



Det Sundhedsvidenskabelige Fakultet



Biofilm og kroniske sår

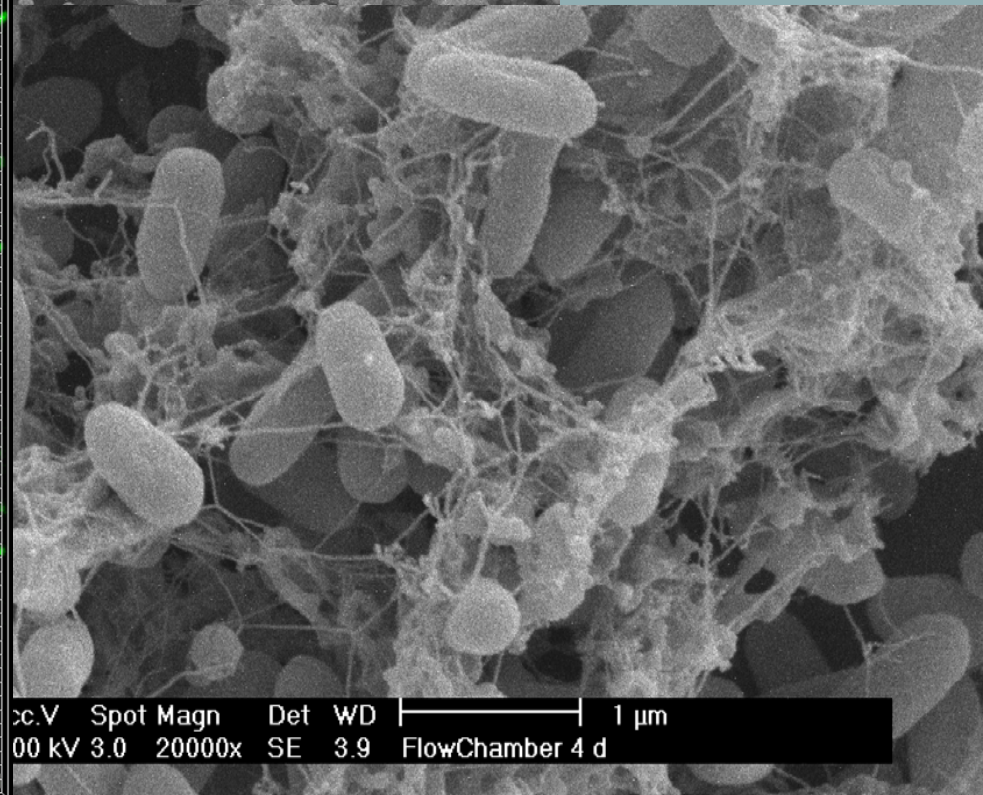
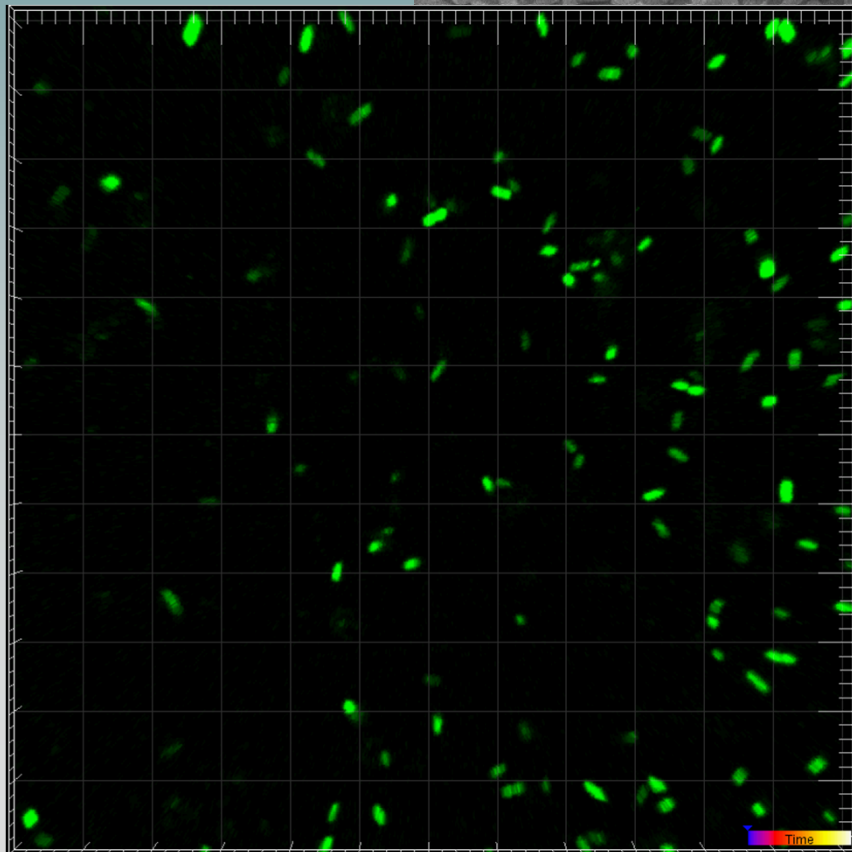
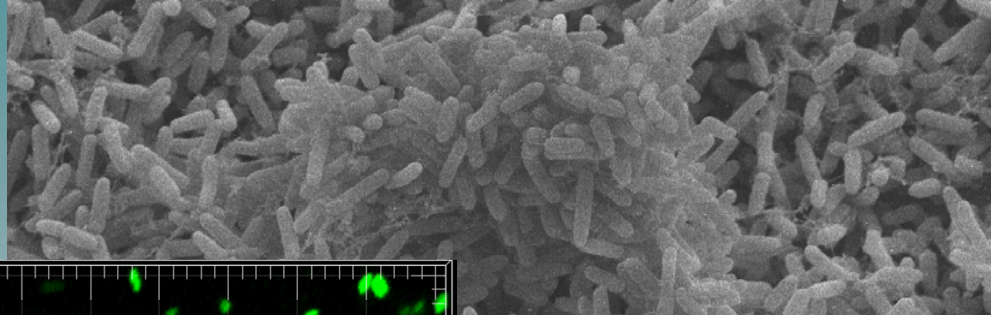
Thomas Bjarnsholt
Professor, DMSc & PhD



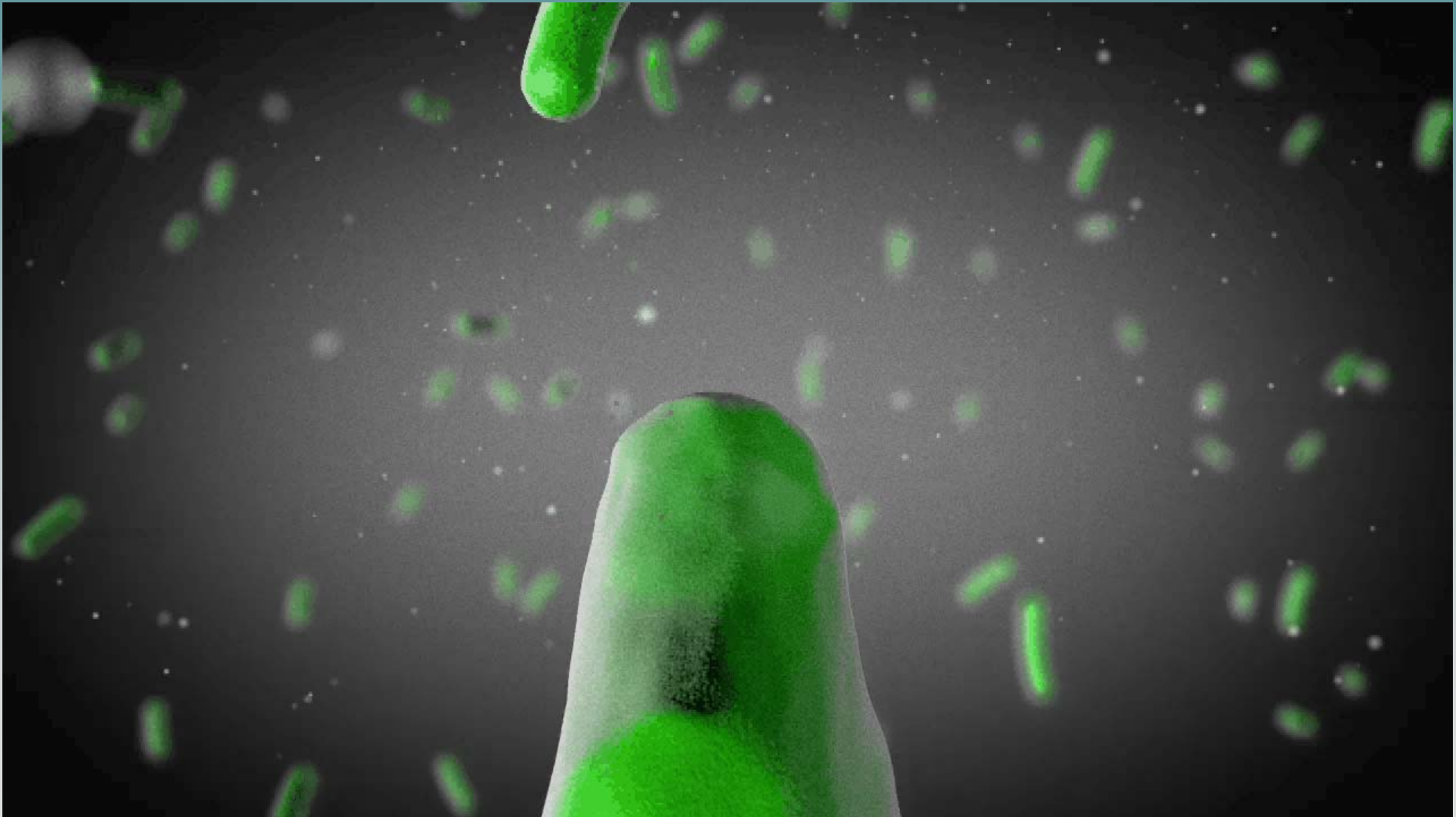
Rigshospitalet



Individuel eller i flok



Forskellen

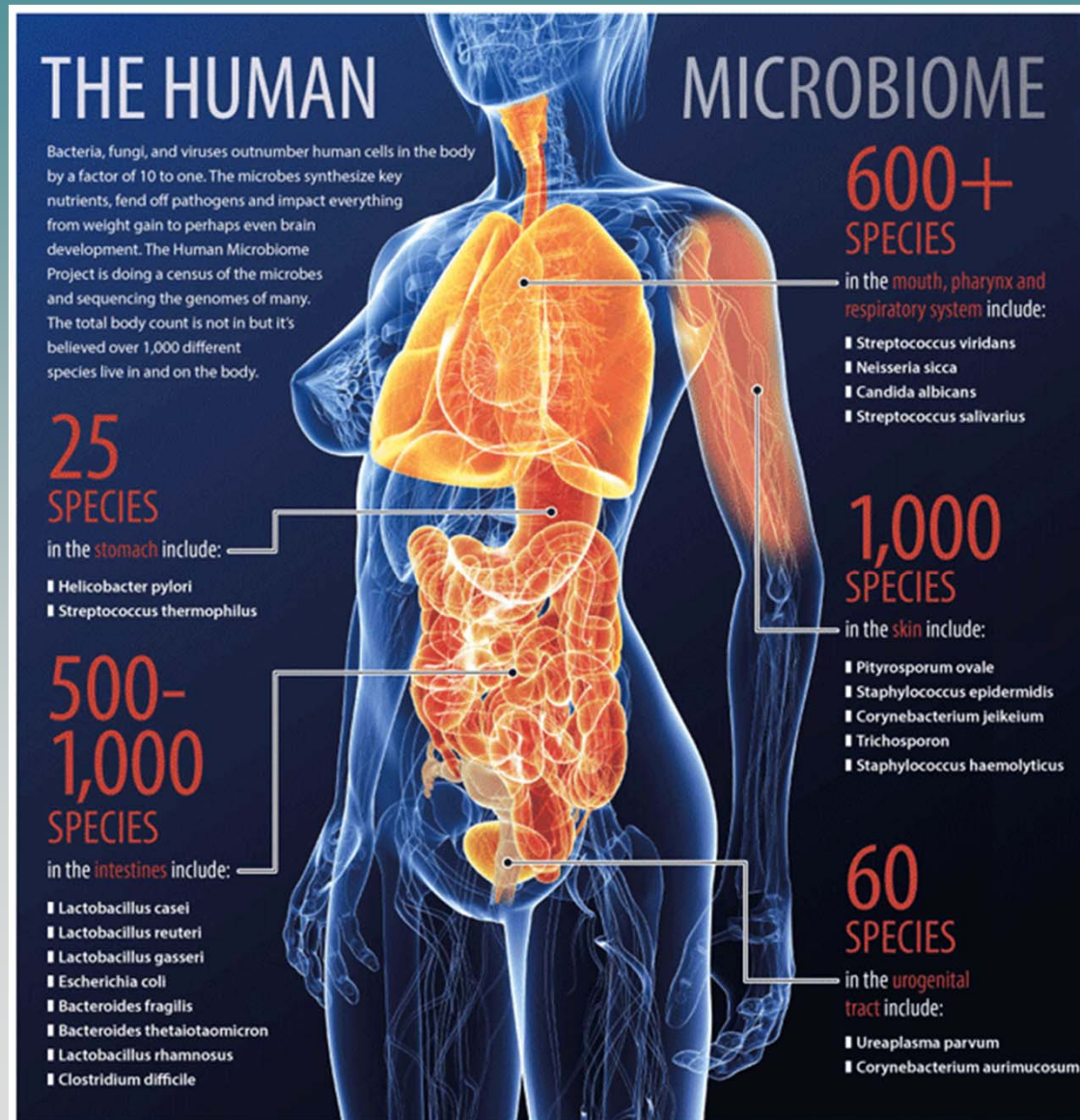


Er biofilm altid dårligt?

A. Yes

B. No

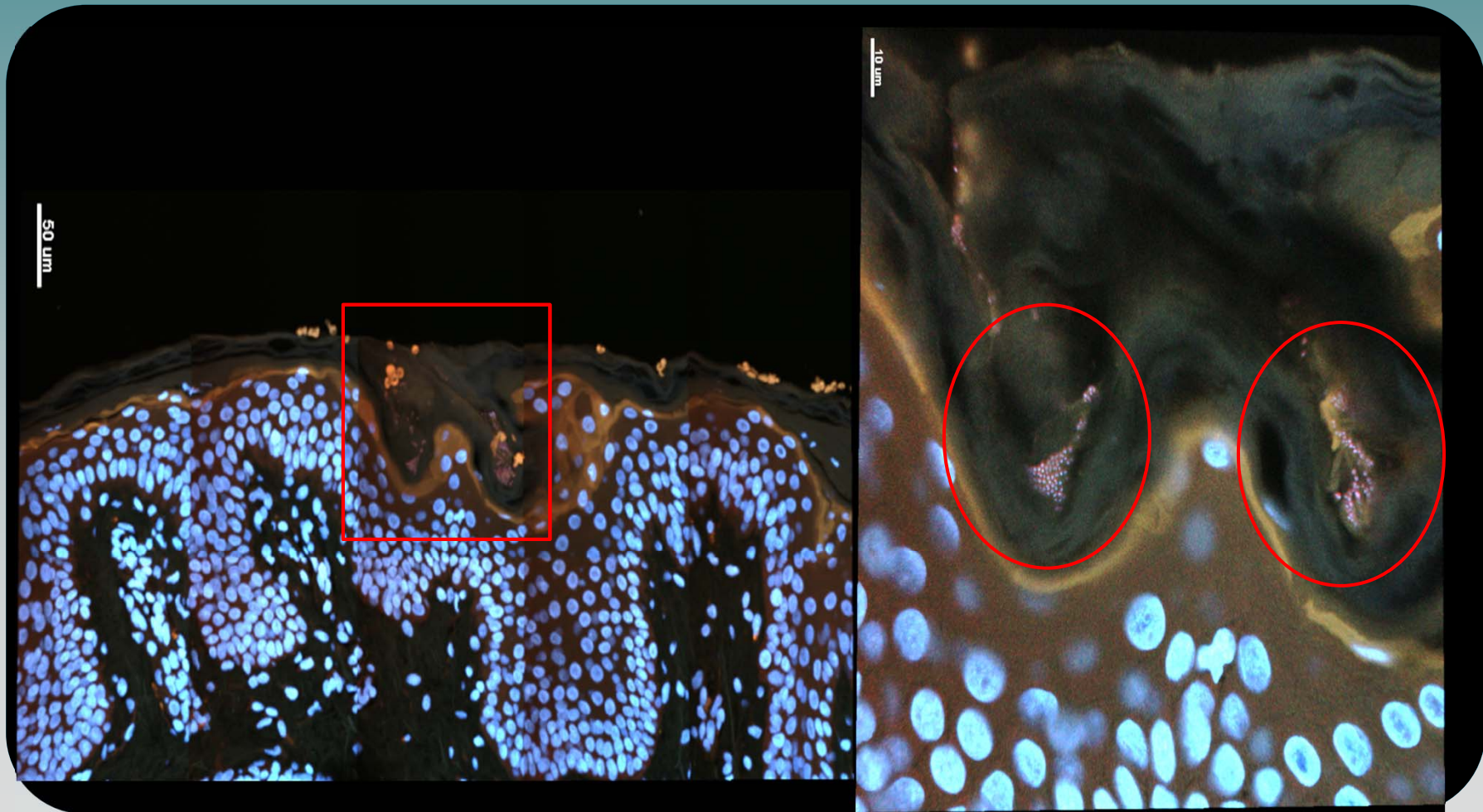
I og på vores krop



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

Dean Tweed • POSTMEDIA NEWS / IMAGE: Fotolia

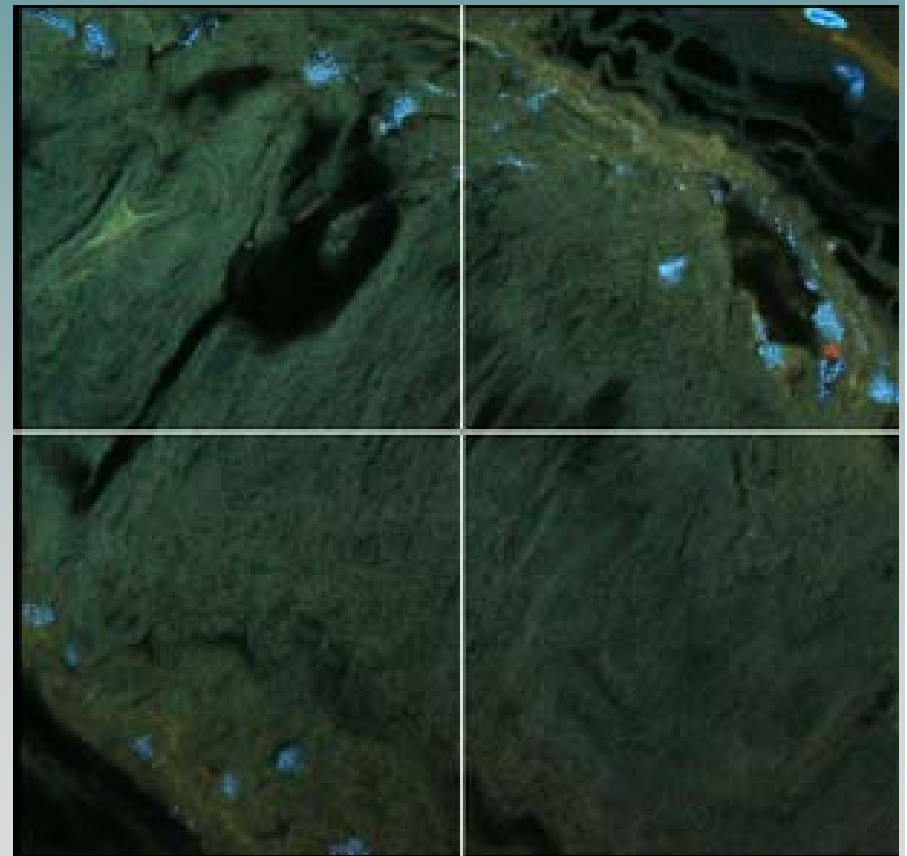
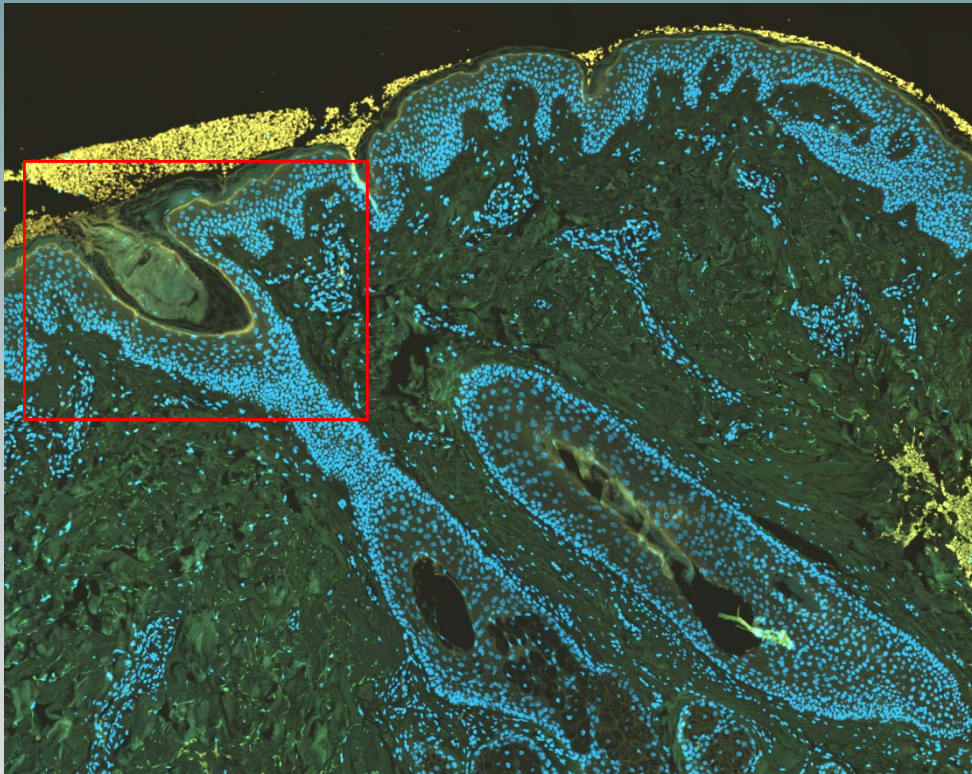
Det humane hud mikrobiom



June 2015

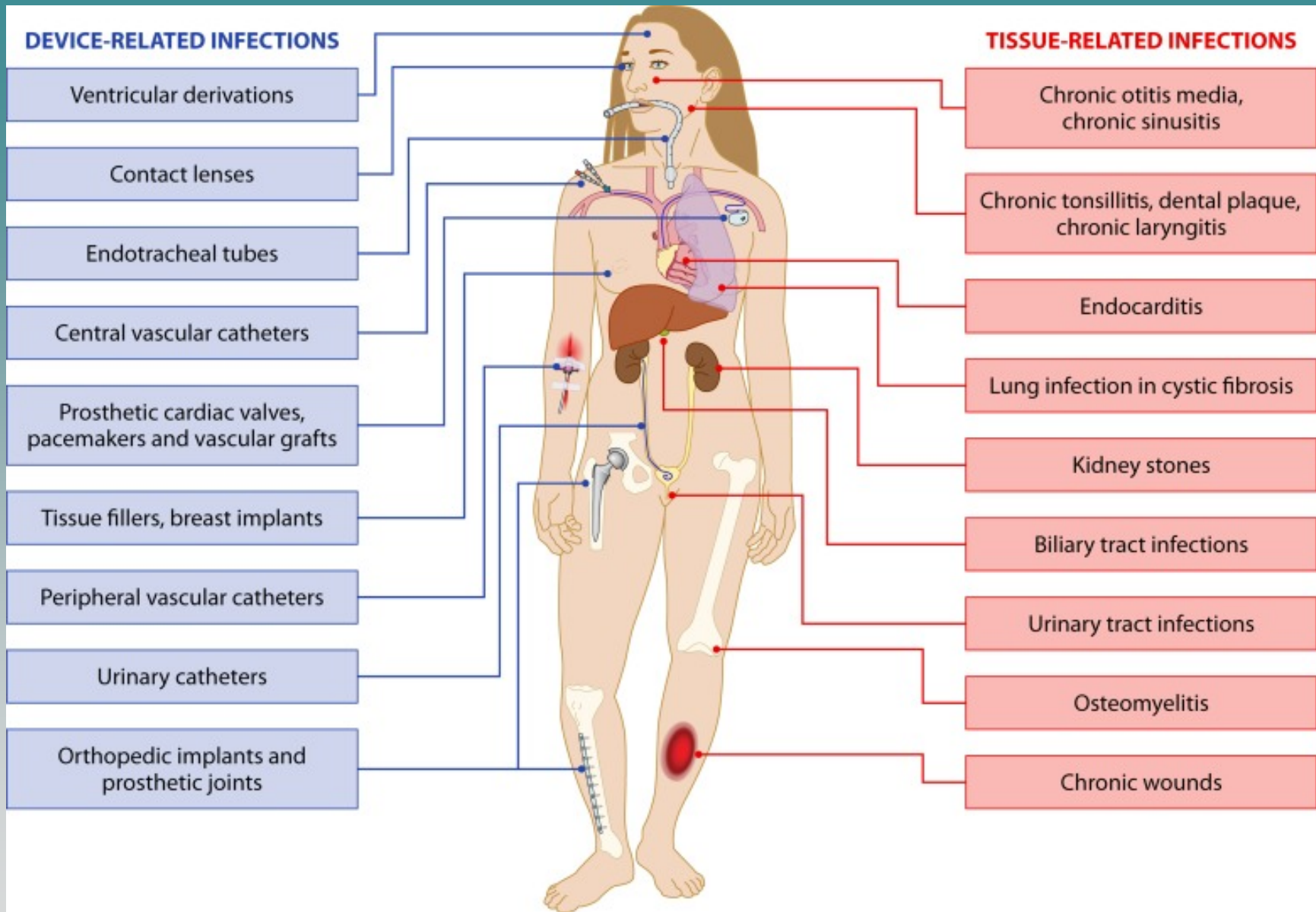
Normal bakterie flora | Stratum Corneum

Hår follikle



Bakterier er flok “dyr”





Lebeaux D, Microbiol Mol Biol Rev. 2014 Sep;78(3):510-43

Individuel eller i flok

- Studie fra 1956.
- 7.500.000 *Staphylococcus aureus* bakterier sprøjtes ind under huden på frivillige = **kun 50% inficeres, alle kommer sig**
- < 100 bakterier på stof under huden = **100% inficeres, INGEN kommer sig**

EXPERIMENTAL STAPHYLOCOCCAL INFECTIONS IN THE SKIN OF MAN

By Stephen D. Elek

St. George's Hospital Medical School, University of London, London, England

The concept of virulence of a microbe is relative to a given host species. The fact that an organism is capable of setting up lesions in 1 experimental animal cannot be used as a basis for comparing the virulence of microbial strains in relation to another species of host. The traditional approach of using any convenient laboratory animal in virulence studies may yield completely fallacious findings if different metabolic products in other species of animals contribute to varying degrees of virulence in those species. There is evidence that the virulence of coagulase positive staphylococci does vary when tested on rabbits, mice, or other animals, but there is no information concerning the existence of similar differences in man (Frappier *et al.*, 1955).

The carrier rate of coagulase-positive staphylococci and their distribution in dust, clothing, and elsewhere is very high, but clinical staphylococcal infection in man is comparatively rare. This suggests that either a minority of nasal strains are endowed with special virulence or that the circumstances of the infection determine the occurrence of disease. Since virulence testing on animals could not be expected to provide the correct answer, it was decided to compare a number of nasal strains with staphylococci isolated from human lesions by injecting volunteers intradermally. All the strains tested were coagulase-positive but, as the other biological properties of such material have frequently been described, no further details were regarded as relevant. Virulence testing in man being necessarily restricted in extent, our primary aim was to establish, within the limitations of the approach, whether or not differences in the resulting lesions could be demonstrated between randomly selected nasal strains of *Staphylococcus pyogenes* and other strains obtained from human lesions, and therefore presumed to be virulent. The indicator effect chosen was pus formation. Since staphylococci typically produce purulent lesions, the minimum inoculum surviving and progressing to pus formation can be used as the basis of comparison between the virulence to man of different strains.

Experiments on man with *Staph. pyogenes* have been carried out before (Garré, 1885; Bumm, 1885; and Bockhart, 1887), but only to show that they cause disease. No information was available concerning the minimal pus-forming dose or concerning differences between strains. Since the number of volunteers available was insufficient to test an adequate number of strains with different sizes of inocula it was decided to determine the minimal pus-forming dose for a strain freshly isolated from a severe lesion and then to compare the effects of the same dose of nasal and of some further known pyogenic strains. If the hypothesis that only a minority of nasal strains are virulent is correct, these strains would be expected not to cause pus formation at that dose.

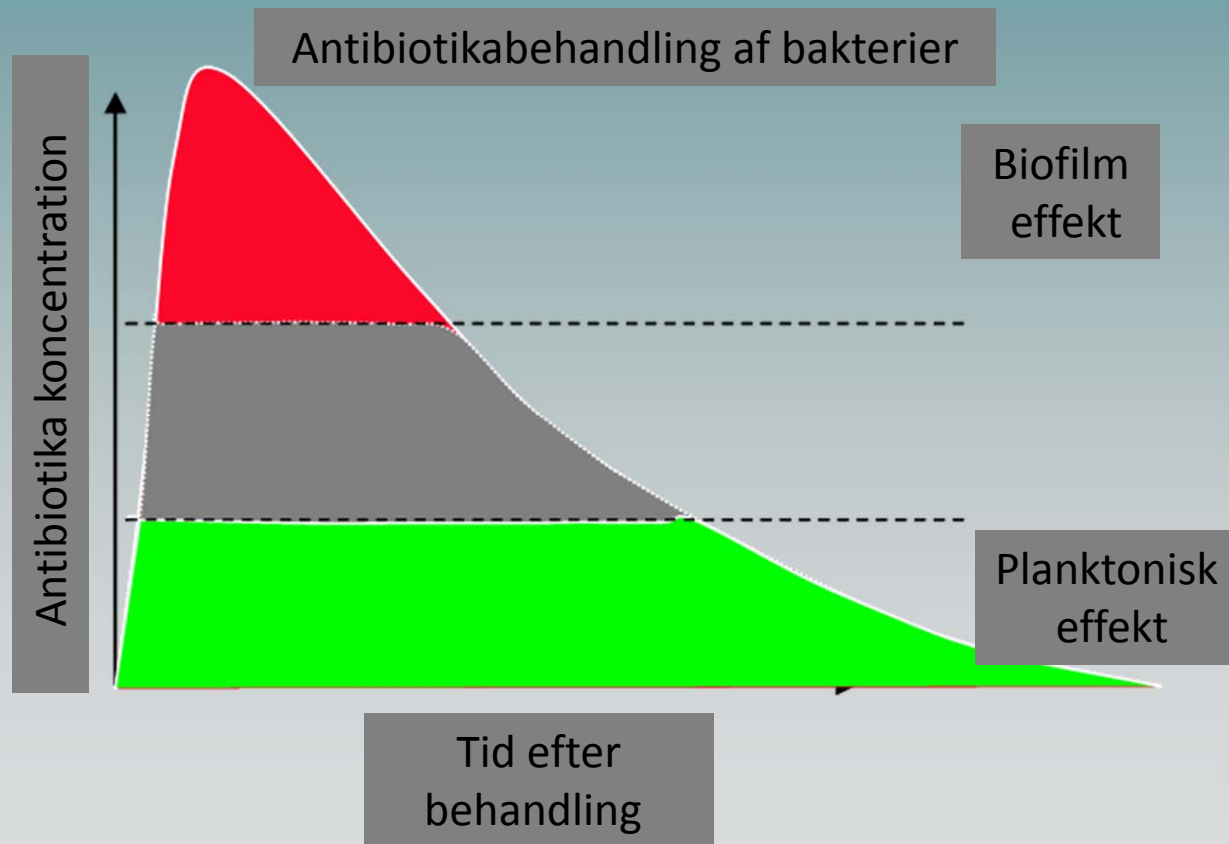
A strain obtained from an abscess of the neck was injected in varying dilu-

Akutte infektioner



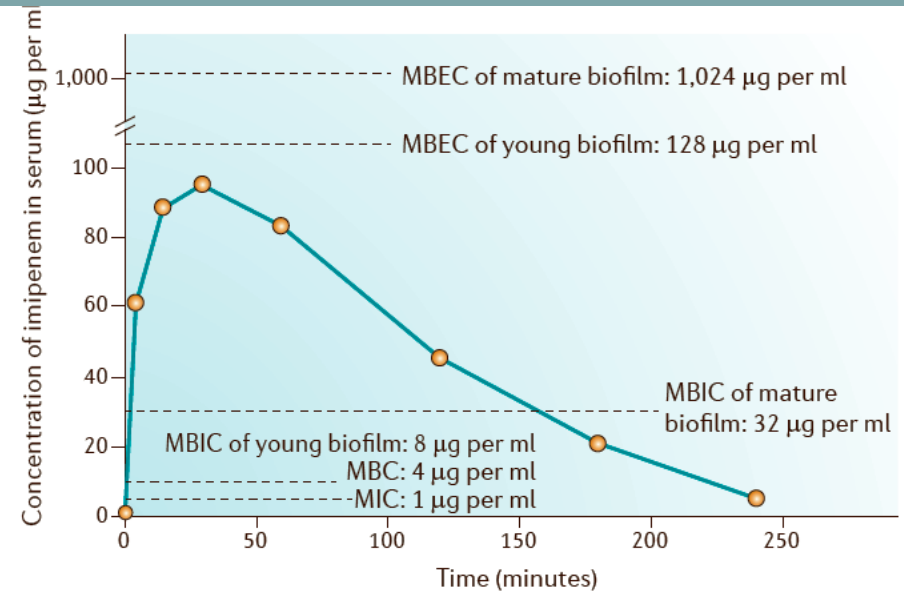
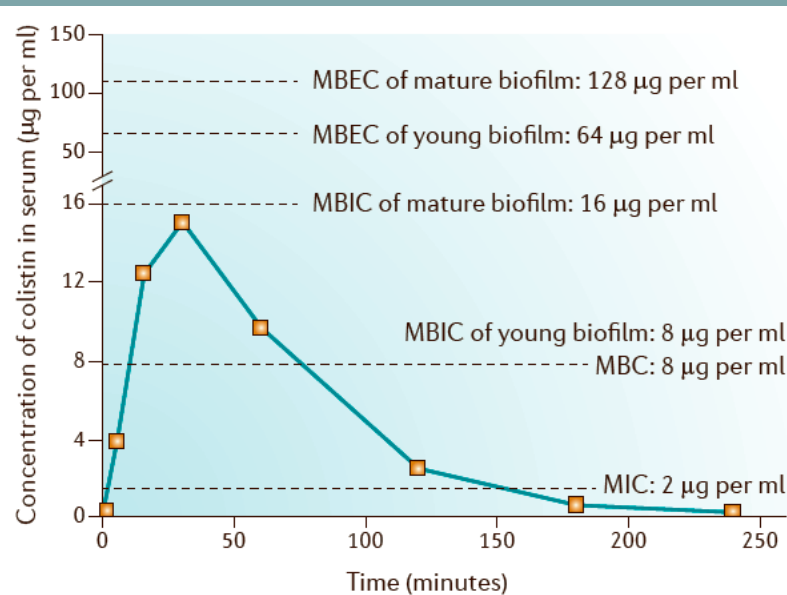
Necroticerende fasciitis
af
S. pyogenes gruppe A

Antibiotika



MBEC and MBIC

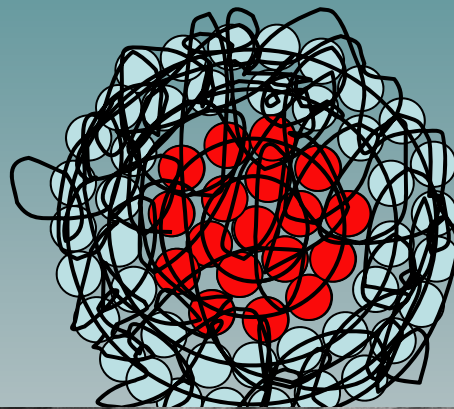
- MBIC – Minimal Biofilm Inhibitory Concentration
- MBEC – Minimal Biofilm Eradication Concentration



Mus behandlet intraperitonalt med 1x colistin (16 mg per kg) eller imipenem (64 mg per kg).

Bjarnsholt et al Nature Review Drug Discovery.
2013 Oct 1;12(10):791-808

Aggregering



- Voksende celler
- Stationære celler (tolerante)

Biofilm

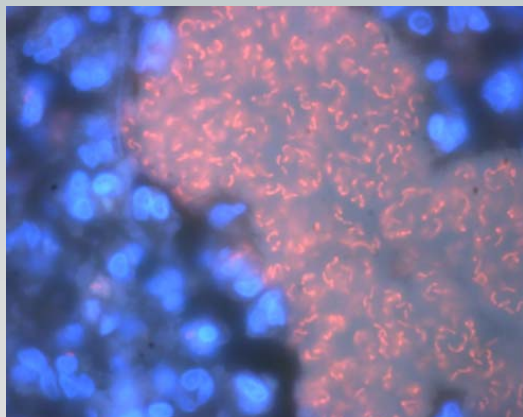
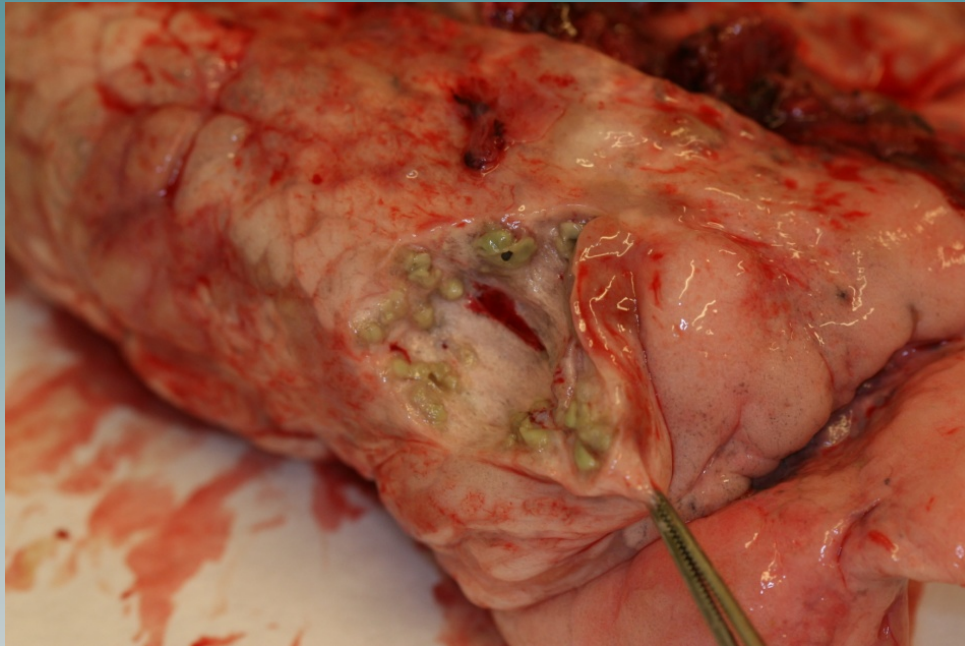


Frie bakterier

Immunforsvaret og virknings
orsvaret og antibiotika
er (for det meste)

Cystisk fibrose – det klassiske eksempel

CF mand, 28 års kronisk bakterieinfektion



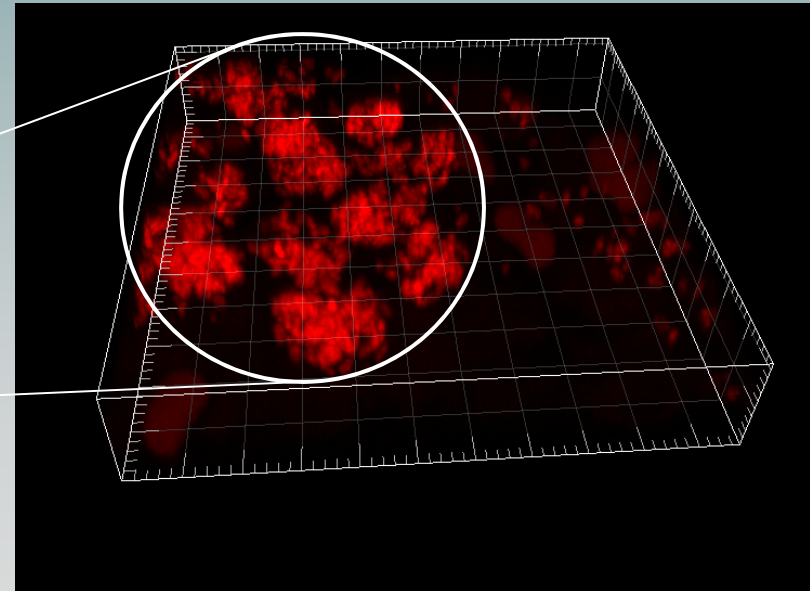
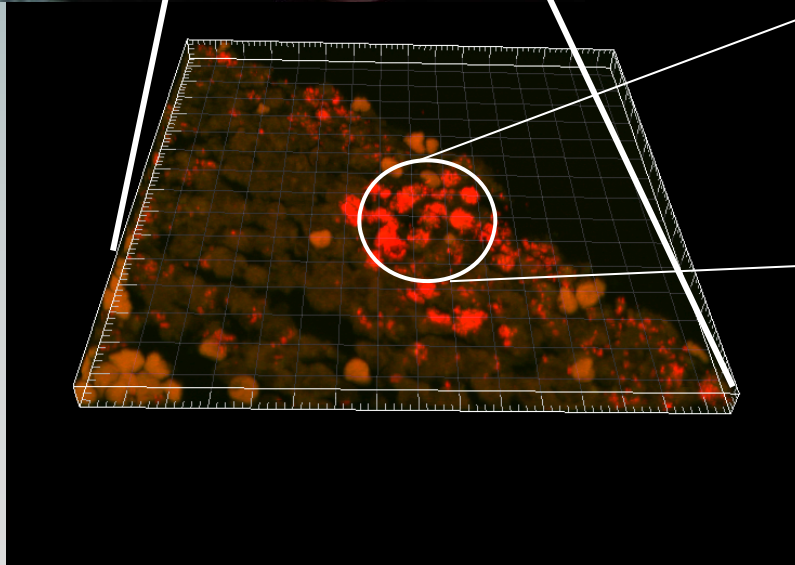
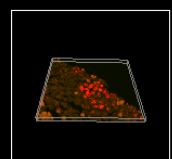
4 x 2 ugers/år IV antibiotika behandling
20 års daglig inhalering af antibiotika

1 kg tobramycin,
10 kg beta-lactam antibiotika
og 1 kg inhaleret colistin

Biofilm i kroniske sår



**Biofilm og Bakterier er ikke synlige
Makroskopisk**



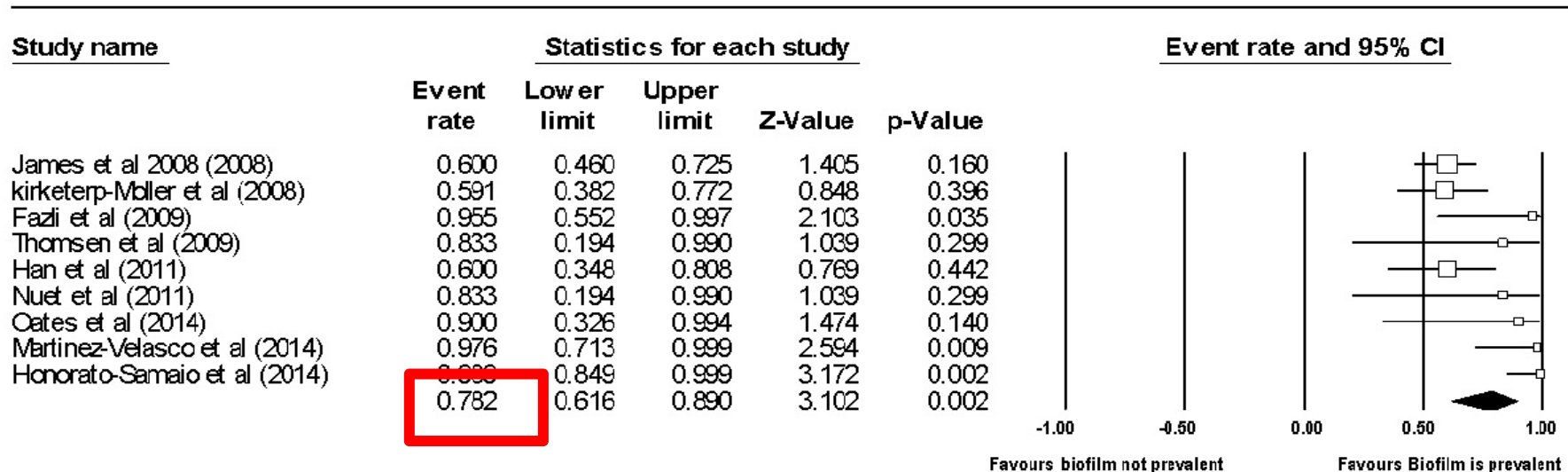
Biofilm i Kroniske sår

Author/s	Study design	Number of participants	Visualisation methods with or without molecular	Chronic wound aetiologies	N° of samples with confirmed biofilm (%)
James et al (2008)	Prospective study case vs control	66	Light microscopy, SEM 16S rRNA with DGGE	13 DFUs, 21 PUs 8 VLUs, 24 NHSW	30 out of 50 (60%)
Kirketerp-Moller (2008)	Prospective cohort study	22	PNA-FISH, CLSM	Un-specified chronic wounds	13 of 22 (60%)
Fazli et al (2009)	Prospective cohort study	9	PNA-FISH, CLSM	10 VLU	10 of 10 (100%)
Thomsen et al (2009)	Prospective cohort study Sub analysis	2	PNA-FISH, 16S rRNA	2 VLU	2 of 2 (100%)
Han et al (2011)	Prospective cohort study	15	PNA-FISH, CLSM 16S rRNA	4 DFUs, 5 PUs, 2 VLU 4 NHSW	9 of 15 (60%)
Neut et al (2011)	Case report	2	CLSM	2 DFUs	2 of 2 (100%)
Oates et al (2014)	Prospective cohort study Sub analysis	4	FISH, SEM,	4 DFUs	4 of 4 (100%)
Martinez-Velasco et al (2014)	Prospective cohort study conference abstract	20	SEM, LM	Un-specified chronic wounds	20 of 20 (100%)
Honorato-Sampaio et al (2014)	Prospective cohort study	45	TEM	45 VLU	45 of 45 (100%)

Meta analysis - 78% chronic non-healing wounds contain biofilm

Global wound biofilm expert panel – 100%

Meta Analysis random-effects model



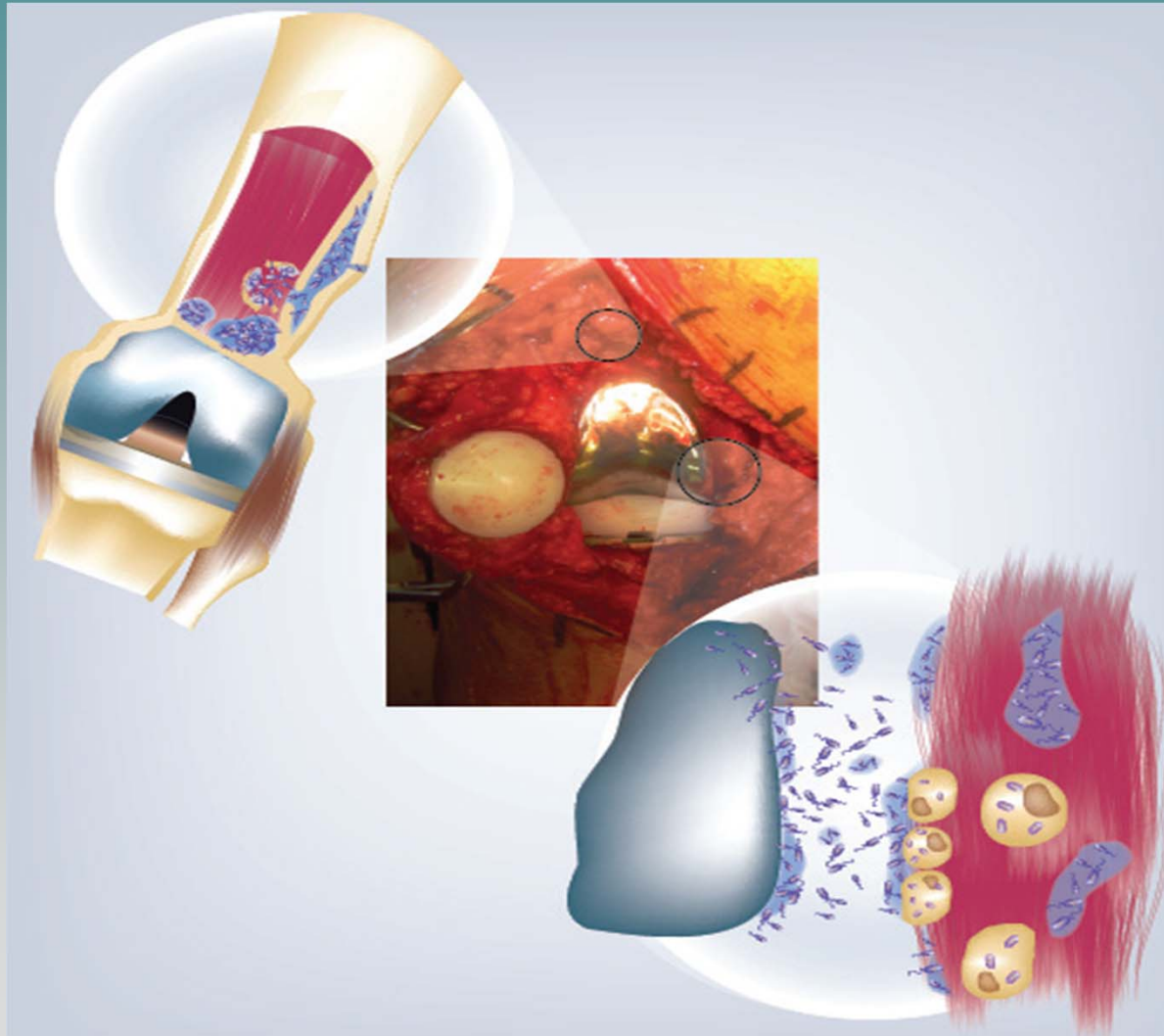
[The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data.](#)

Malone M, **Bjarnsholt** T, McBain AJ, James GA, Stoodley P, Leaper D, Tachi M, Schultz G, Swanson T, Wolcott RD. J Wound Care. 2017 Jan 2;26(1):20-25

Biofilm infektioner

- Kronisk langstrakt infektion
- Ofte virker antibiotika ikke
- Modstår immunforsvaret
- Behandlingssvigt eneste mulighed er ofte at fjerne implantatet
- Svært at diagnosticere

Hvor?

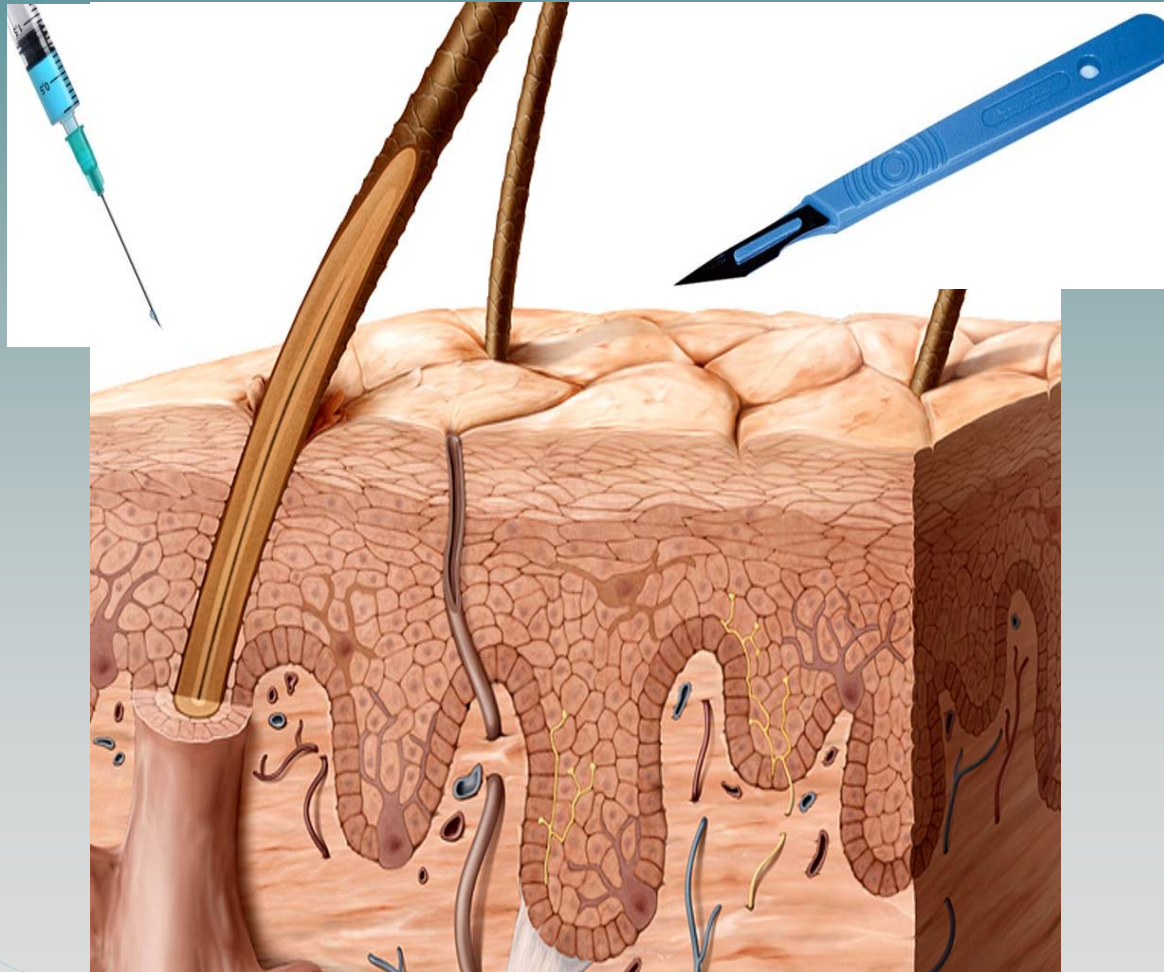


McConoughey *et al.* 2014

Human skin Microbiome

Beneficial and nuisance

Courtesy of Lise Christensen



June 2014 <http://tangentindinc.com>

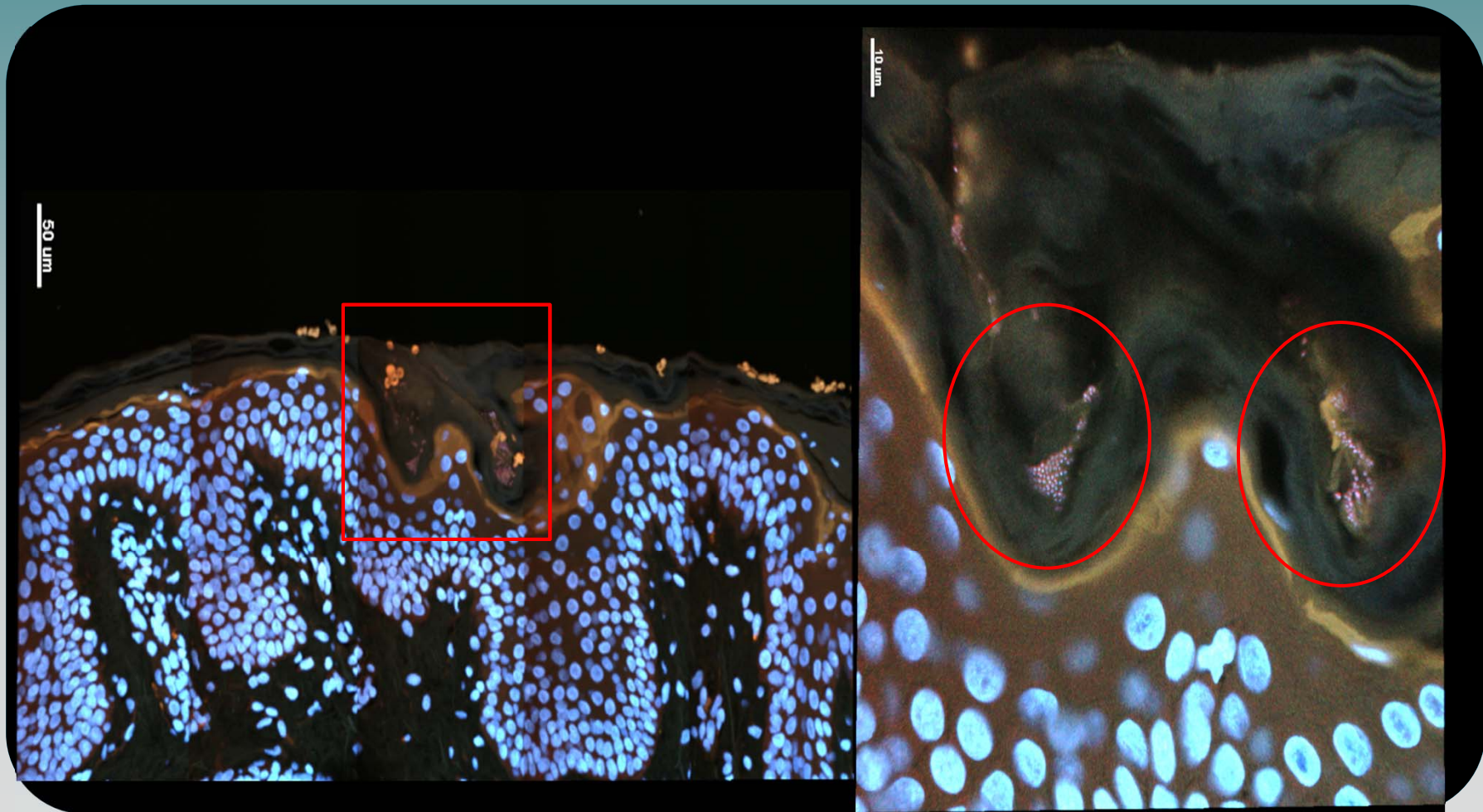
<http://medgraphik.com>

<http://www.growell.co.uk>

Bjarnsholt *et al.* 2008

Human skin Microbiome

Beneficial and nuisance



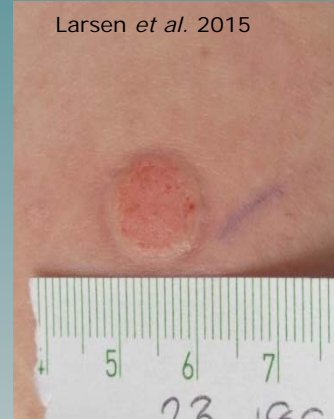
June 2015

Normal bacterial flora in Stratum Corneum

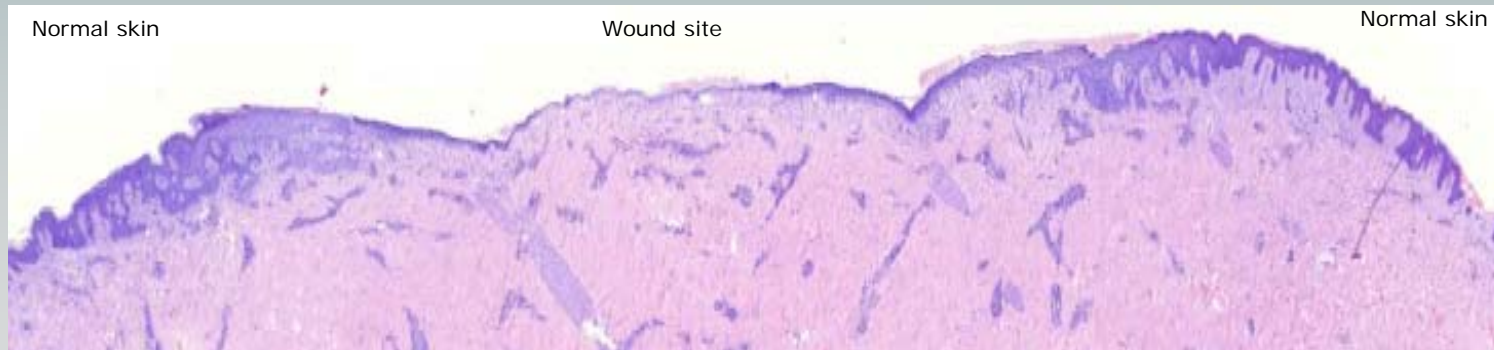
Human skin Microbiome

Beneficial and nuisance

Day 0



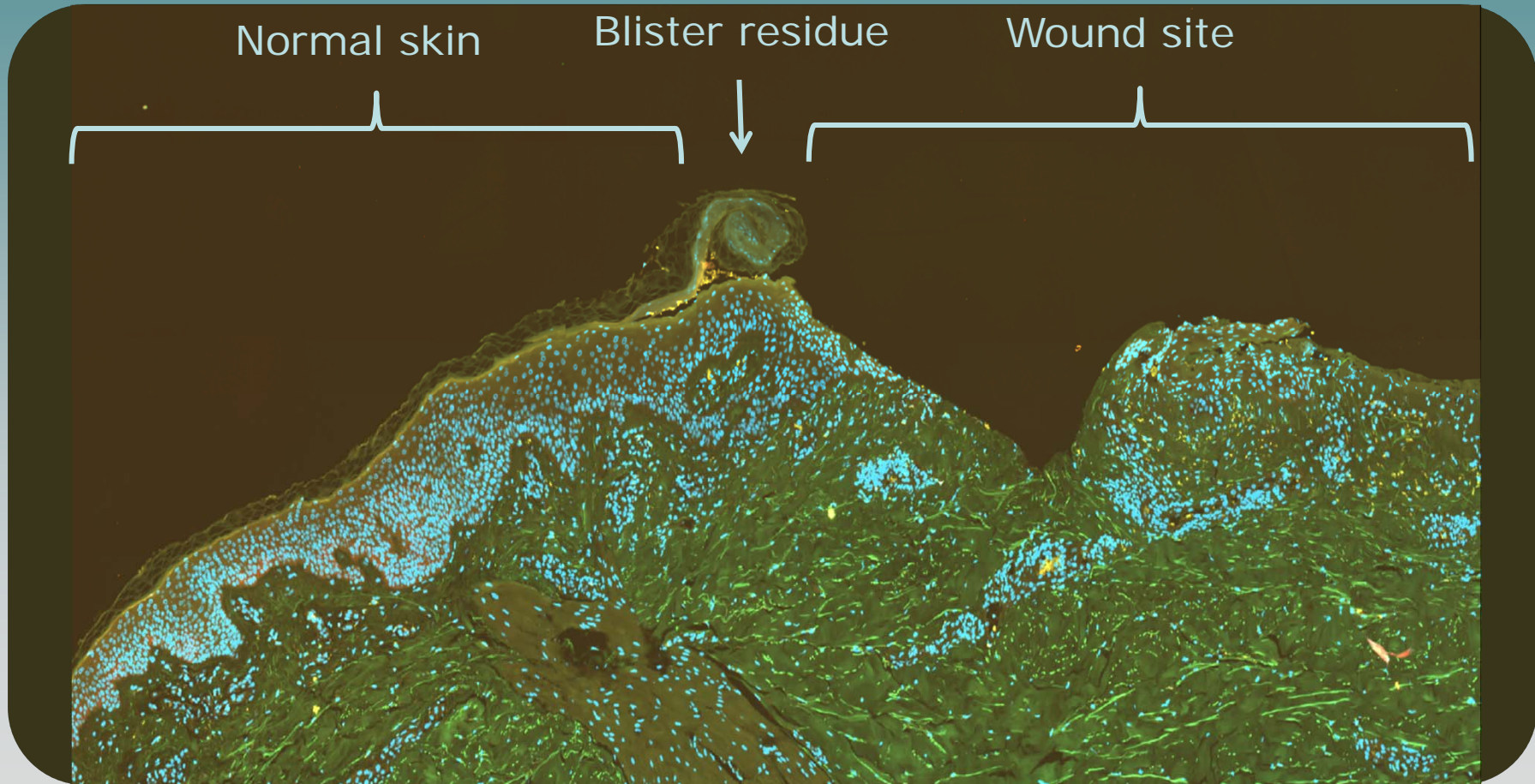
Day 4



Ågren *et al.* 2014

Human skin Microbiome

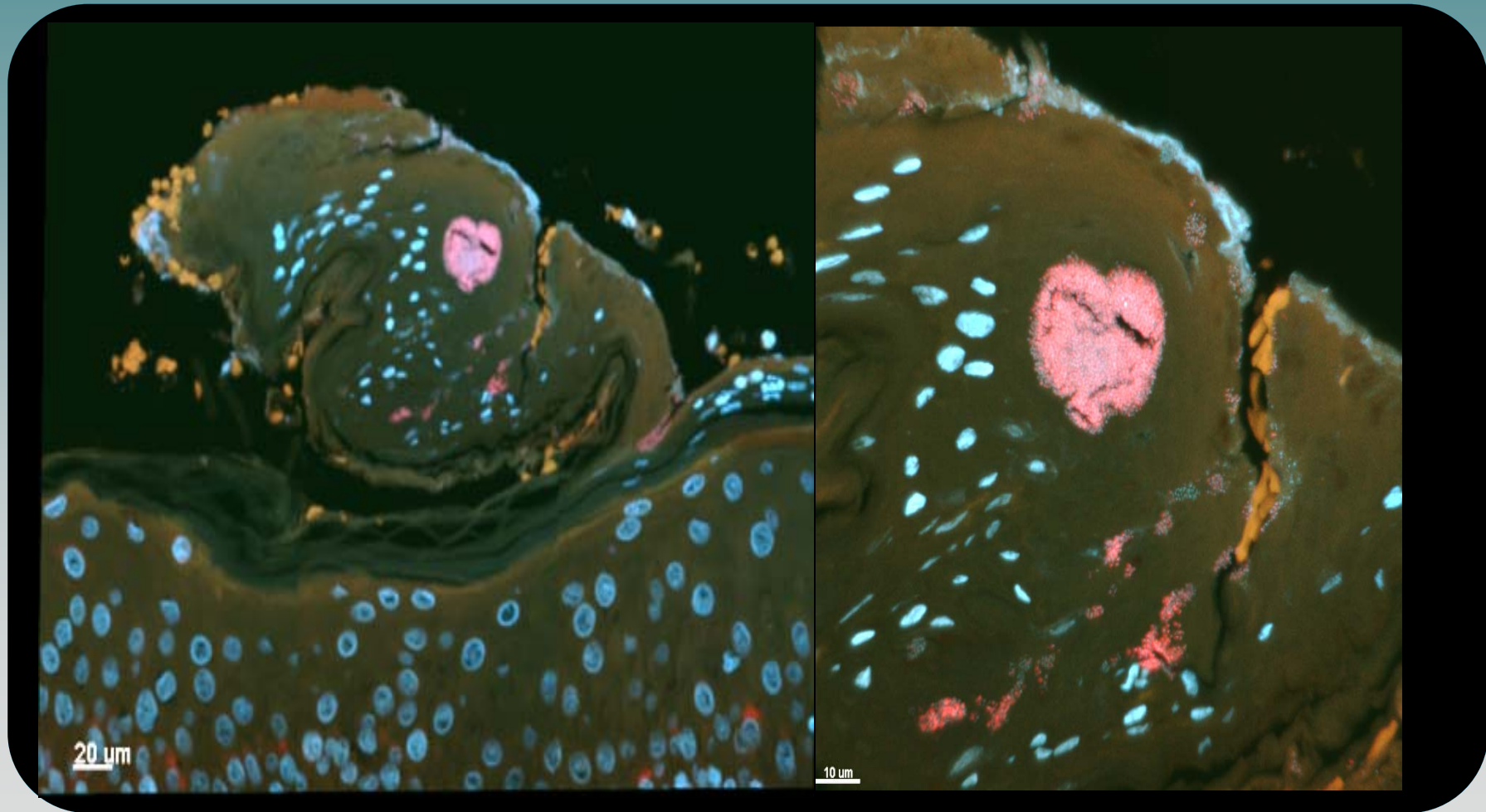
Beneficial and nuisance



June 2011
Fluorescent slide scan of full-thickness skin biopsy – zoom at wound edge

Human skin Microbiome

Beneficial and nuisance

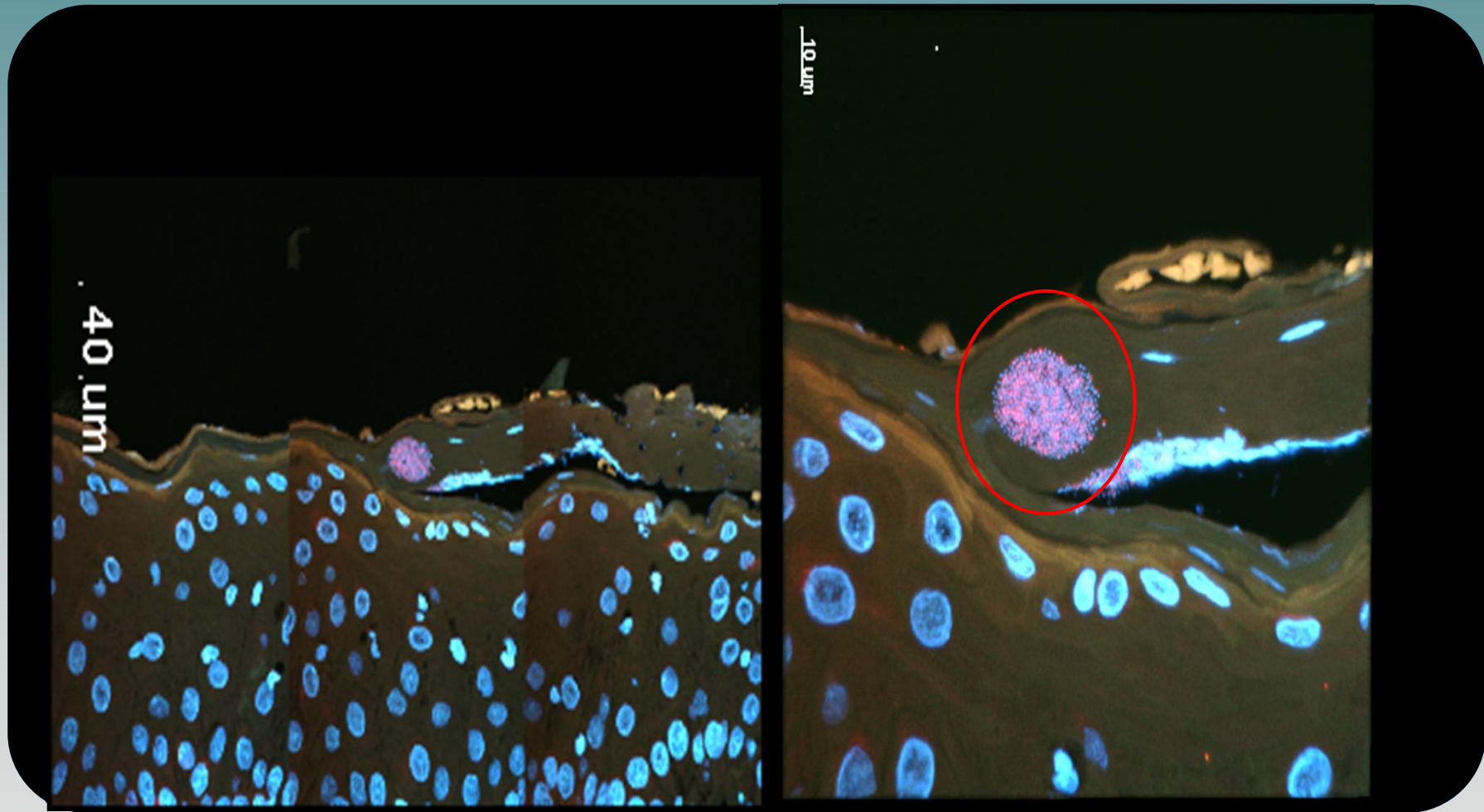


June 2015

Biofilm aggregates at wound edges

Human skin Microbiome

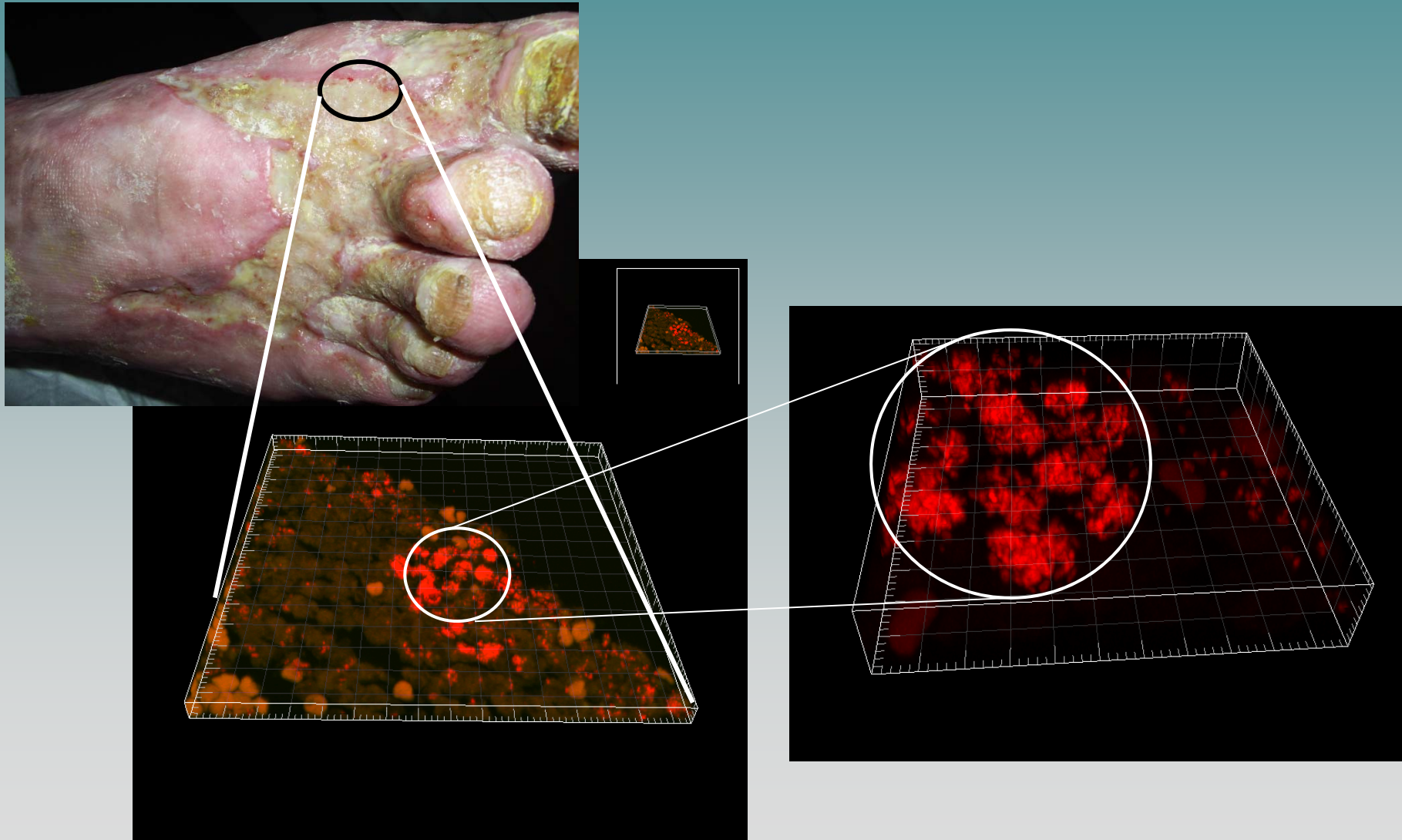
Beneficial and nuisance



June 2015

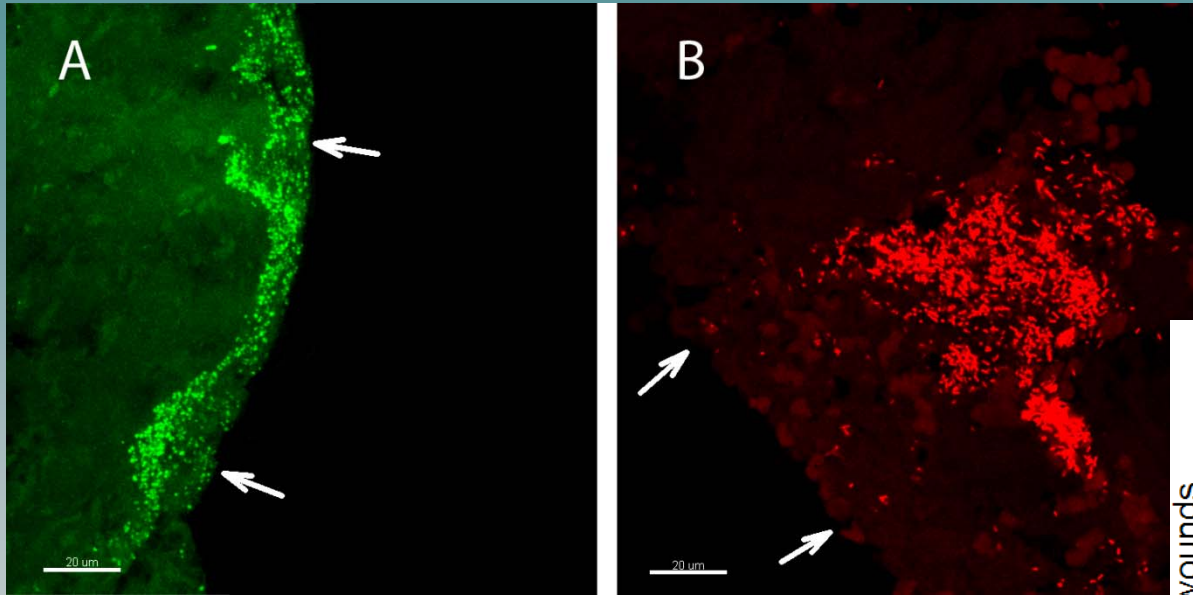
Biofilm aggregates at wound edges

Biofilm i kroniske sår



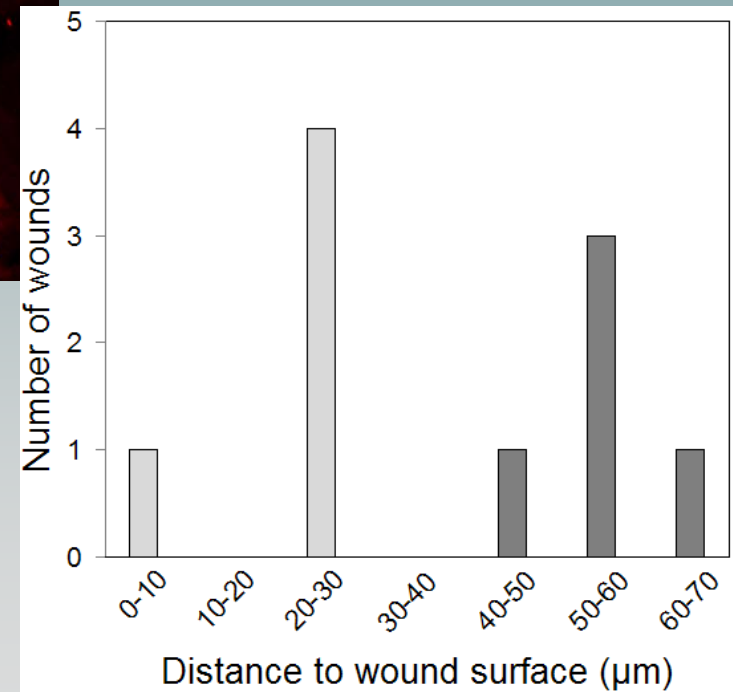
Bjarnsholt et al; Wound Repair and Regeneration, 2008 Jan-Feb;16(1):2-10.

Fordeling af specier



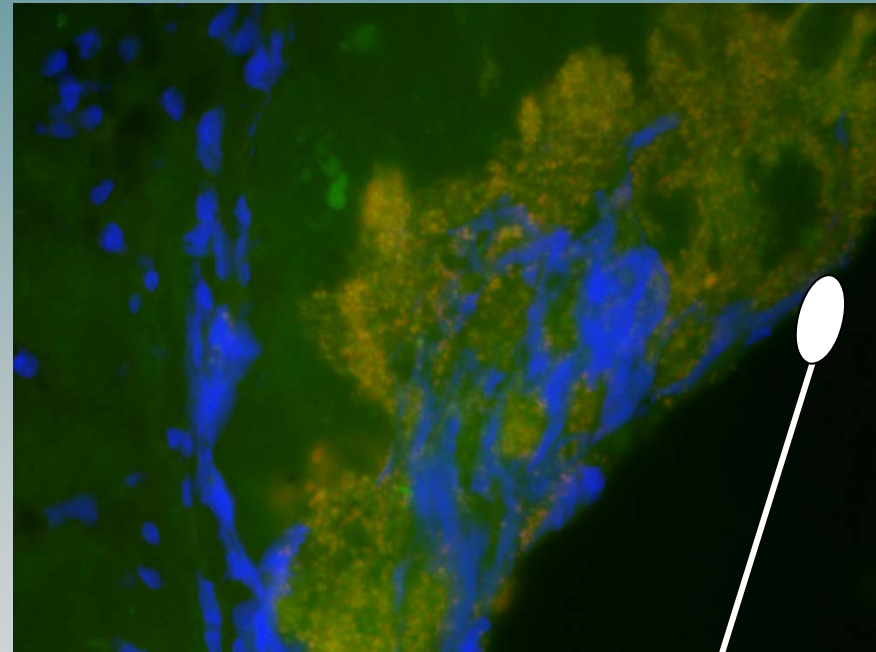
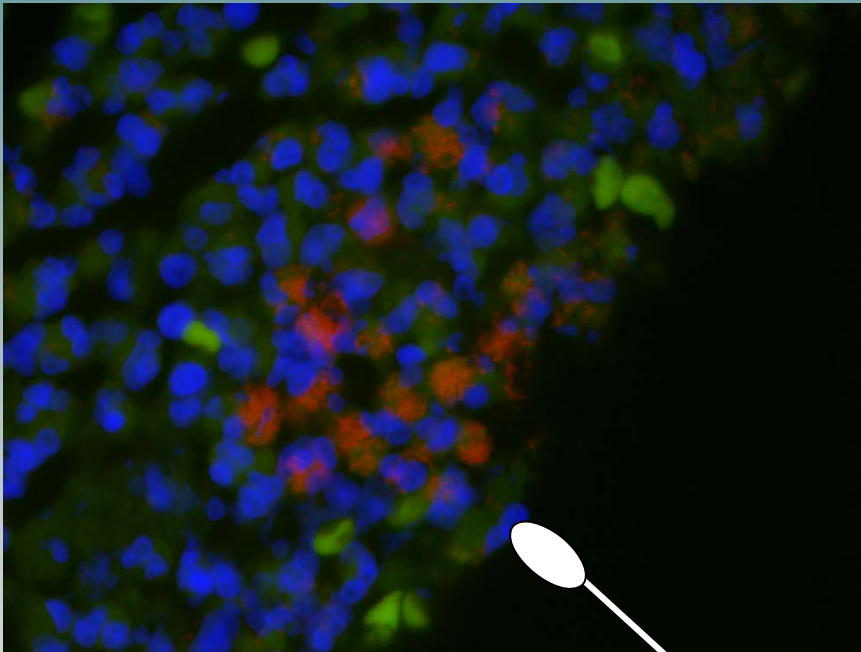
S. aureus

P. aeruginosa



S. aureus biofilm □
P. aeruginosa biofilm ■

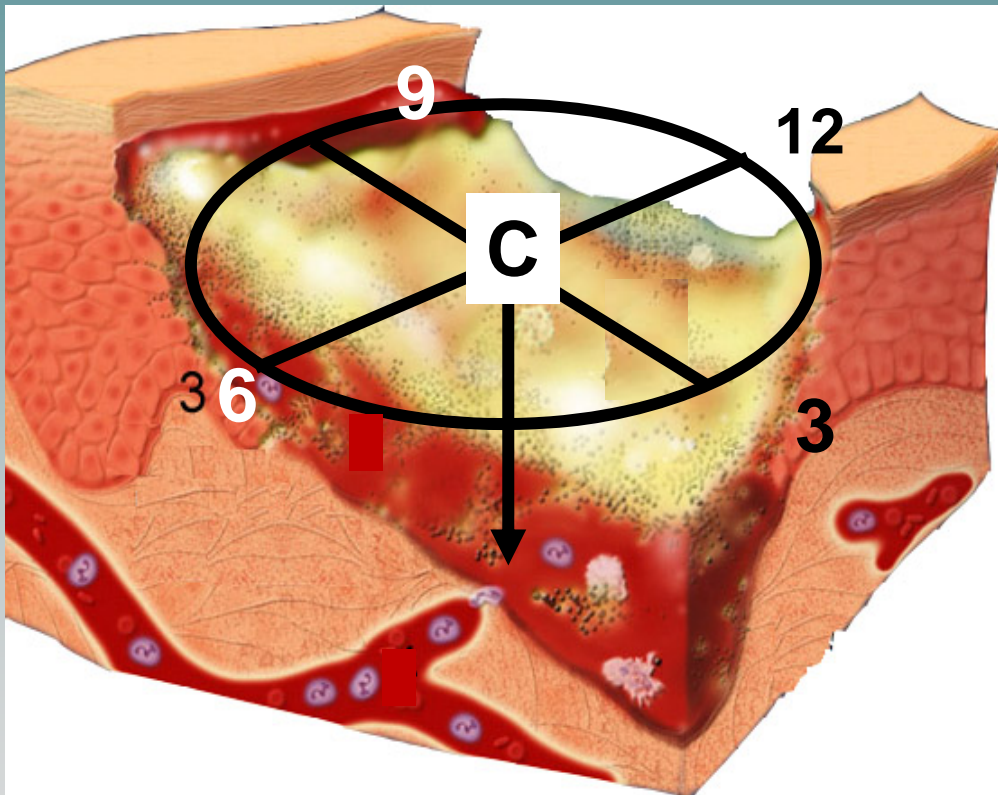
Forskellige områder



Podninger

Heterogen fordeling af bakterier i kroniske sår

qPCR *Pseudomonas aeruginosa*



Position	Wound 1	Wound 2
C	510±18%	920±9%
3	No sample	300±13%
6	760±7%	8200±8%
9	47±9%	800±10%
12	280±3%	15±5%

Thomsen TR, Aasholm MS, Bjarnsholt T, Givskov M, Kirketerp-Møller K, and Nielsen PH. 2010. The bacteriology of chronic venous leg ulcer examined by culture-independent molecular methods. *Wound Repair and Regeneration*: Jan-Feb;18(1):38-49

Picture from homepage of Montana State University

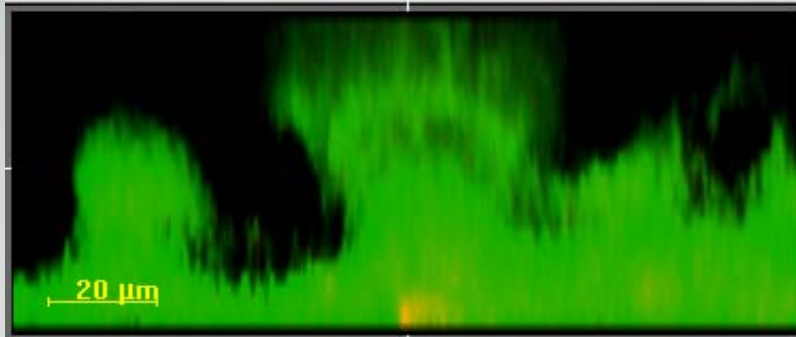
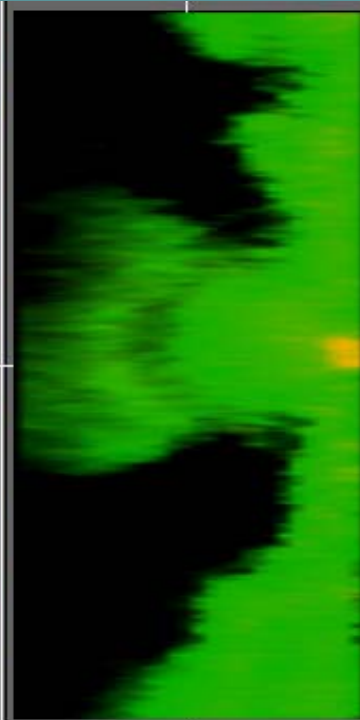
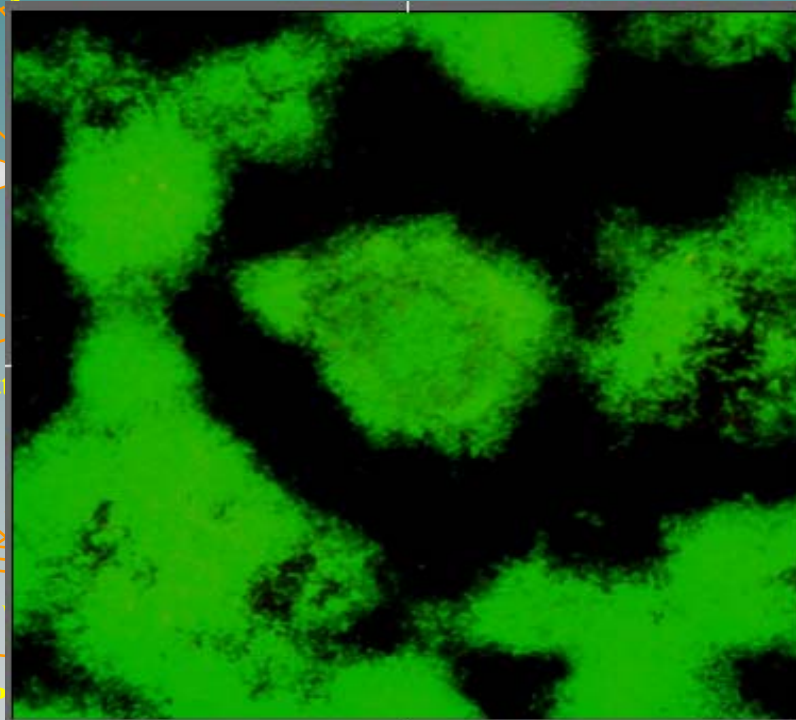
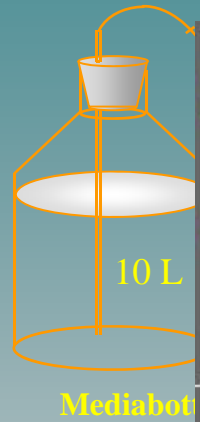
Hvordan genkender man en biofilm?

- A. Bakterier på en overflade
- B. Store biofilm
- C. Bakterier og inflammation

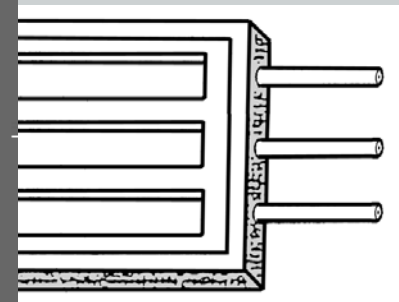
Biofilm klinisk vs. in vitro



in vitro biofilmen



mm →

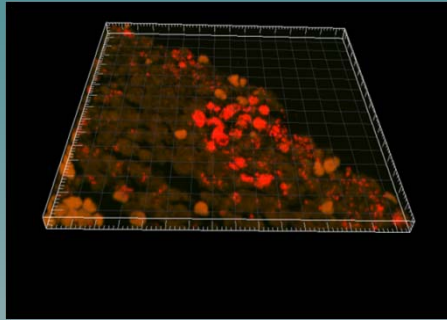


4 mm

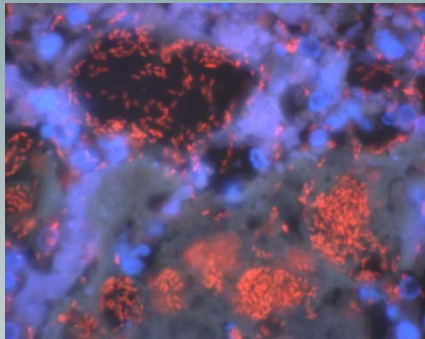
Coverglass

Janus Haagensen

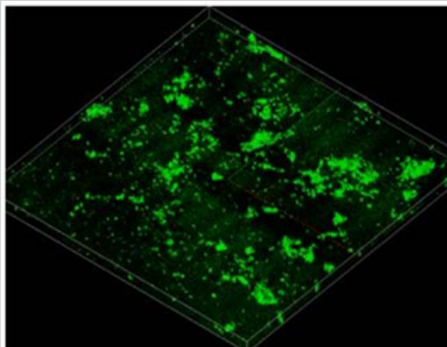
in vivo Biofilmen



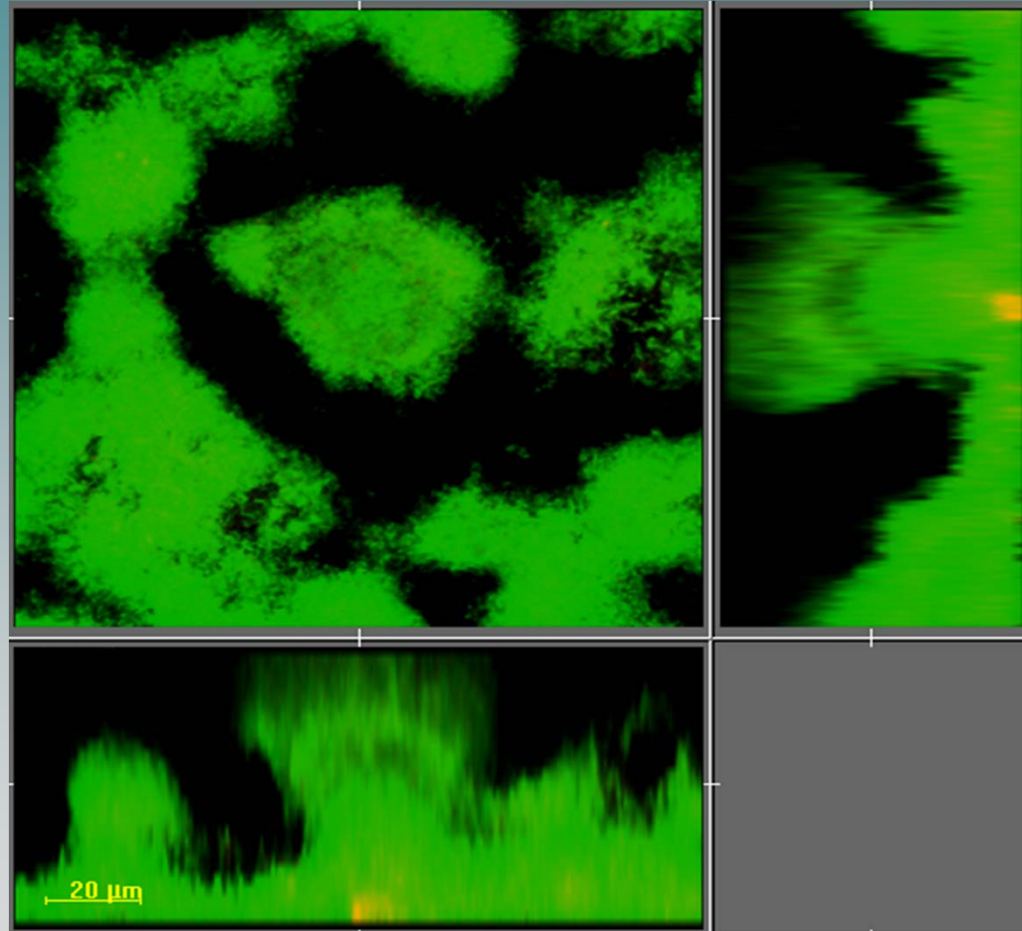
Sår



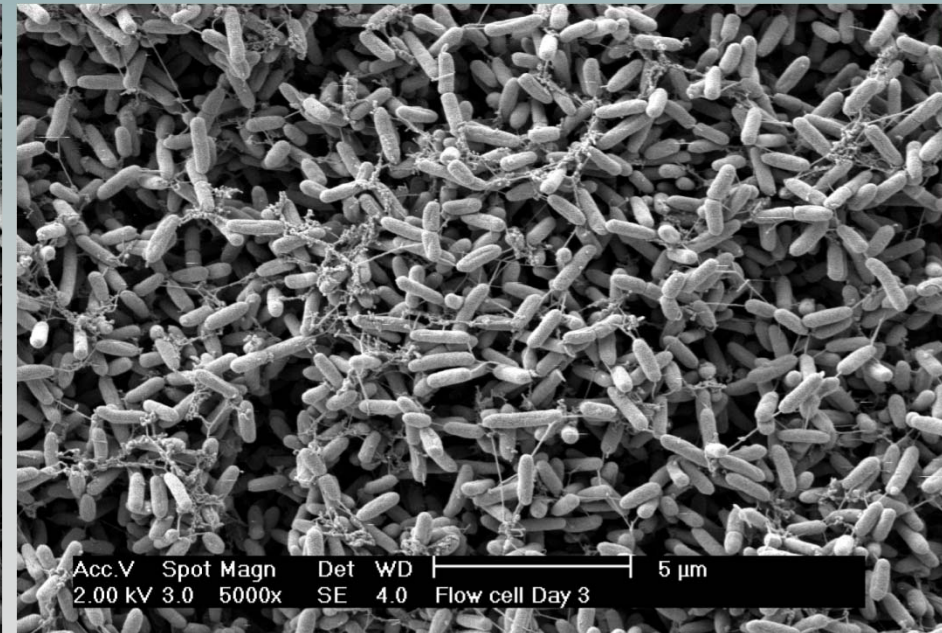
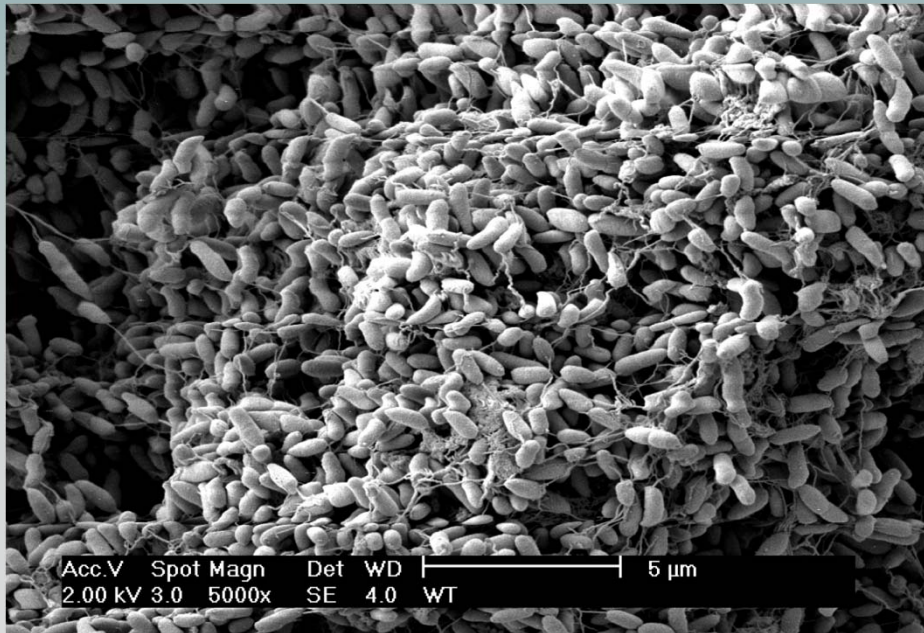
CF lunge



Implantat muse model



Surface or not to surface that is not the question



Alhede M et al.; PLoS One. 2011;6(11):e27943

Diagnose

Method	Advantages	Pitfalls and difficulties
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

Hvad hvis man ikke kan finde noget?

Meta analysis - 78% chronic non-healing wounds contain biofilm

Global wound biofilm expert panel – 100%

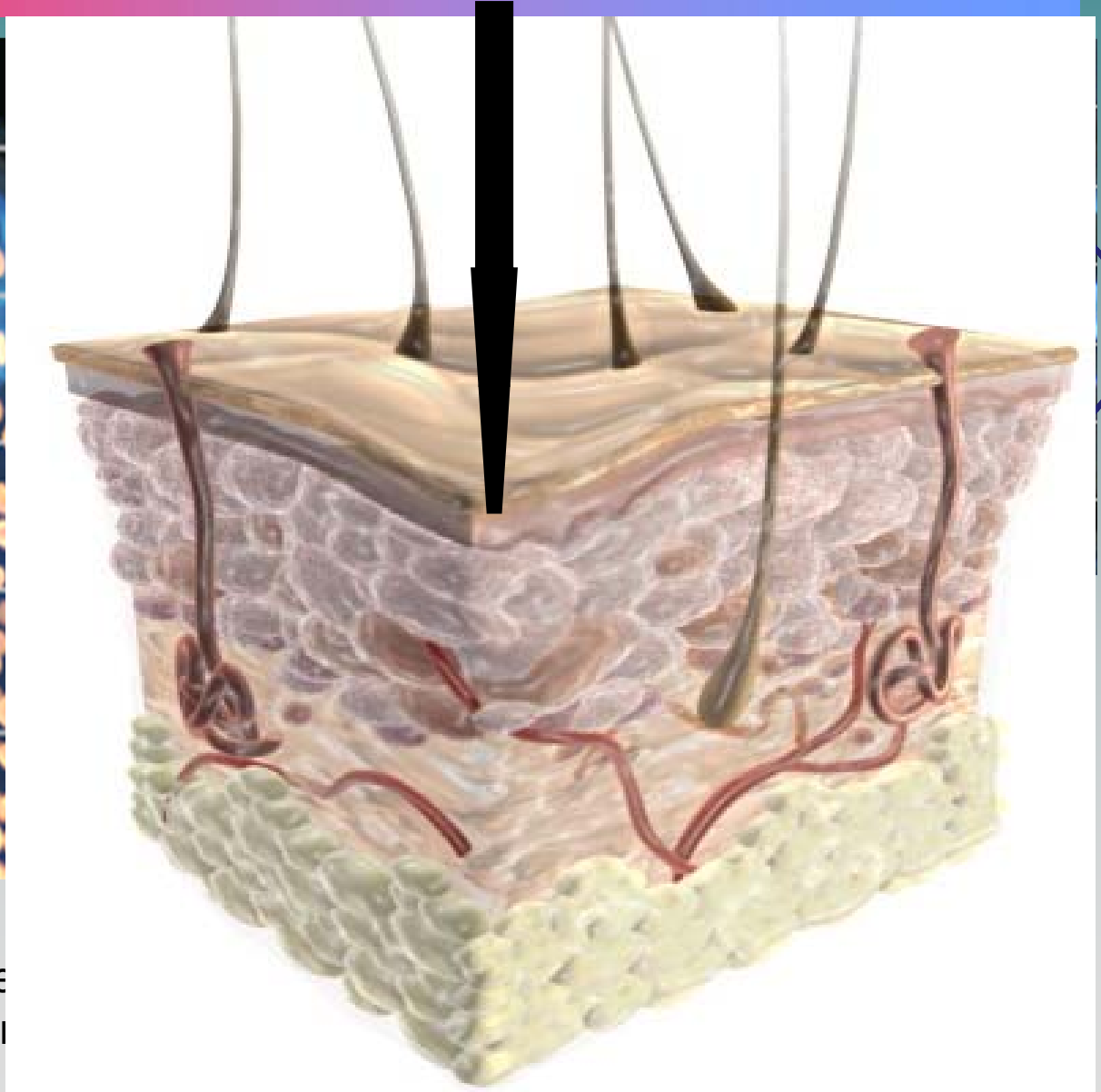
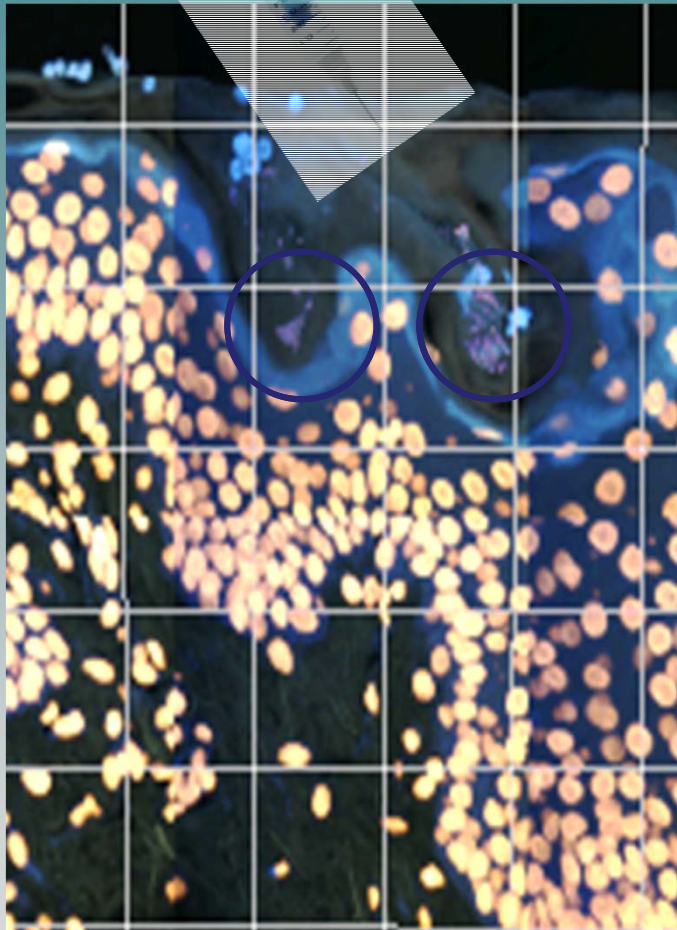


Consensus guidelines for the identification and treatment of biofilms in chronic non-healing wounds.

Schultz G, Bjarnsholt T, James GA, Leaper DJ, McBain AJ, Malone M, Stoodley P8, Swanson T, Tachi M, Wolcott RD; Global Wound Biofilm Expert Panel.

Wound Repair Regen. 2017 Sep 29.

Forebyggelse?



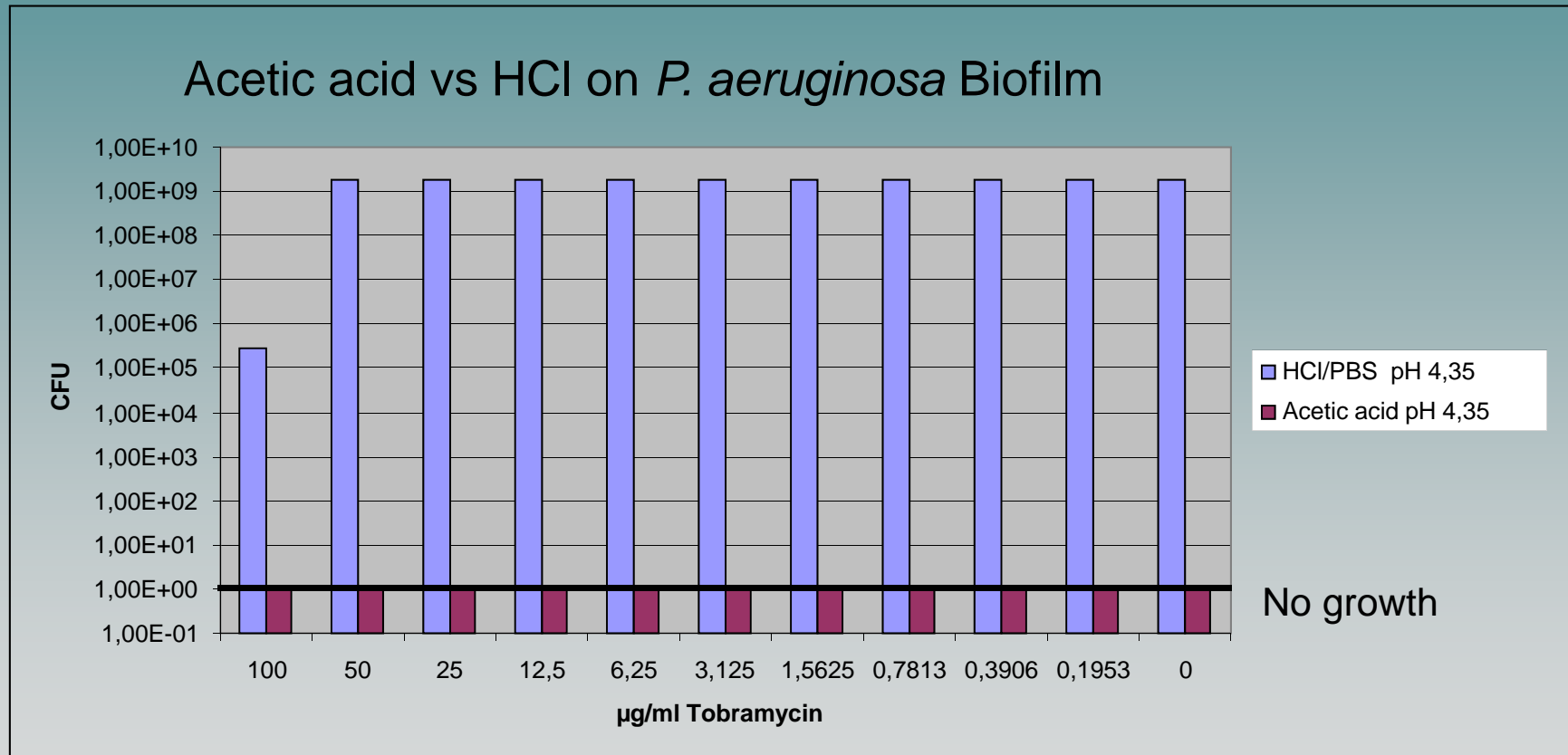
- Bacteria primarily found in the
• (1 exsample of bacteria in

Acetic acid

Acetic acid

- Well characterized natural anti-biofilm agent with novel pH dependant antimicrobial activity
- 0.5-2 % acetic acid at pH ~3-5
- $\text{CH}_3\text{COOH} \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+$ (pKs 4,76)
Active Not active

In Vitro - HCl vs. Acetic Acid



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



Klaus Kirketerp-Møller

Hvad nu?



Acknowledgments

University of Copenhagen:

Maria Alhede

Kasper N Kragh

Steffen R Eickhardt-Sørensen

Anne K Nielsen

Stephanie G Crone

Majken Sønderholm

Blaine Fritz

Camilla Stavnsbjerg

Marie Thaysen

Lasse Kvich

Lene Bay

Anne Hesselvig

Michael Givskov, Klaus Qvortrup

Oana Ciofu, Søren Sørensen

Michael Kühl, Mette Burmølle

Hans Petter Hougen, Henrik Elvang Jensen

Louise Kruse Johansen

Rigshospitalet:

Niels Høiby

Peter Østrup Jensen

Claus Moser

Kim Thomsen

Lars Christophersen

Michael Tvede

Claus B Andersen

Preben Homøe

Others:

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

Funding Sources



LUNDBECKFONDEN

-LEO-Fonden

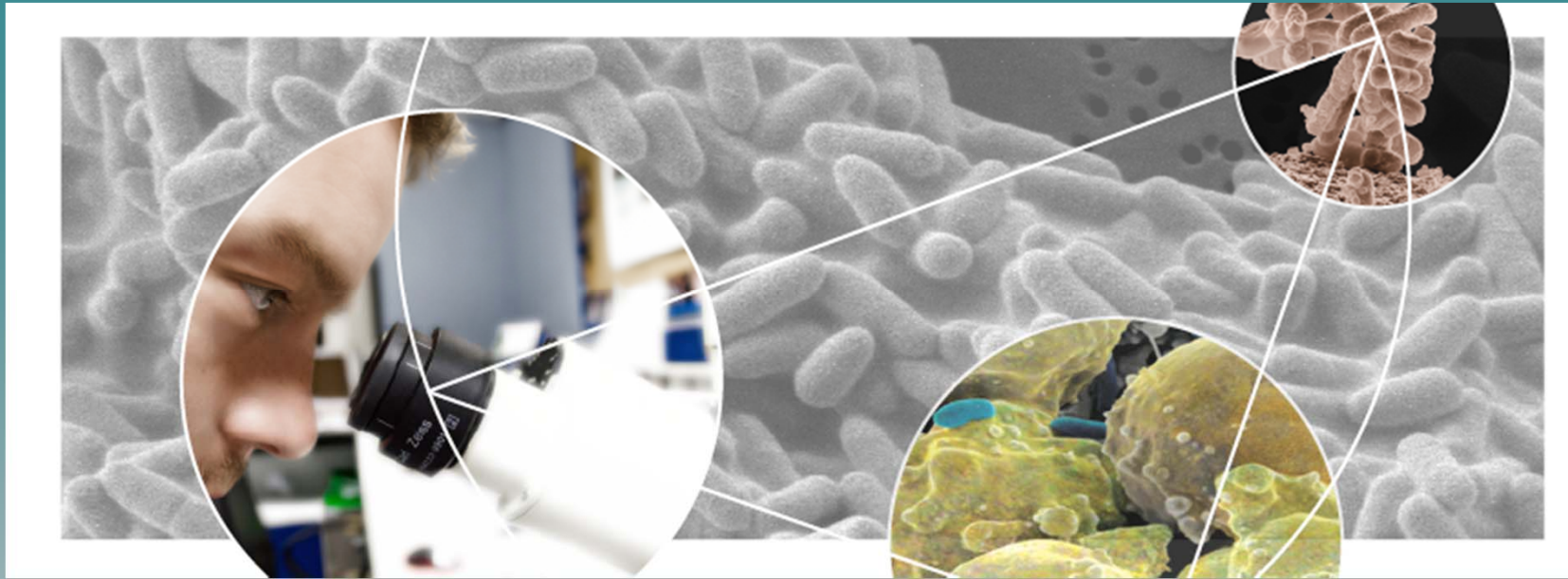
-Human Frontier Science project

-Capital Region Research Foundation for Health Research

-Novo Nordisk A/S

-AdvanDx Inc.

-Contura



Would you like to learn about Biofilms in chronic infections?

Sign up for our biofilm online courses at:

- www.biofilmcourse.ku.dk
- www.coursera.org/course/bacteria