*
 - *S. aureus*
 - Spatial organization of microcolonies within the wound
 - Quantitative analysis of inflammatory response in chronic wounds with *P. aeruginosa*

- ### Methodology
- Sample collection from Copenhagen Wound Healing Center
 - In situ investigation of biofilm on wound samples by:
 - Fluorescent in situ hybridization (FISH)
 - Confocal laser scanning microscopy (CLSM)
 - Quantitative image analysis
 - Immunohistochemistry with specific antibodies against immune cells



To see whether this is a general characteristic:

- Five *S. aureus* detected wound
- Five *P. aeruginosa* detected wound
- Obtained slices at different intervals
- Performed FISH and CLSM
- Analysed 15 images of each wound with ImageJ software to locate the center of mass of cell aggregates
- Measured the distance from the center of mass of aggregates to the wound surface

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Nonrandom Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in Chronic Wounds[†]

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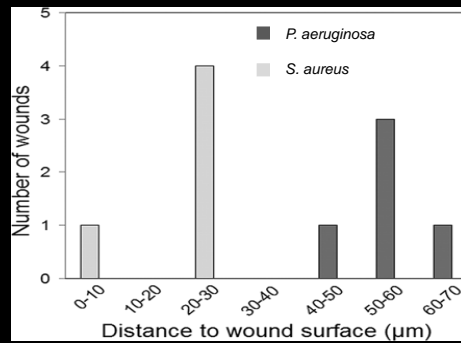
Results

Average distance of bacterial aggregates to the surface of wound samples

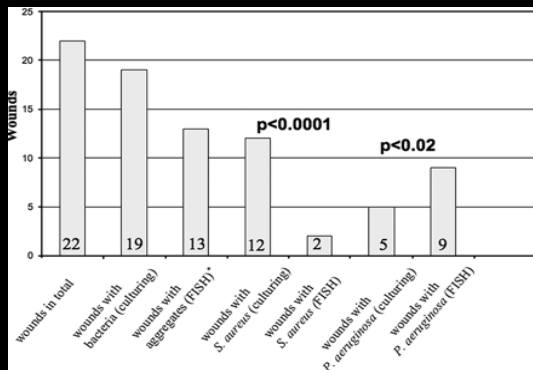
Wound biopsy specimen	Bacterial species detected by PNA-FISH	Average distance to wound surface (µm)
LGA 02	<i>S. aureus</i>	28.3 (6.6)
BIJ 04	<i>S. aureus</i>	8.8 (1.7)
HAH 08	<i>S. aureus</i>	28.1 (5.0)
M 2 ^a	<i>S. aureus</i>	26.1 (5.1)
Pt 17	<i>S. aureus</i>	23.7 (3.7)
M 3 ^a	<i>P. aeruginosa</i>	57.5 (9.4)
Pt 11	<i>P. aeruginosa</i>	50.0 (13.4)
Pt 20	<i>P. aeruginosa</i>	53.5 (9.9)
Pt 23B	<i>P. aeruginosa</i>	68.7 (11.2)
Pt 31	<i>P. aeruginosa</i>	46.1 (6.0)

Fazli, et al., 2009

Number of wounds in each distance category



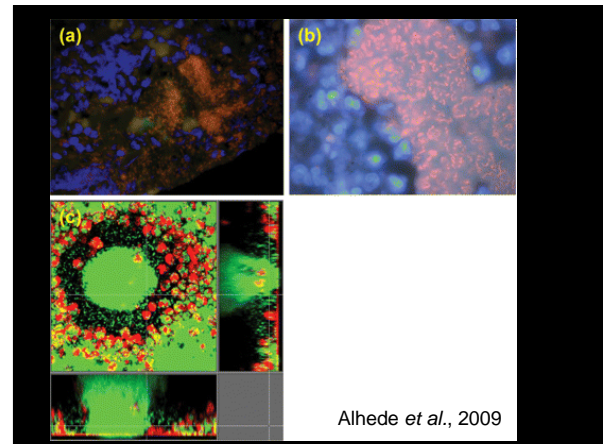
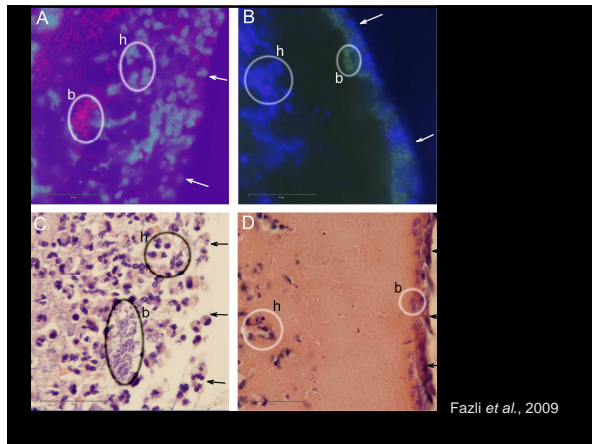
Fazli, et al., 2009



Kirketerp-Møller et al., 2008

Summary and conclusion

- Bacteria in chronic wounds reside as aggregates
- Distribution of *P. aeruginosa* and *S. aureus* is non-random
- Non-random distribution might indicate the underestimation of *P. aeruginosa* by culturing
- *P. aeruginosa* being at deeper regions of the wounds can make it the causative agent



Current work

- Quantitative analysis of the inflammatory response in infected chronic wounds with:
 - *P. aeruginosa*
 - *S. aureus*
 - Unidentified bacteria
- Comparison with the inflammatory response in CF lung
 - Immunohistochemistry
 - Neutrophils
 - Macrophages
 - T- and B-Cells

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