Pathogenesis and clinical importance of biofilms

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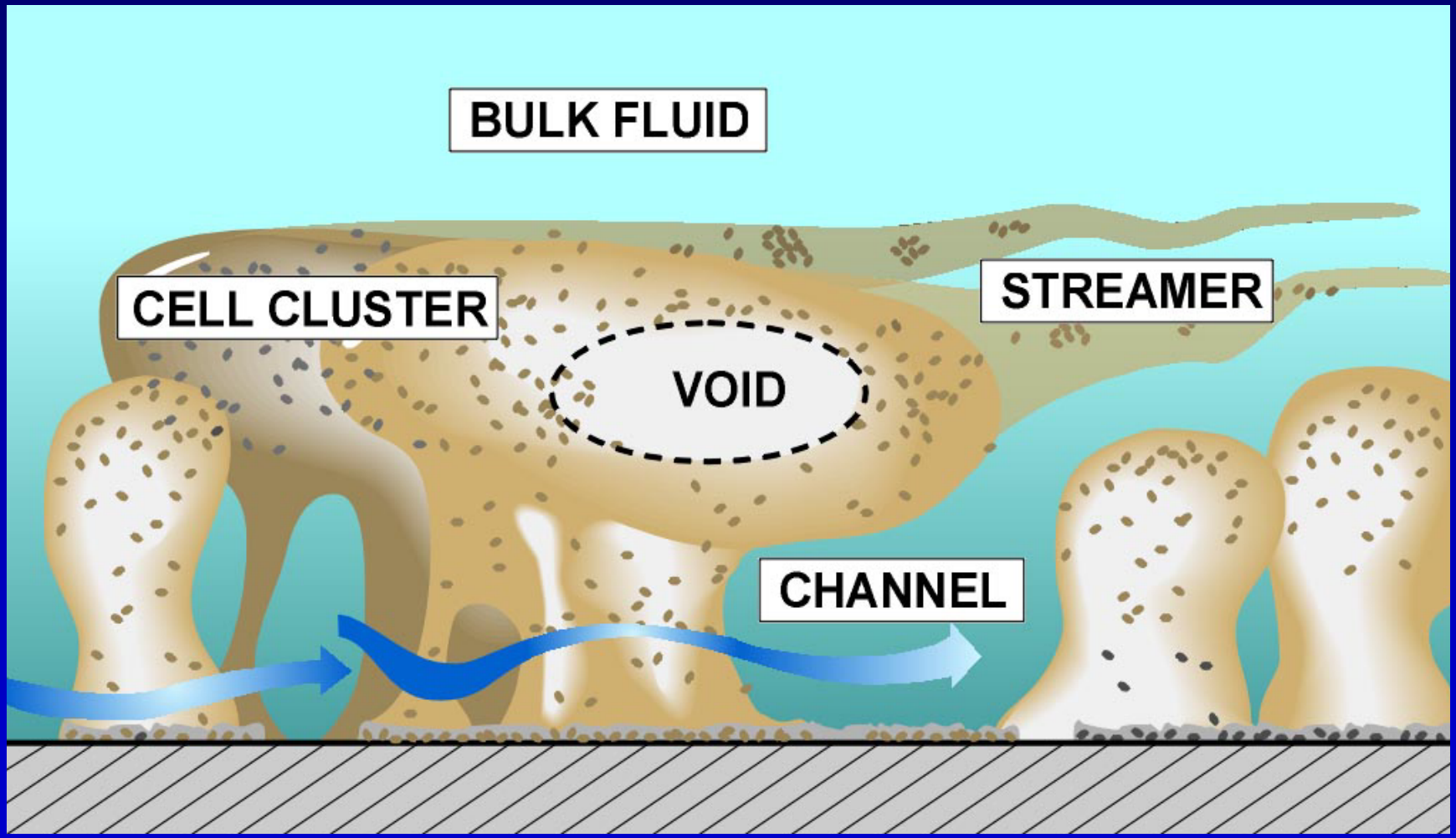
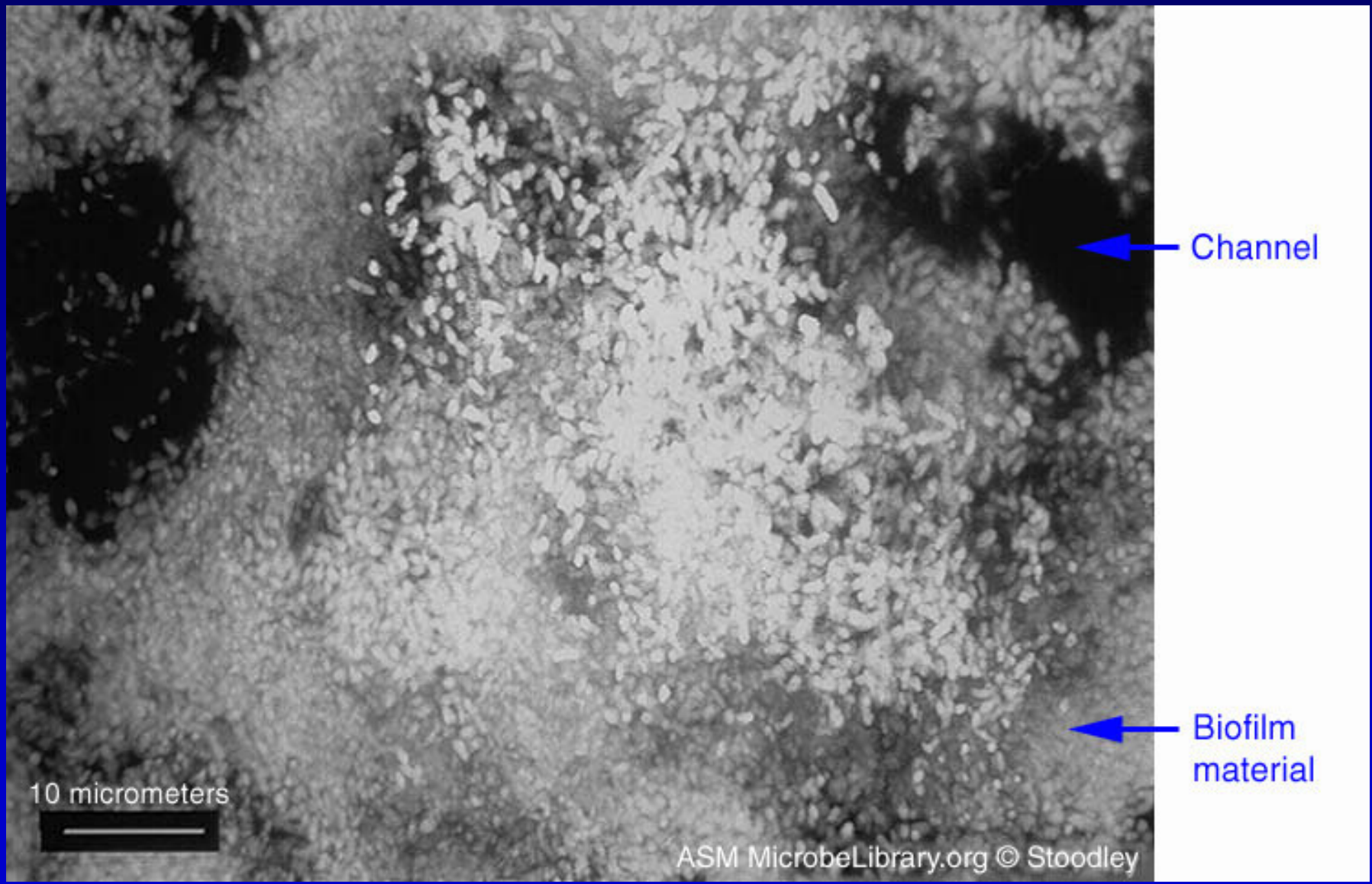
contents

- What is a biofilm
- Biofilms in infections
- Formation of a biofilm
- Characteristics of a biofilm

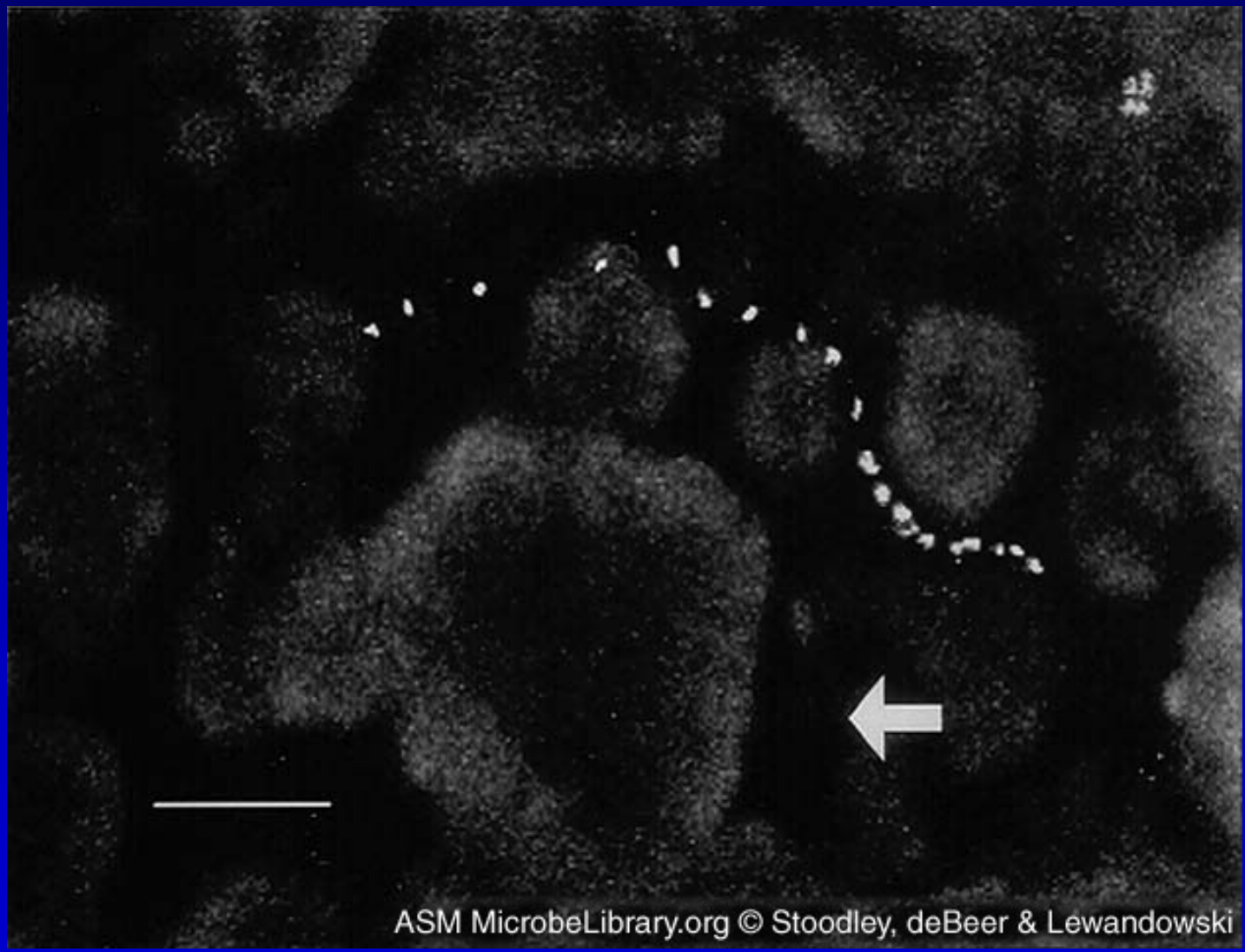
Structure of a Biofilm



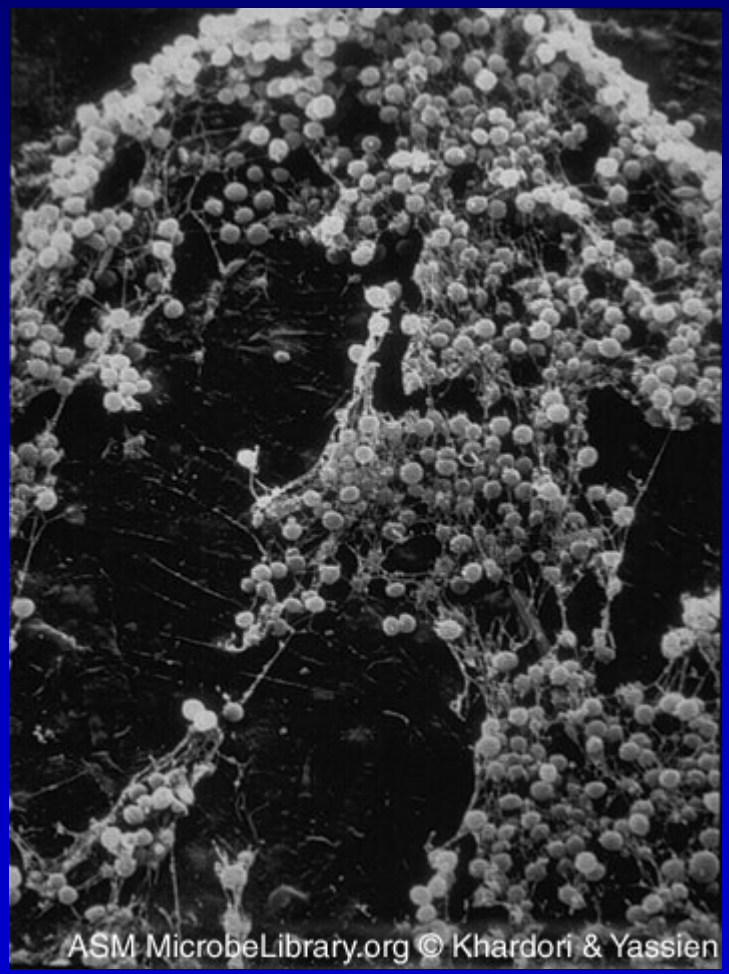
Structure of a Biofilm

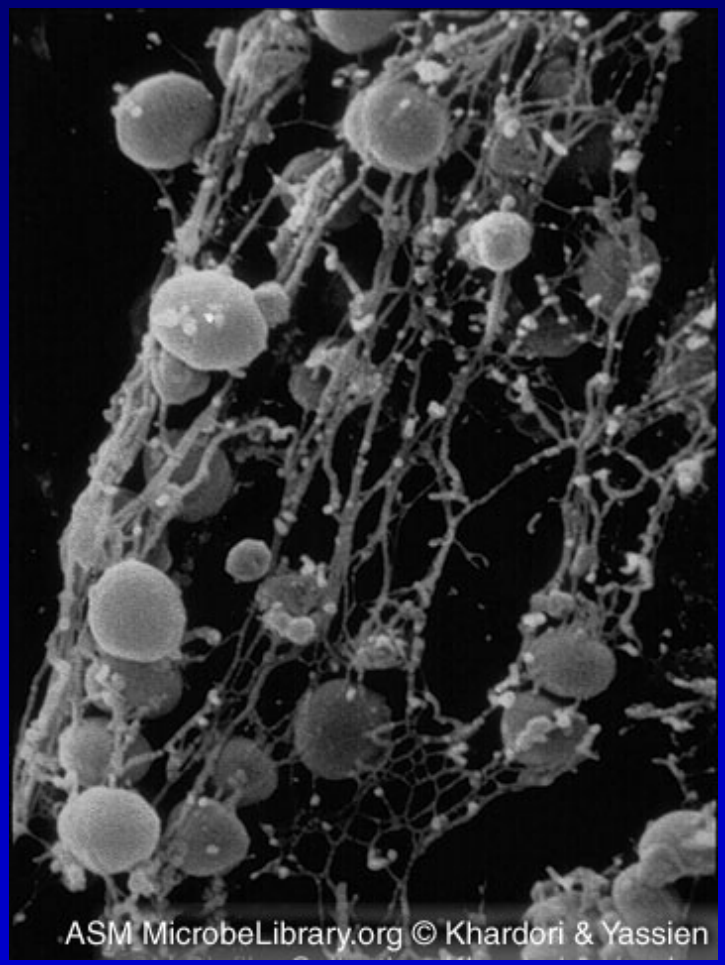


Biofilm ultrastructure

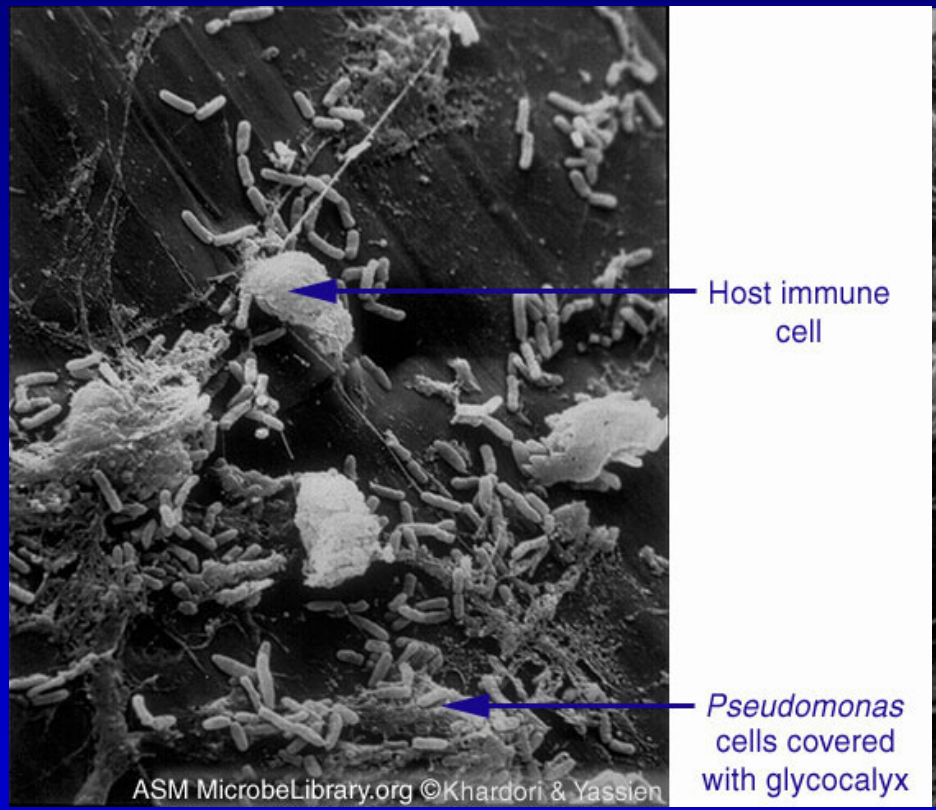


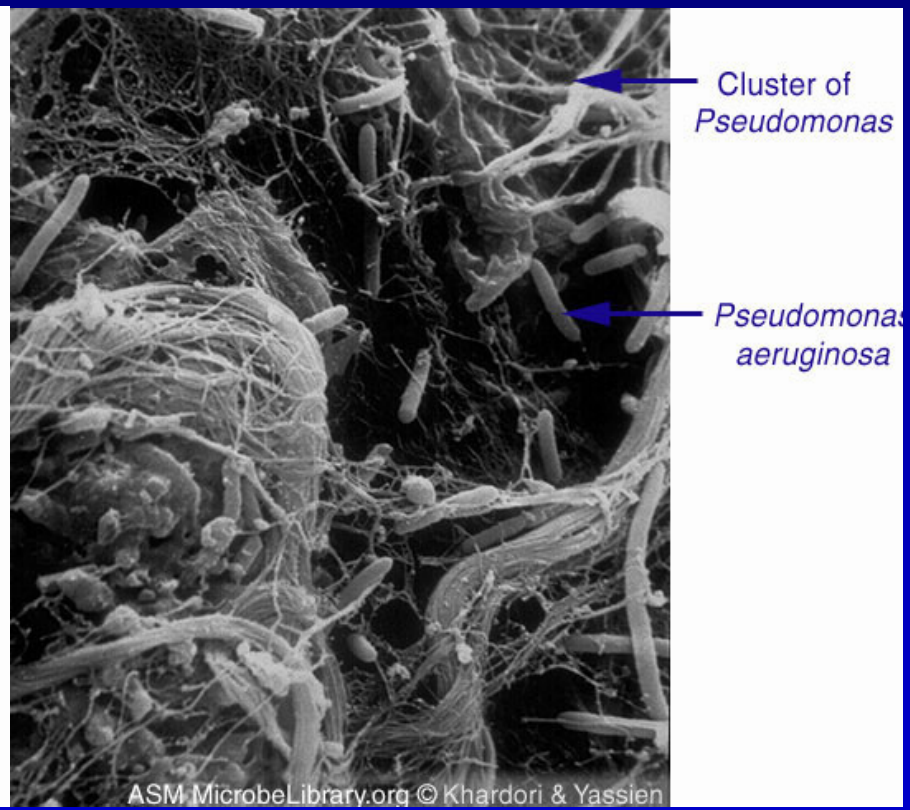
Staphylococcal biofilms



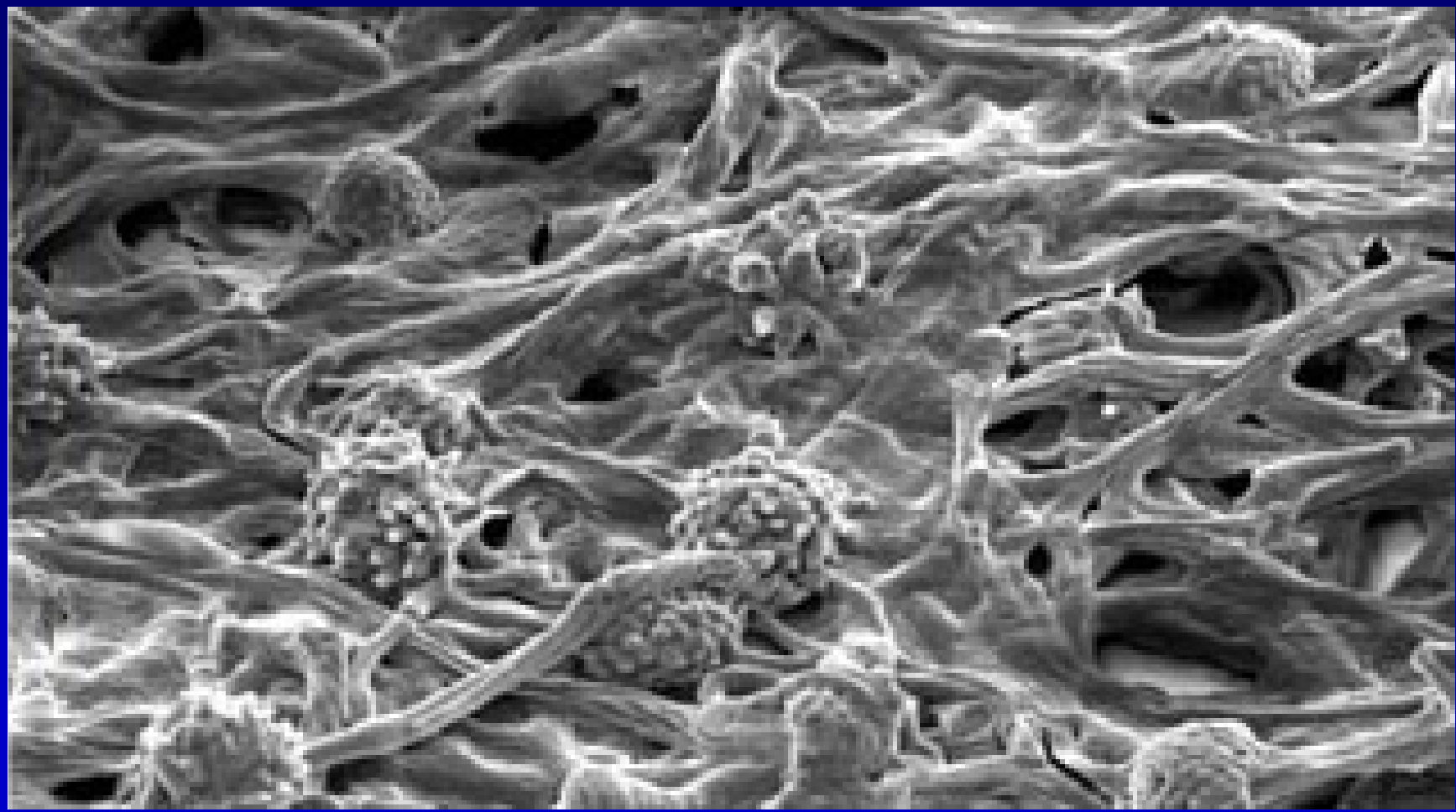


Pseudomonas biofilms





Mixed biofilms : bacteria + fungi



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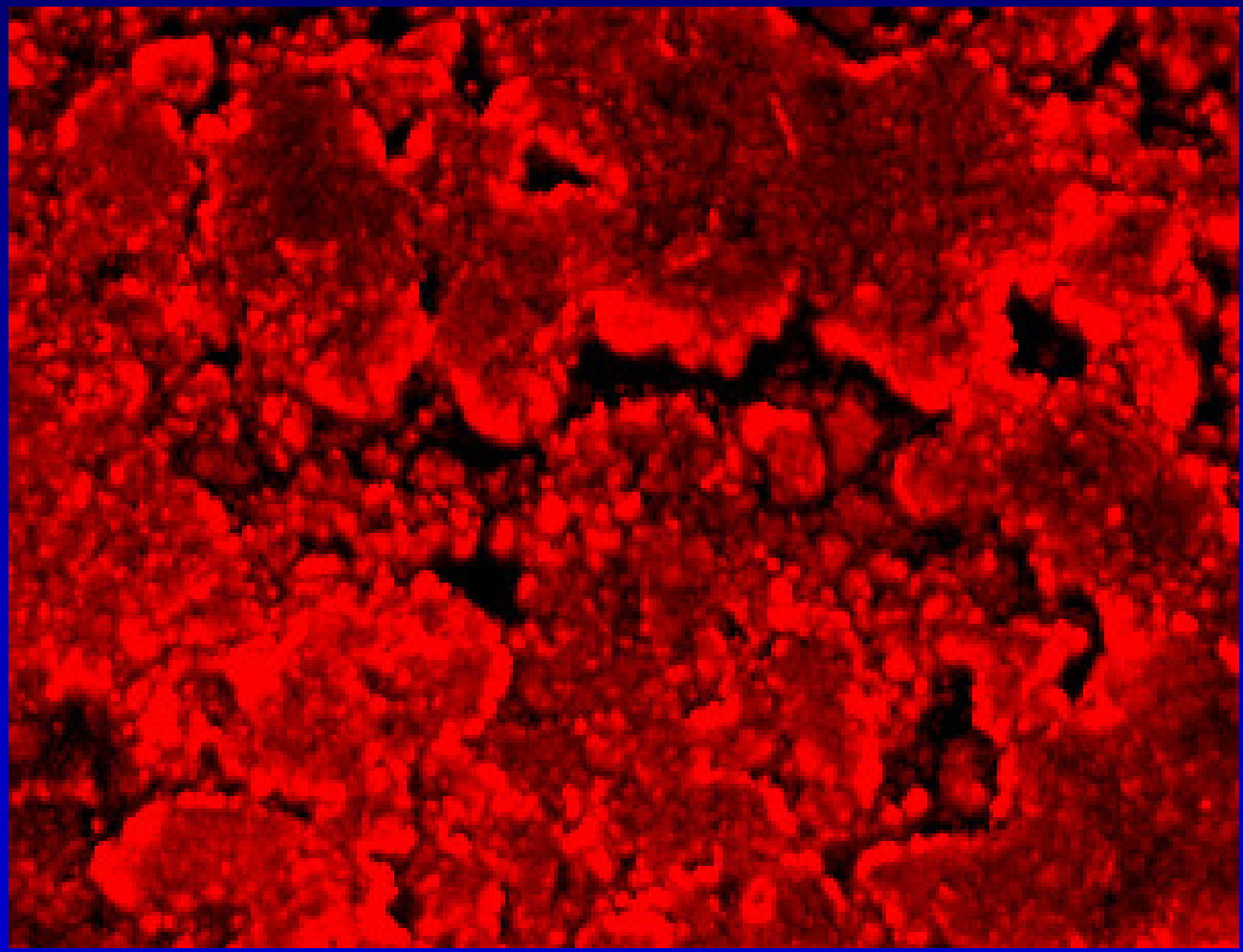
Biofilms in infections

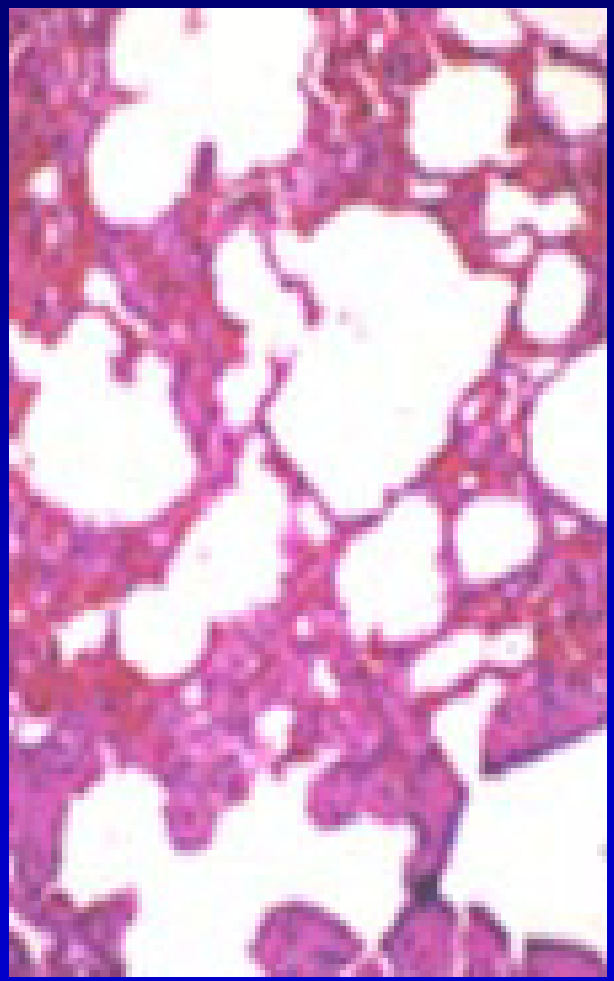
- Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections (NIH 2002).
- Infectious processes in which biofilms have been implicated
  - urinary tract infections,
  - catheter infections,
  - middle-ear infections,
  - formation of dental plaque, gingivitis,
  - coating contact lenses,
  - endocarditis
  - infections in cystic fibrosis
  - infections of permanent indwelling devices such as joint prostheses and heart valves.

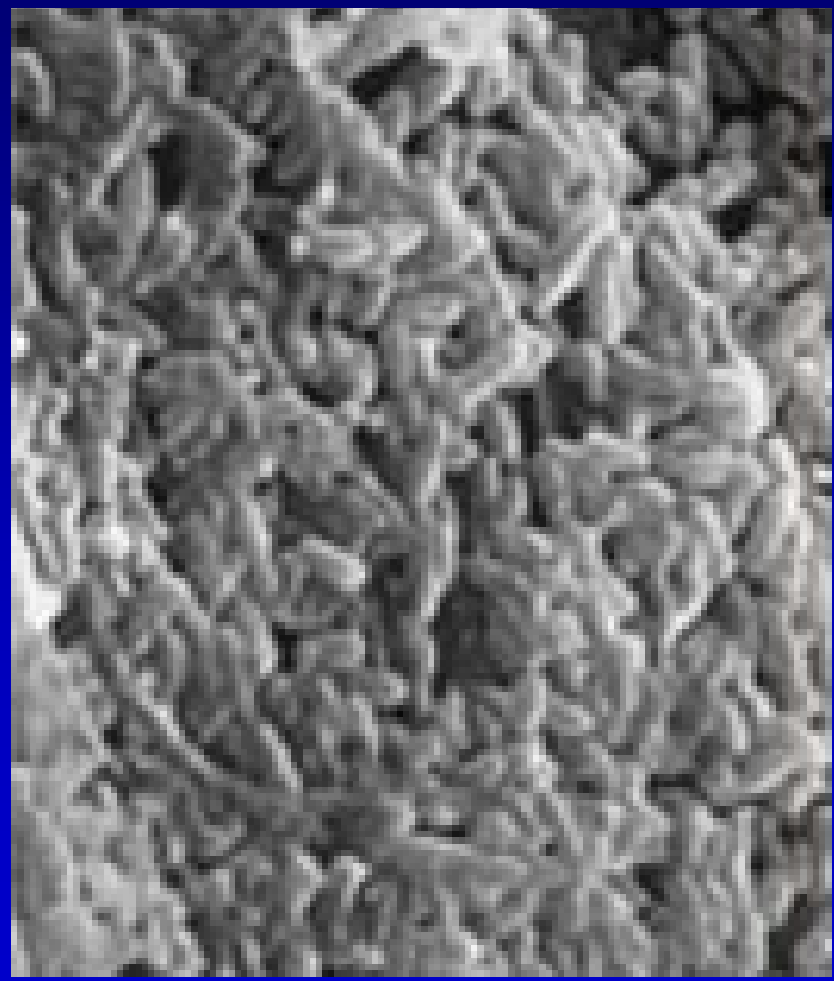
Biofilms in infections: dental plaque

Biofilms in infections: dental plaque

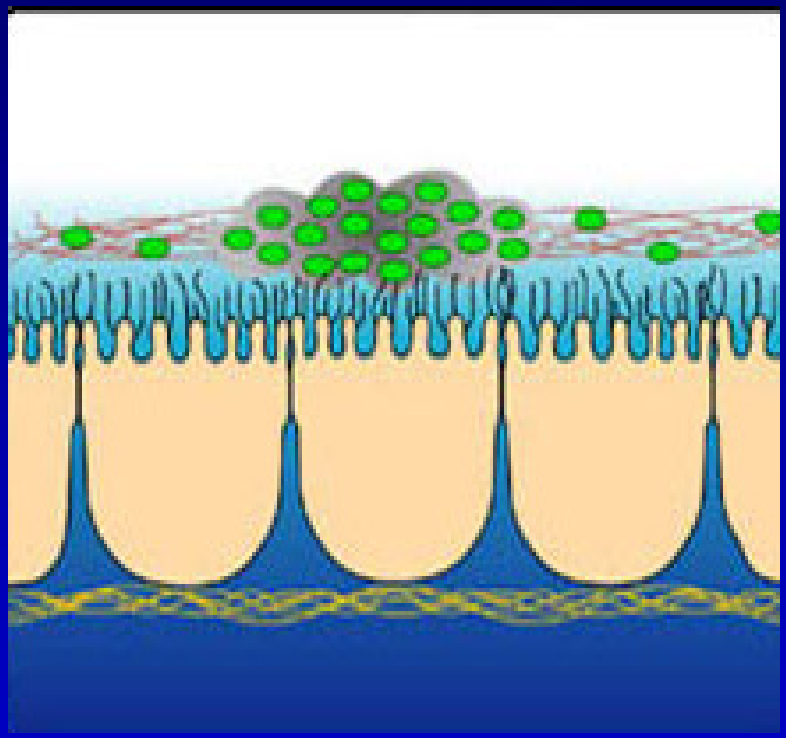


Biofilms in infections: cystic fibrosis

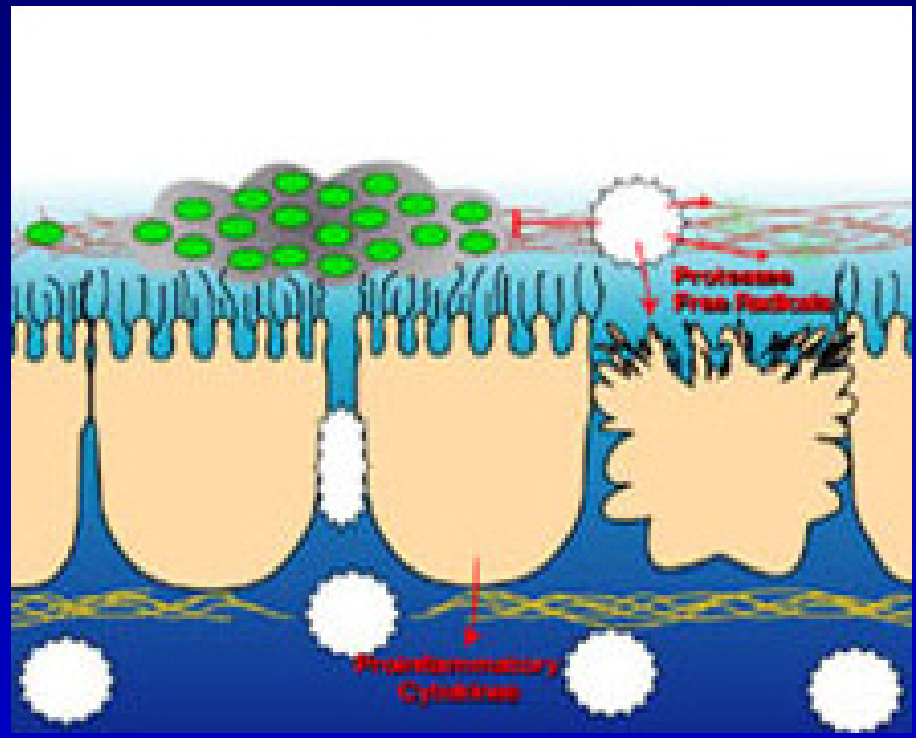




Biofilms in infections: cystic fibrosis



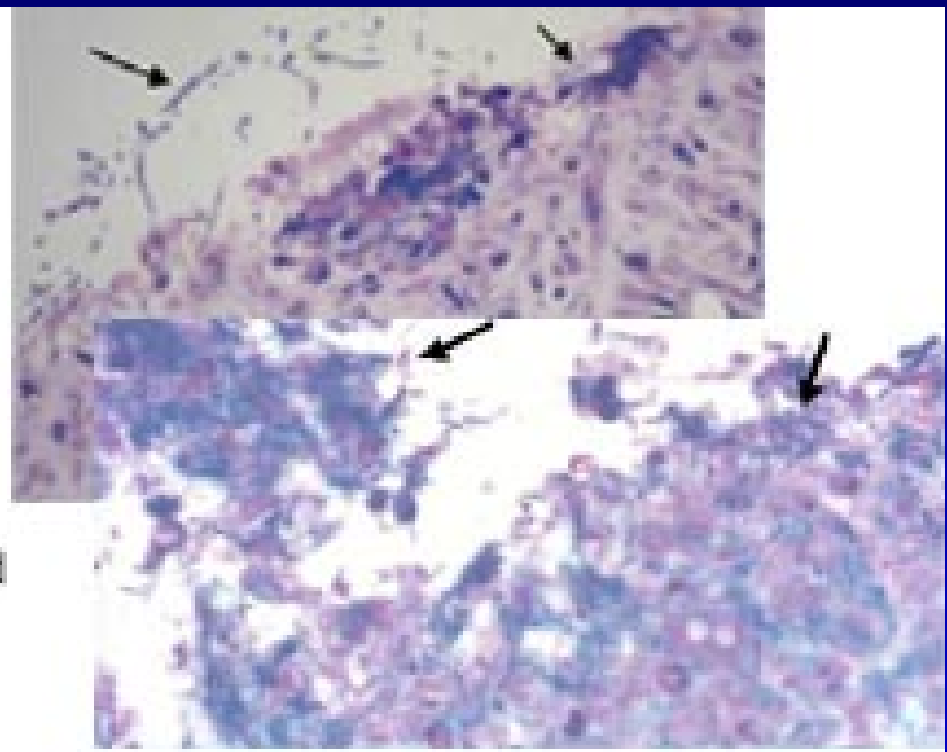
Colonization of CF Lung: Altered airway lumen promotes P aeruginosa colonization. Biofilm protects bugs and promotes hypermutation.



Immune Attack: "Hyperinflammation" as recruited neutrophils unable to eradicate bugs, instead damage lung tissue.

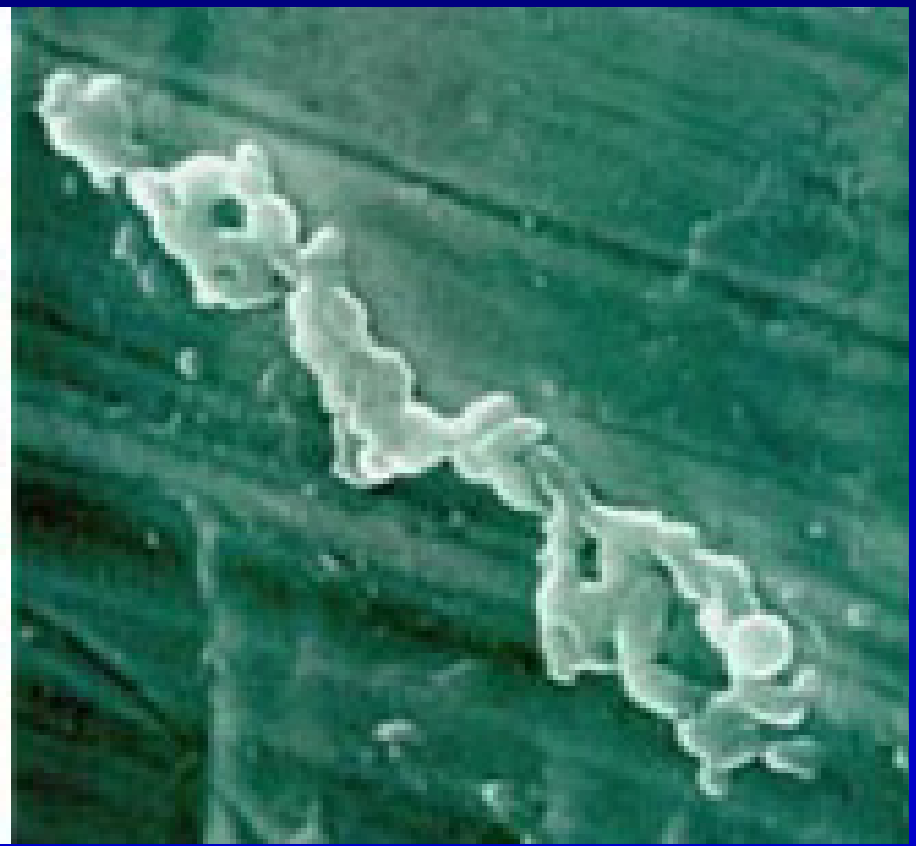
Biofilms in infections :wound infections

Biofilms in delayed wound healing: bacterial infected venous ulcer (above left); infected open wound (left).

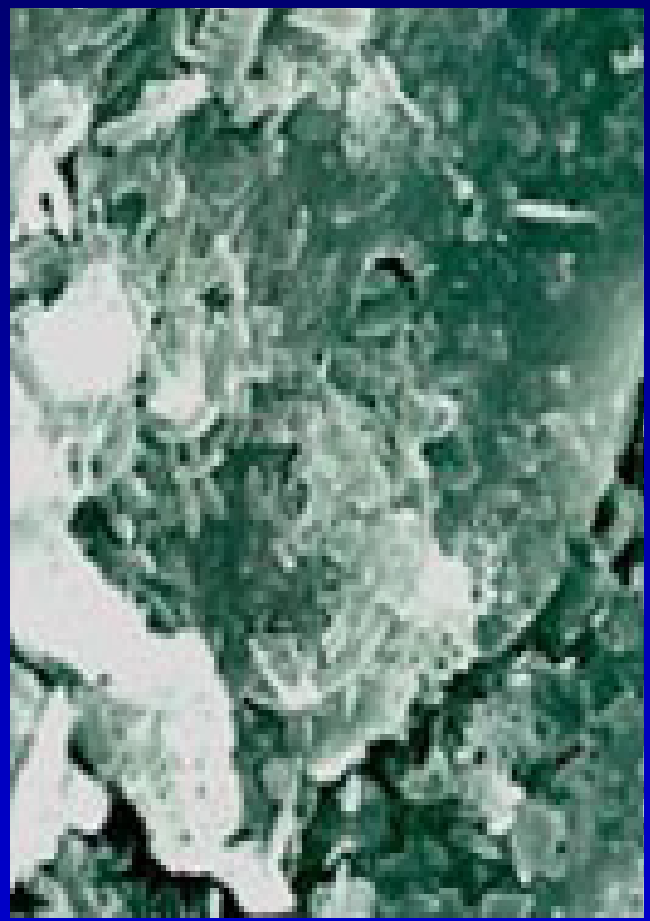


Bacteria on the surface of a contaminated wound (above right); Bacterial microcolonies encased in protective bacterial exopolysaccharide and host exudate.

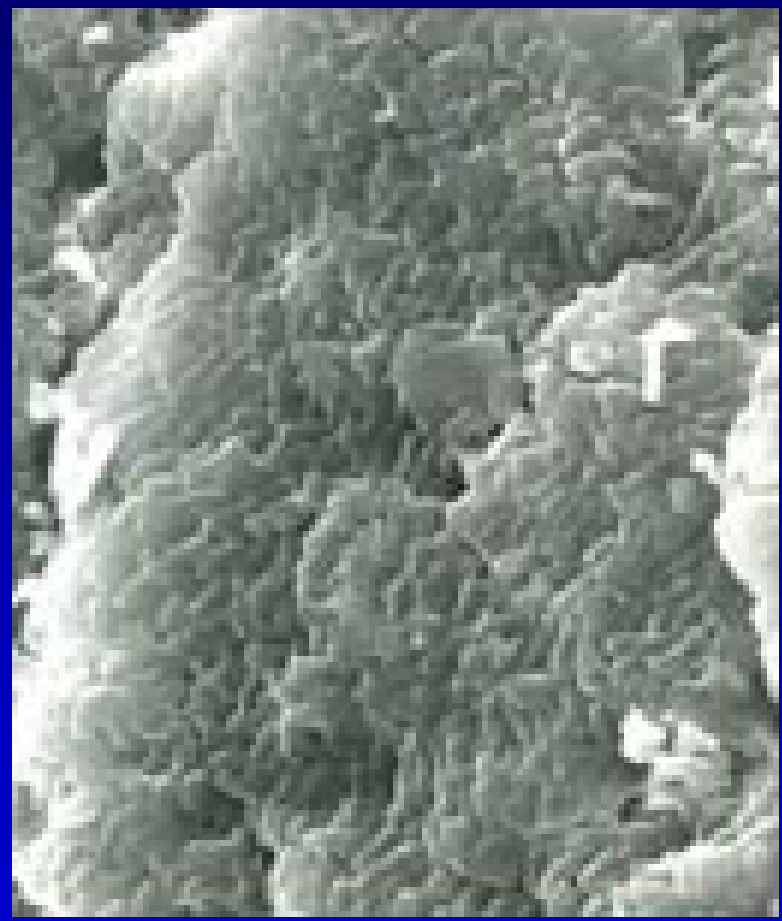
Biofilms in infections:
IUD & pelvic inflammatory disease



Biofilms in infections: urinary catheters

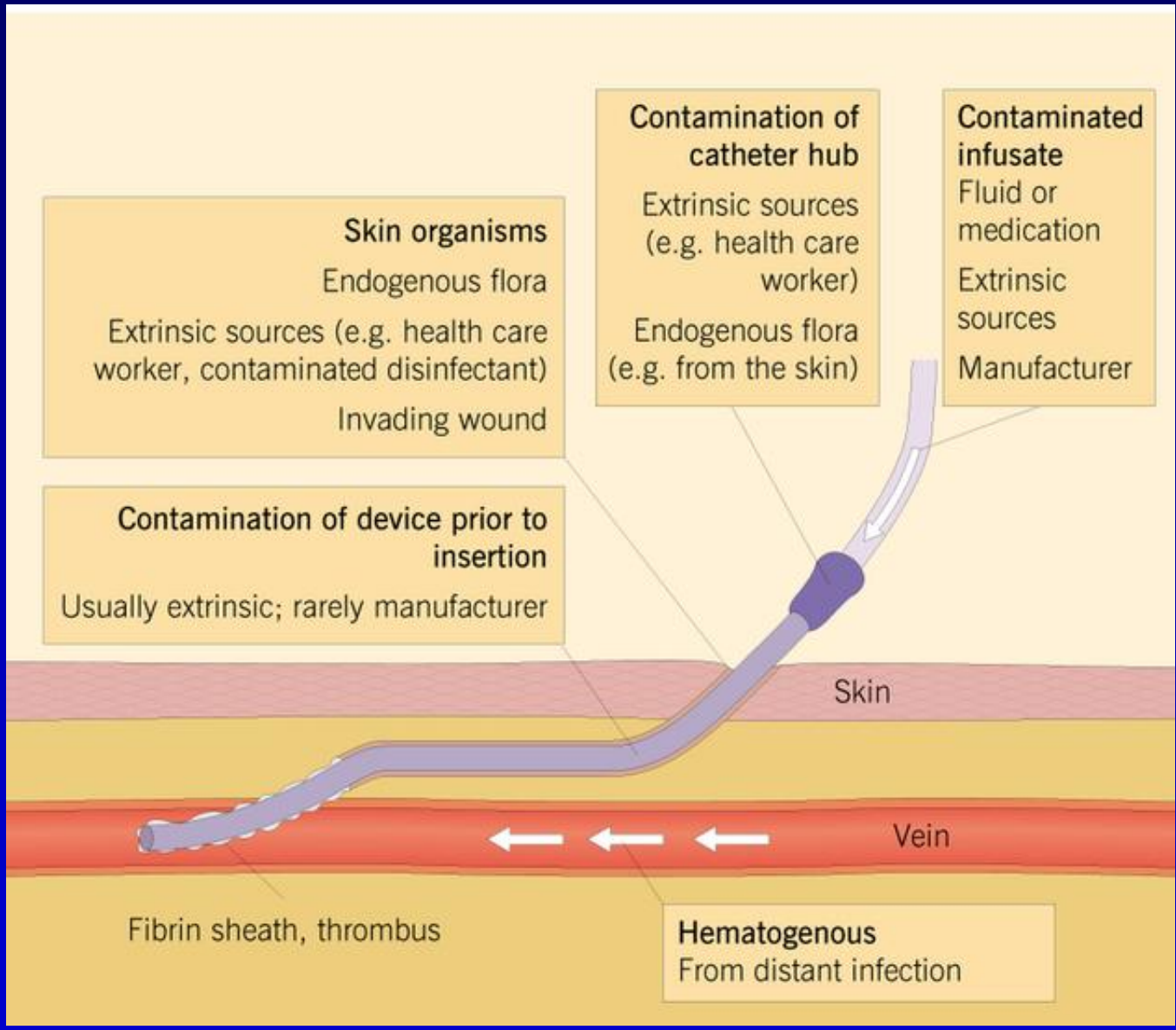


Exterior surface



Interior surface

Biofilms in infections: catheters



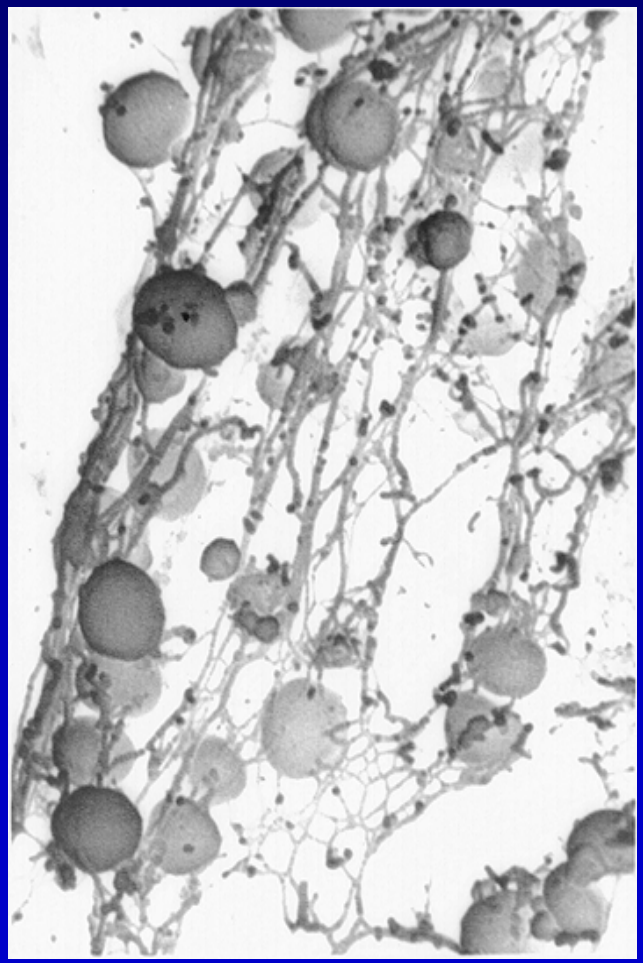
Biofilms in infections: catheters

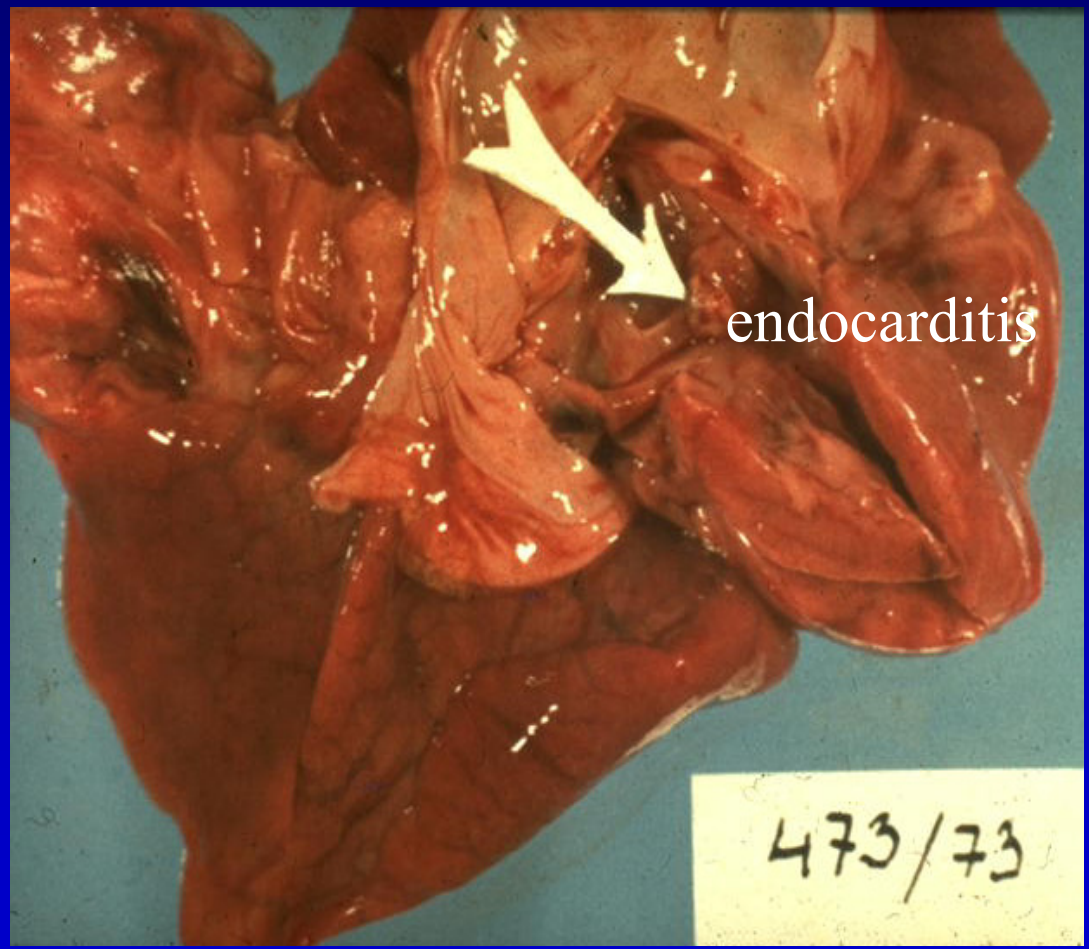
- Colonisation of catheters
  - Short term (<8 days)
    - Micro-organisms from the skin (70-90%)
    - Bacteria from the hub/lumen (10-50%)
    - Bacteria from bloodstream (3-10%)
    - Bacteria from infusate (<3%)
  - Long term (>8 days)
    - Most frequent : hub
    - Also frequent : skin

Biofilms in infections: catheters

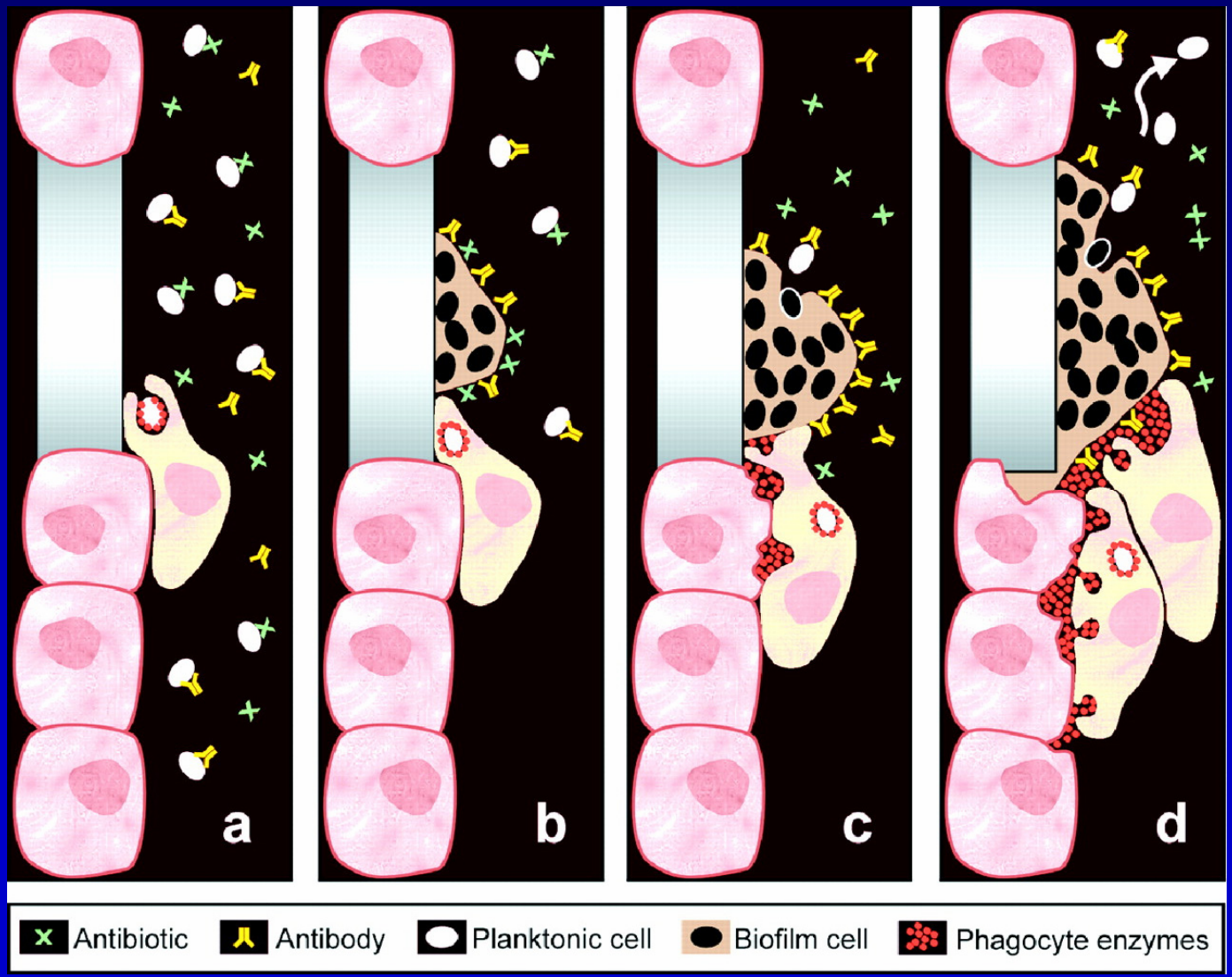
- Common micro-organisms
  - *Staphylococcus aureus*
  - Coagulase-negative staphylococci
  - Enterococci
  - *Candida* spp.
- Uncommon micro-organisms
  - *Enterobacter* spp.
  - *Acinetobacter* spp.
  - *Serratia marcescens*
  - *Pseudomonas* spp.
  - *Malassezia furfur*

Biofilms in infections: prosthetic material

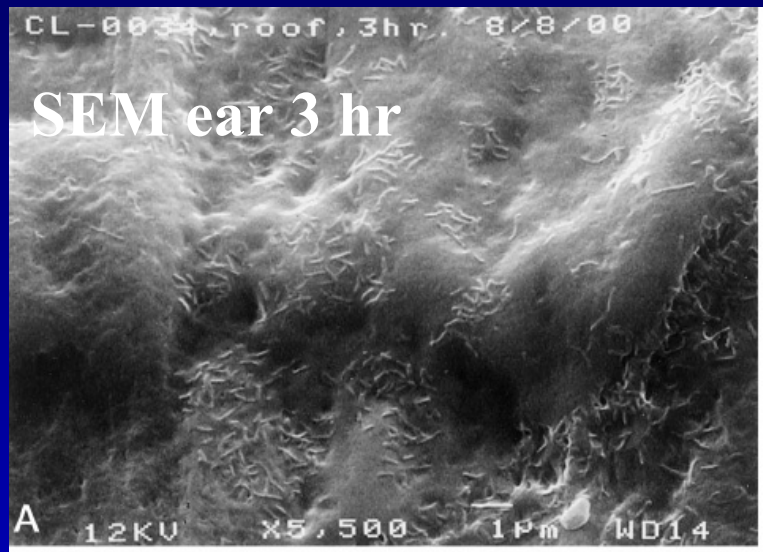


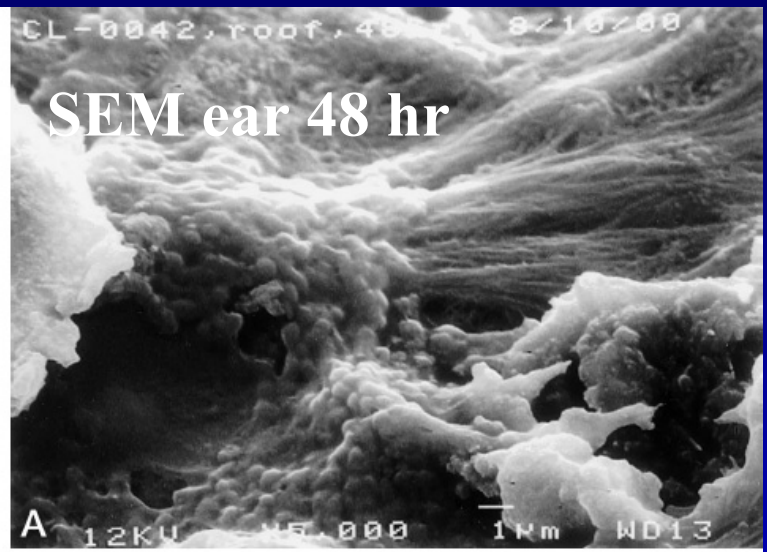


Biofilms in infections: prosthetic loosening



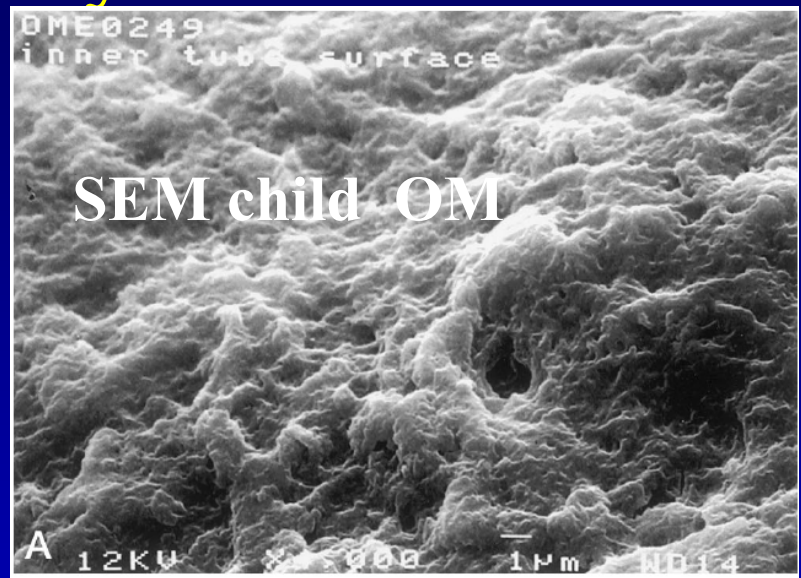
Biofilms in infections:
otitis media – chinchilla model

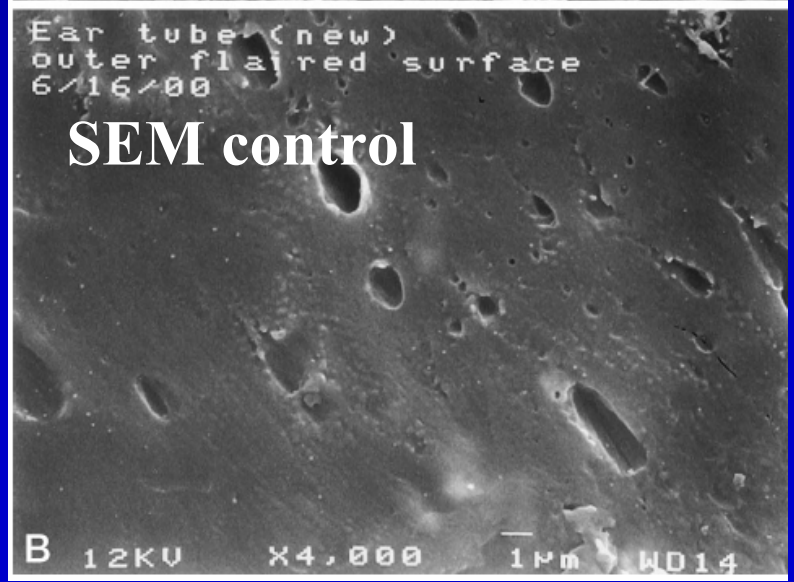




Post, '01

Biofilms in infections: otitis media – tympanostomy tubes





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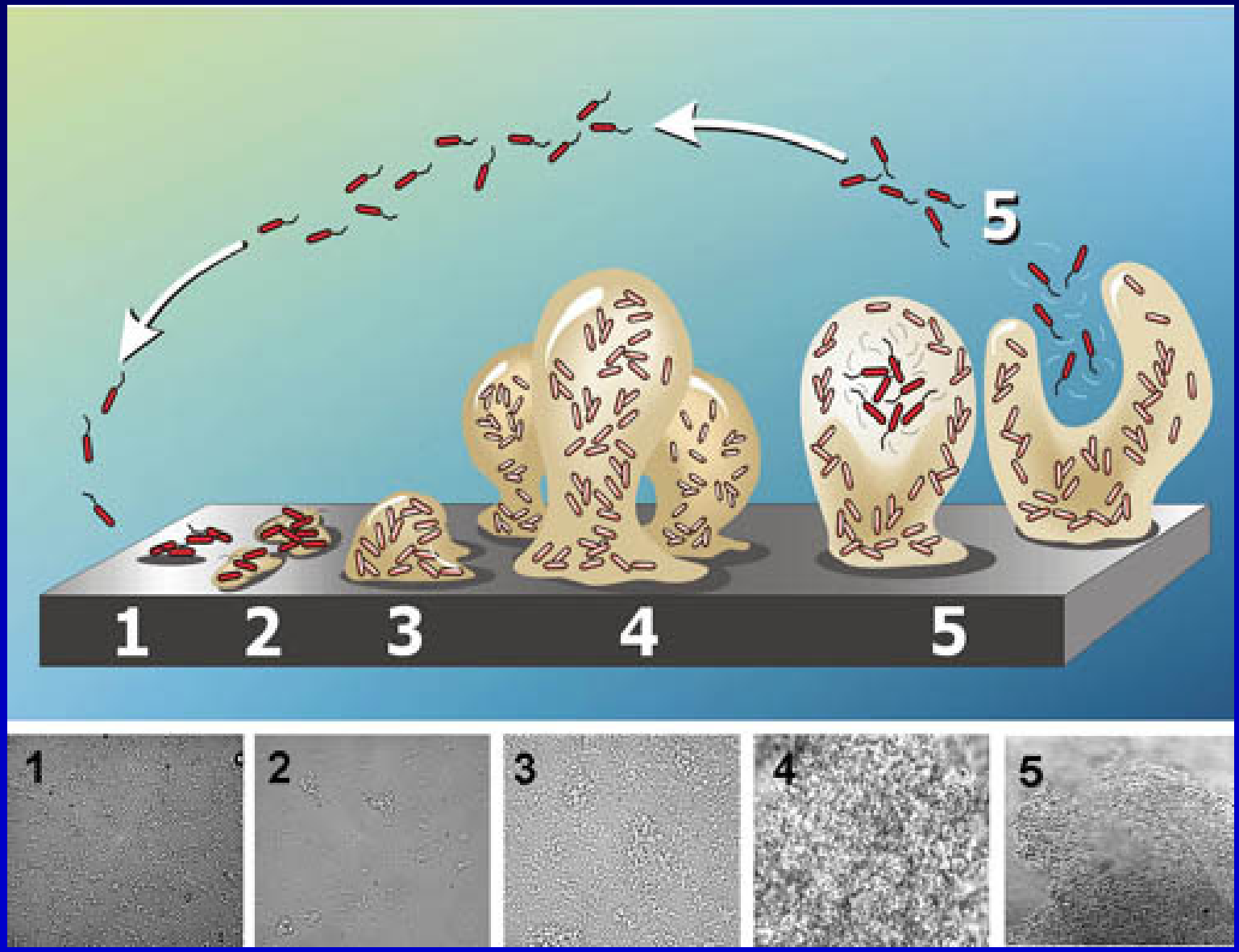
Formation of a biofilm



Formation of a biofilm

- Three stages:
  - Attachment:
    - Uncoated plastic material
    - Material coated with host-derived proteins
  - Biofilm formation
    - Intercellular adhesion and accumulation of multi-layered cell clusters
    - Generation of slime glycocalix
  - Biofilm persistence and detachment of cell-clusters

Formation of a biofilm

- Initial attachment to uncoated plastic material
  - primary adhesion: within seconds, aspecific, dependent on physicochemical interactions (vd Waals, electrostatic) and surface properties of foreign body surface
  - Main bacterial parameter: hydrophobicity of bacterial surface
    - Role of AtlE (autolysin) in surface hydrophobicity, of lipoteichoic-like acids?, of fimbria-like polymers
- Significance for biofilm formation considering rapid (seconds) coating of foreign body?

» Ferreiros, FEMS Microbiol Lett, 89, Vacheethasanee, J Biomed Mat, 98, Vacheethasanee, J Biomed Mat, 00, Heilmann, Infect Immun, 96, Gross, Infect Immun, 01, Lambert, FEMS Immun Med Micro, 00

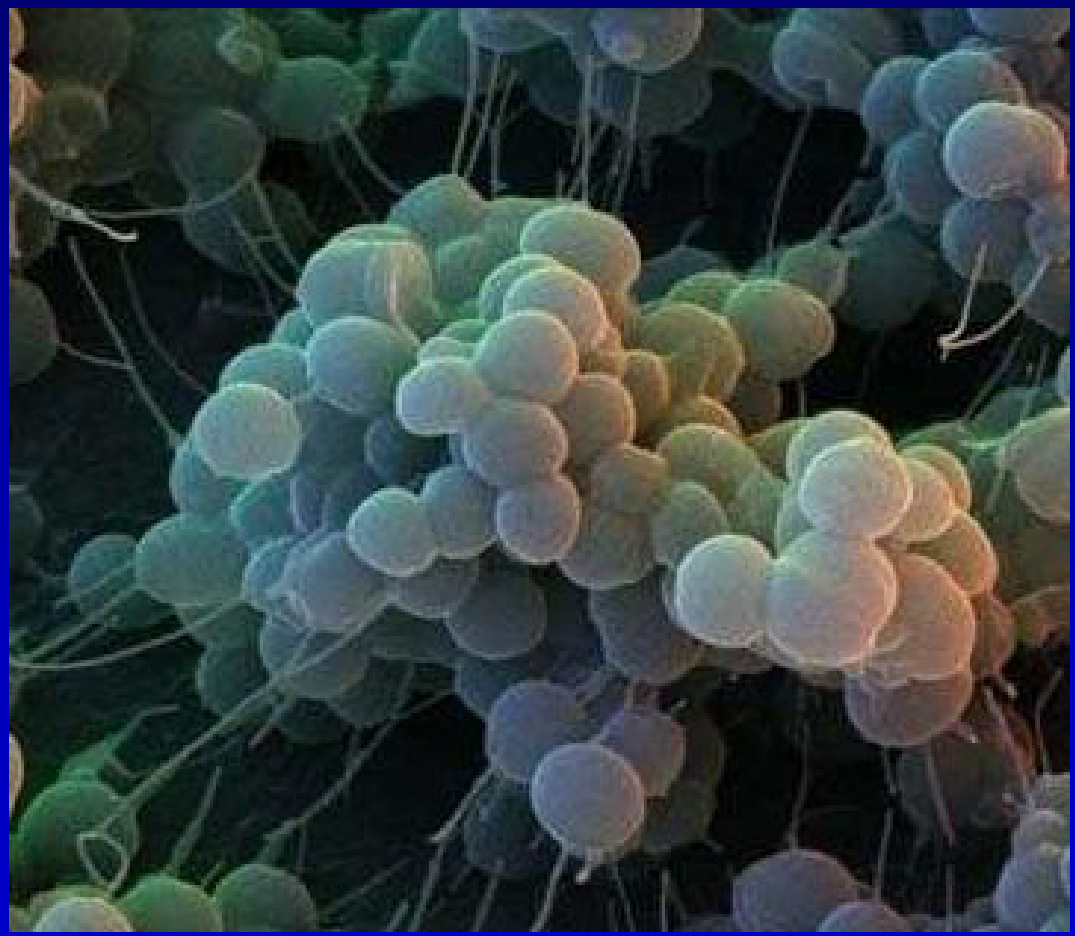
Formation of a biofilm

- (Secondary) attachment to material coated with host-derived proteins
  - Promoted by surface irregularities, host-derived substances (fibronectin, collagen, laminin, vitronectin, fibrinogen, fibrin thrombi, activated platelets)
  - MSCRAMM's:
    - fibrinogen-binding protein (Fbe)
    - Few peptidoglycan-bound surface proteins
    - Role of non-covalently linked surface proteins (AtlE)
  - Capsular polysaccharide adhesin (PS/A)
    - Encoded by *icaABCD*
      - » Gross, Infect Immun, 01, Franson, JCM, 84, Heilman, Mol Microbiol, 97, Timmerman, Infect Immun, 91, Tojo, JID, 88, Mc Kenney, Infect Immun, 98, Dunne, CMR, 02, Nilsson, Infect Immun, 98

Formation of a biofilm

- Intercellular adhesion and accumulation
  - 40 to 60 min after adhesion
  - multi-layered clusters of interconnected cells
  - Several polymeric carbohydrates and proteins involved
    - Accumulation Associated Protein (AAP)
    - Polysaccharide Intercellular Adhesin (PIA) or PS/A?
      - encoded by *icaABCD*
        - » Mack, J Bac, '96, Heilmann, Mol Microbiol, '97, Husain, Infect Immun, '97, Stewart, Lancet, '01, Zimmerli, JID, '82

Staphylococcal biofilm formation: intercellular adhesion

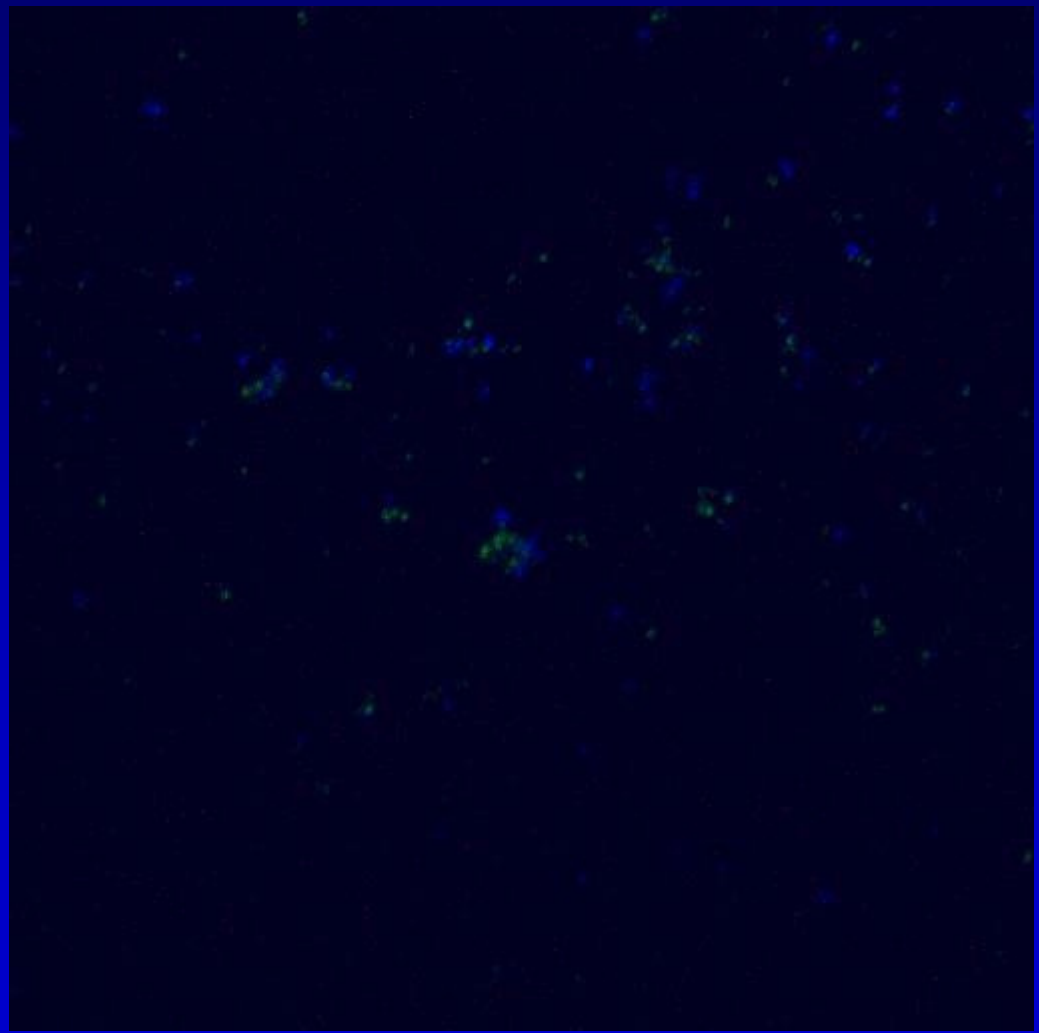


Formation of a biofilm

- Generation of extracellular slime
  - Composition extracellular slime
    - Teichoic acid
    - Bacterial and host proteins
    - Polysaccharide Intercellular Adhesin (PIA)
      - ≈ β-1,6-linked *N*-acetylglucosamine (20% non-acetylated)
    - Capsular polysaccharide adhesin (PS/A)
      - β-1,6-linked *N*-succinylglucosamine
        - » Hussain, FEMS Microbiol Rev, '93, Kojima, JID, '90, Mack, J Bac, '96, McKenney, Infect Immun, '98, Gerke, J Biol Chem, '98,

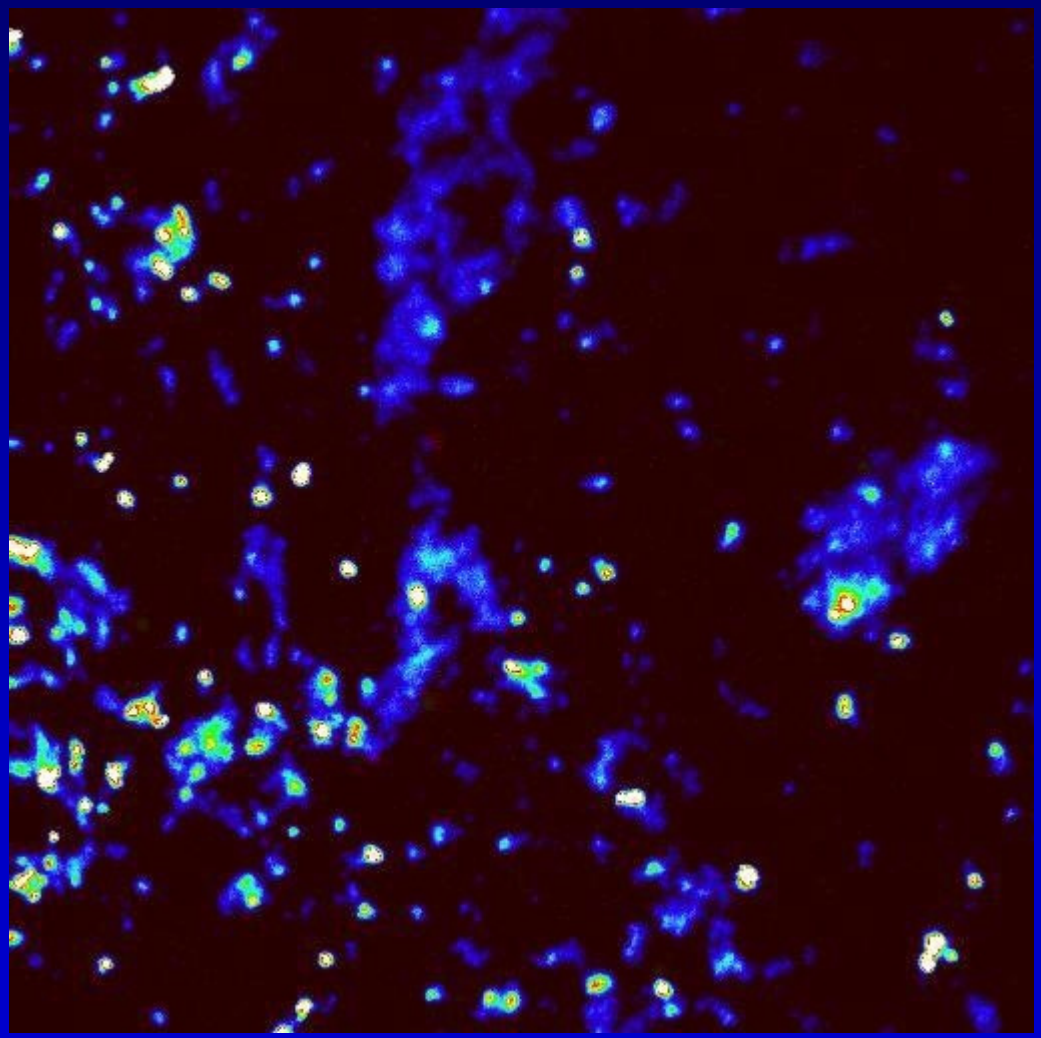
Formation of a biofilm

Confocal microscopy : biofilm after 30 min



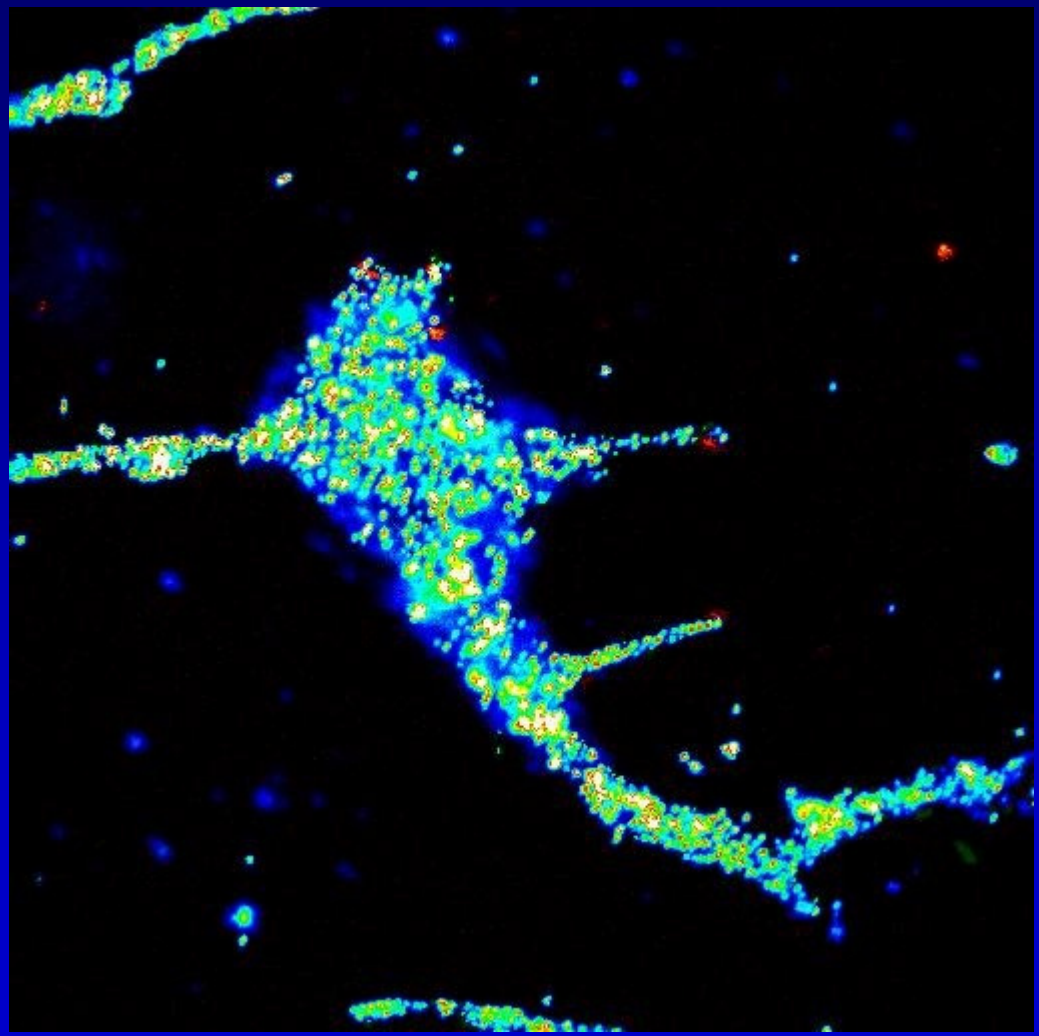
Formation of a biofilm

Confocal microscopy : biofilm after 2 hrs



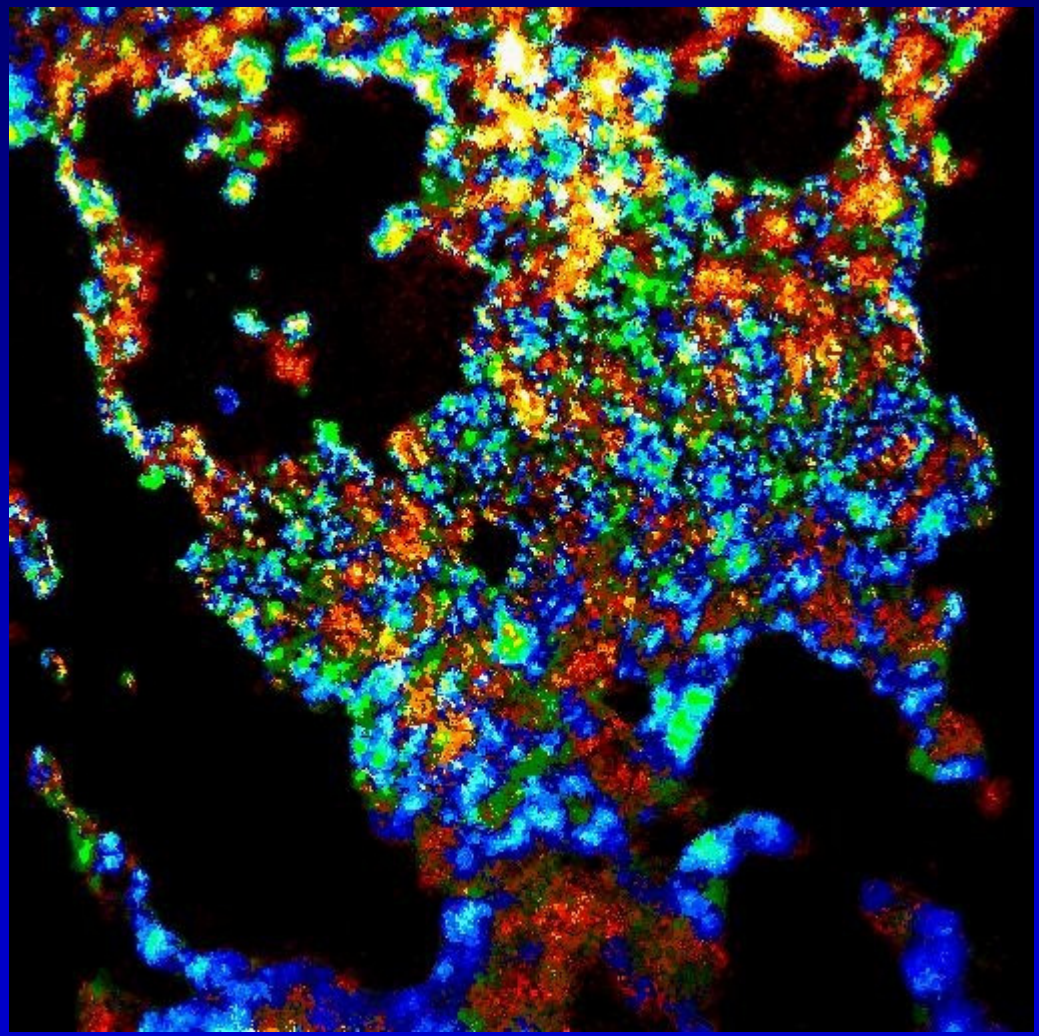
Formation of a biofilm

Confocal microscopy : biofilm after 3 hrs



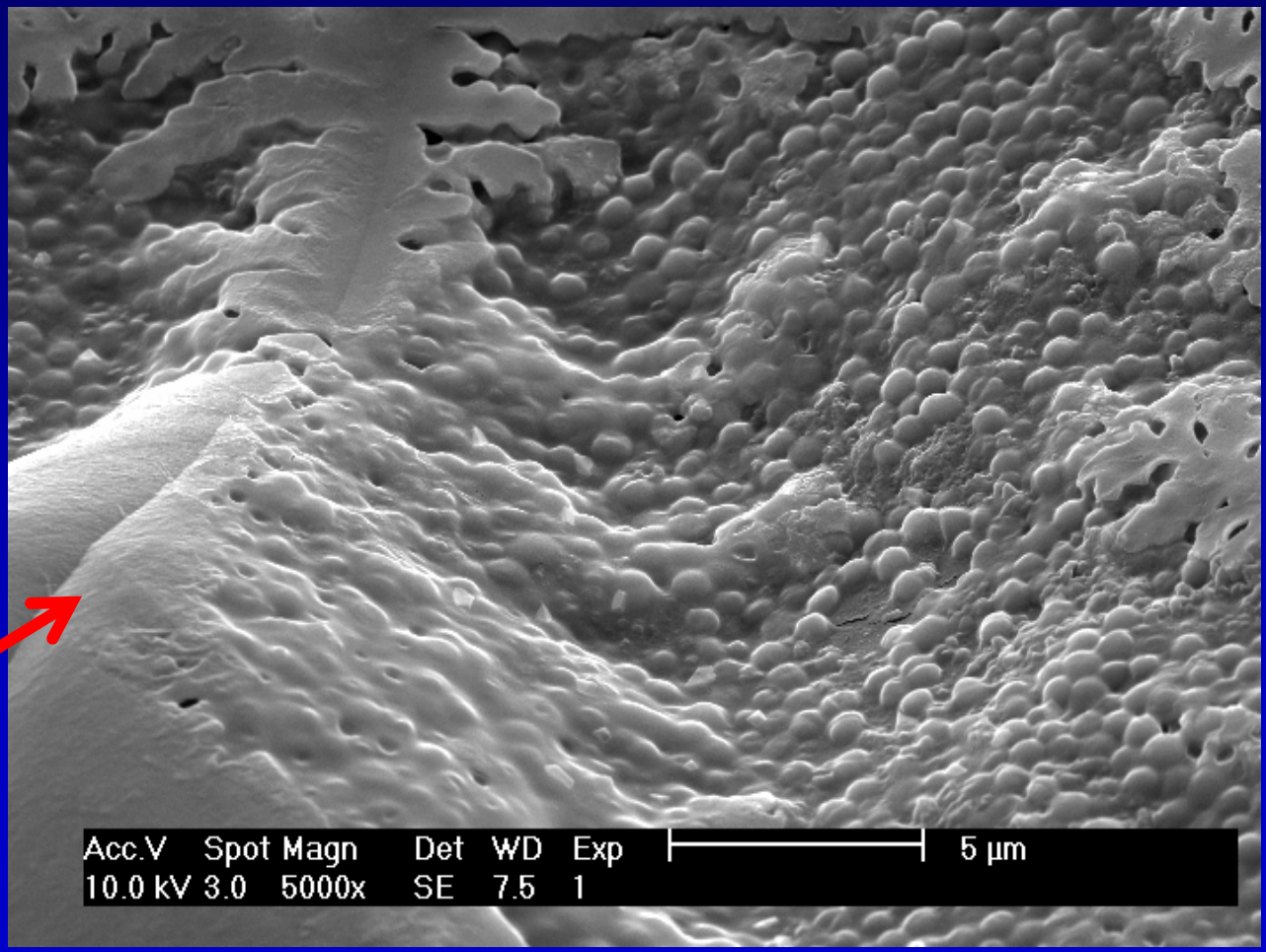
Formation of a biofilm

Confocal microscopy : biofilm after 3 days



Formation of a biofilm

SEM microscopy : biofilm after 3 days



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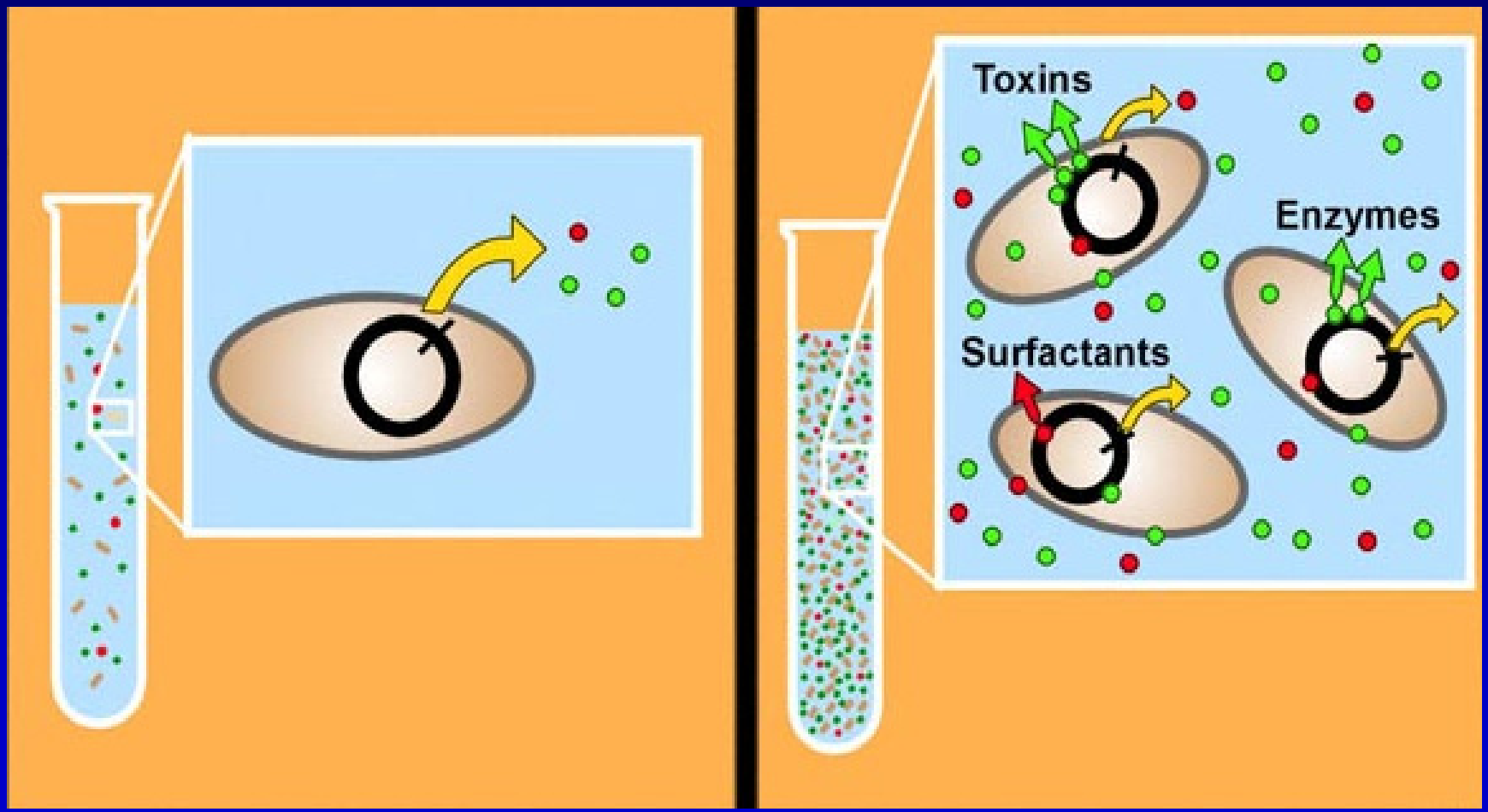
Characteristics of a biofilm

- Cellular control of biofilm formation
  - Role of *agr* quorum-sensing system:
    - Stimulates expression virulence factors
    - Down regulates expression of surface–proteins (including AtlE)
  - Role of additional regulatory loci:
    - *sar*: SarA co-stimulates with AgrA~P transcription RNAIII
    - SigB: stimulates PIA and biofilm production

» Otto, FEBS Lett, '98, Rachid, AAC, '00, Fluckiger, Infect Immun, '98, Vuong, Infect Immun, '00, Otto, Pept, '01

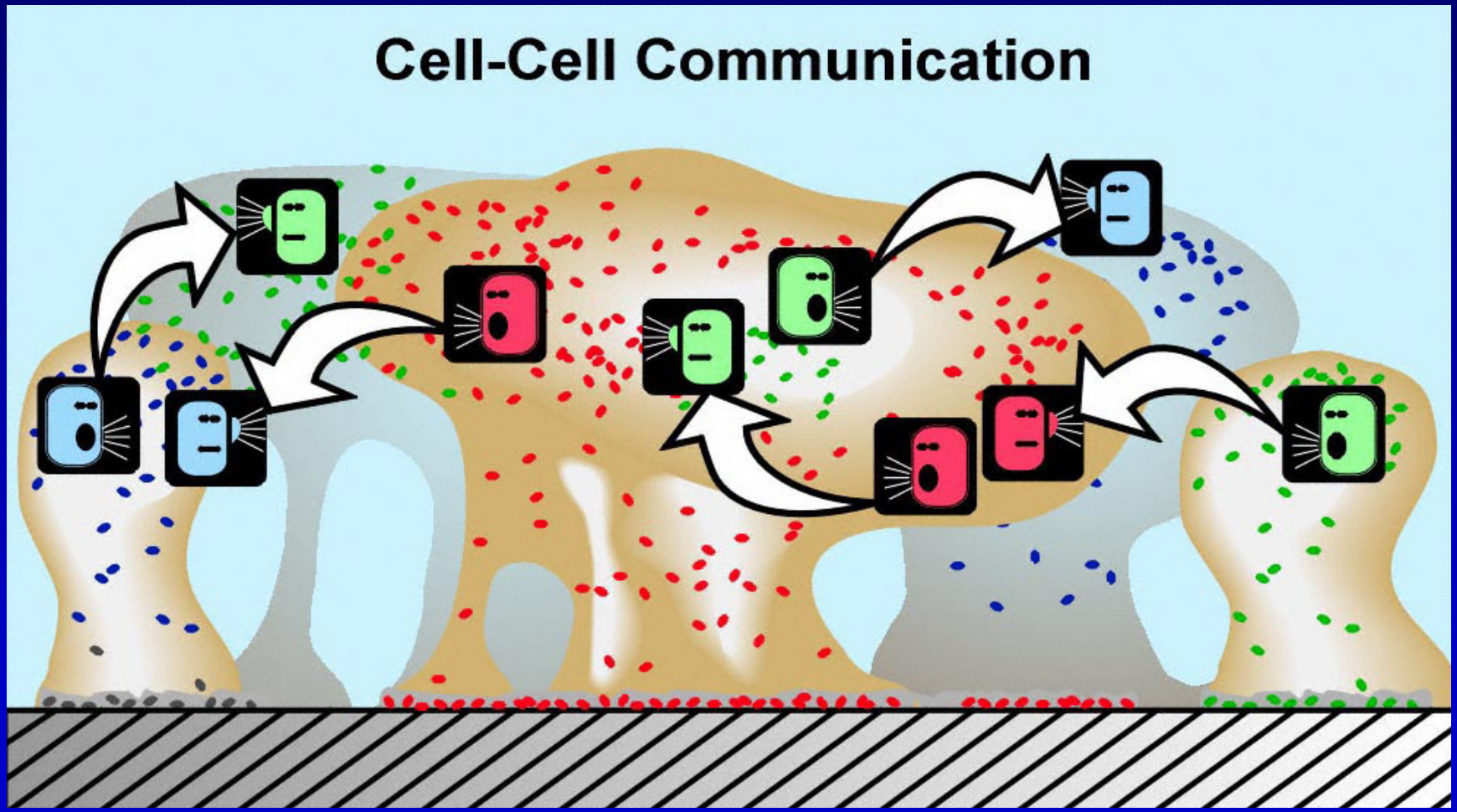
Characteristics of a biofilm

Quorum-sensing



Characteristics of a biofilm

Quorum-sensing & cross-talk



Characteristics of a biofilm : persistence

- Deficiencies/modulation of local host immune response
  - Due to bacterial products
  - Due to the foreign body
    - » Zaat, NTG, ‘02, Chuard, JID, ‘91, Chuard, AAC, ‘93, BaddourJID, ‘88, Zimmerli, JID, ‘82,
- Intrinsic resistance to antimicrobial compounds:
  - increased MBC values

Characteristics of a biofilm :
persistence

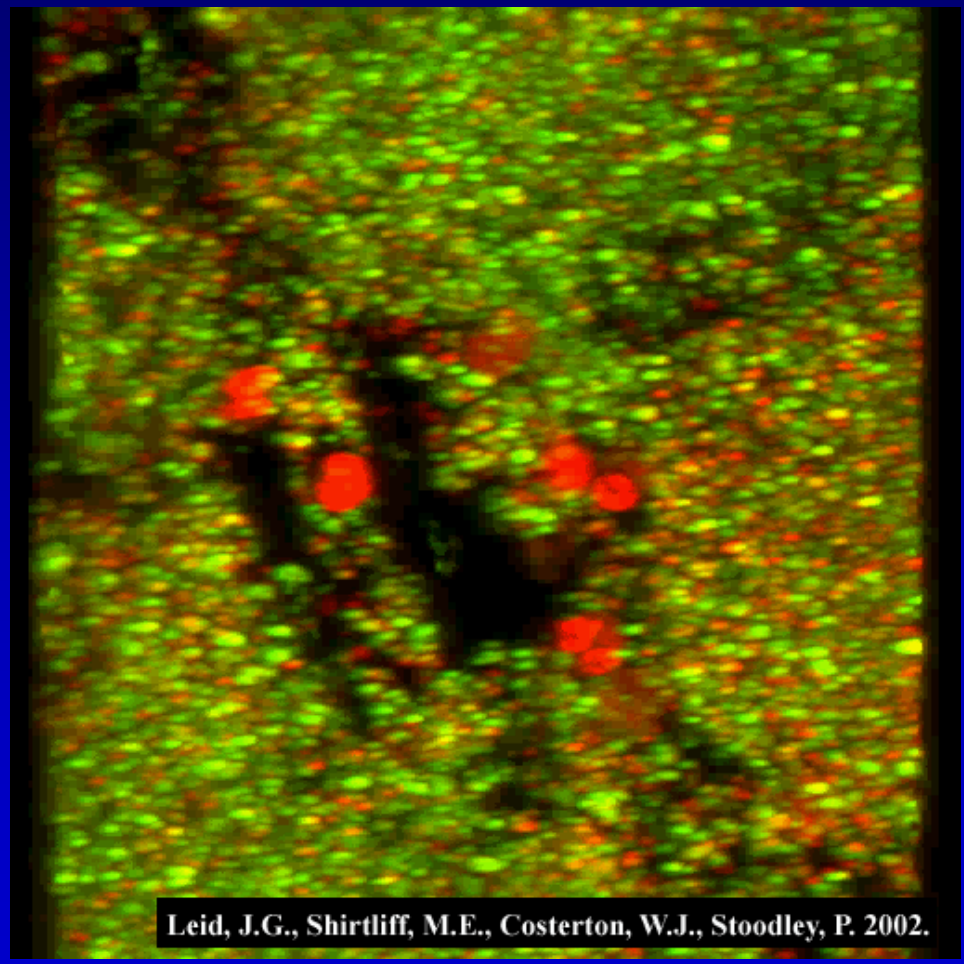
persistence of CoNS biofilms:
- resistance against host immune defences

	blood	foreign body
Numbers log_{10} CFU	6.3 / 1.7	6.2 / 2.1
Chemotactic index	2.4 / 0.2	2.0 / 0.5
phagocytosis	410 / 74	60 / 8.9
respiratory burst activity	12.2 / 0.5	4.4 / 2.8
expression of ICAM-1	3.7 / 2.4	43 / 9.8

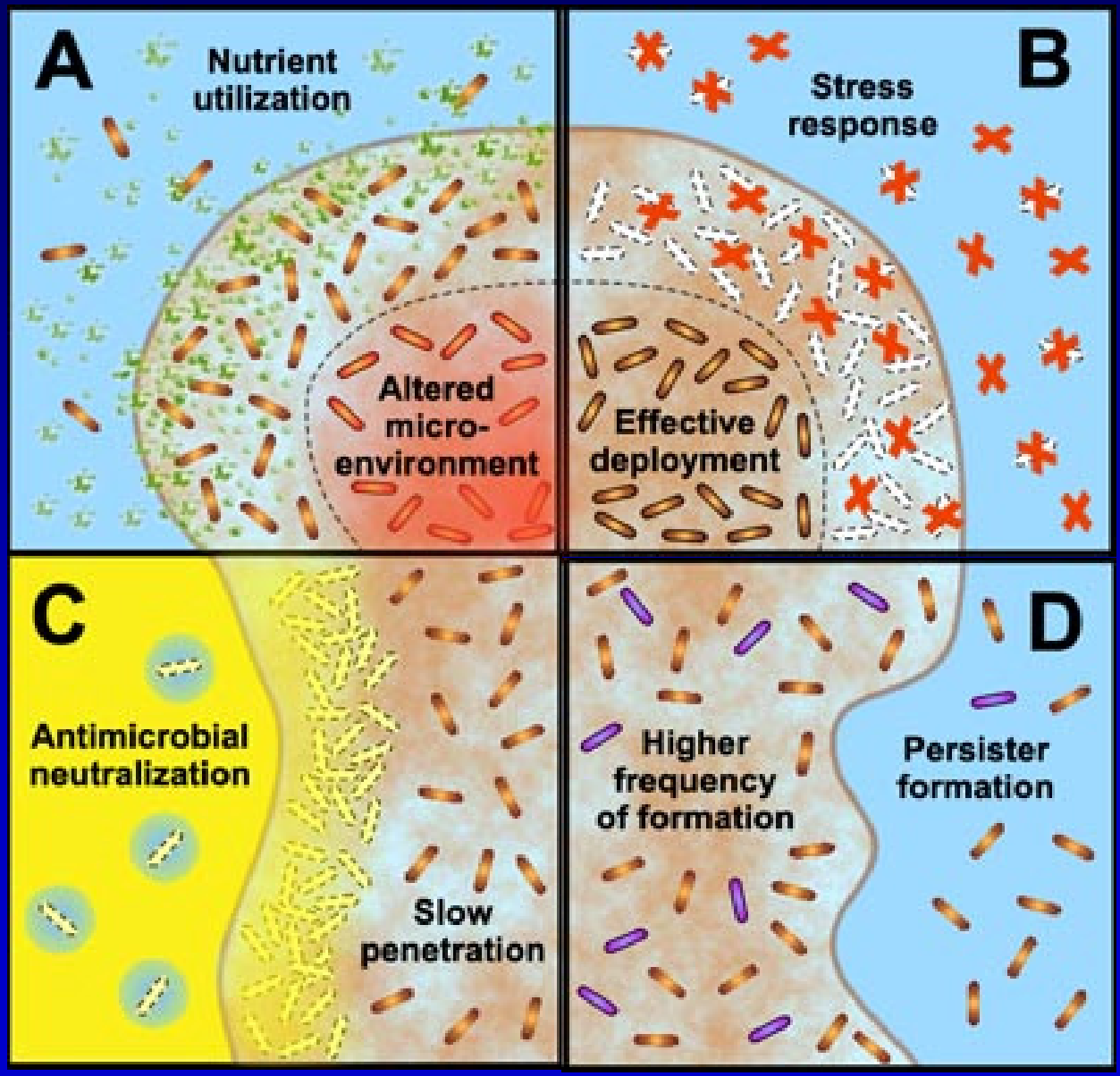
Van Wijngaerden, Acta Biomed Lov, 98, Van Eldere, Micro Ther, 99

Characteristics of a biofilm :

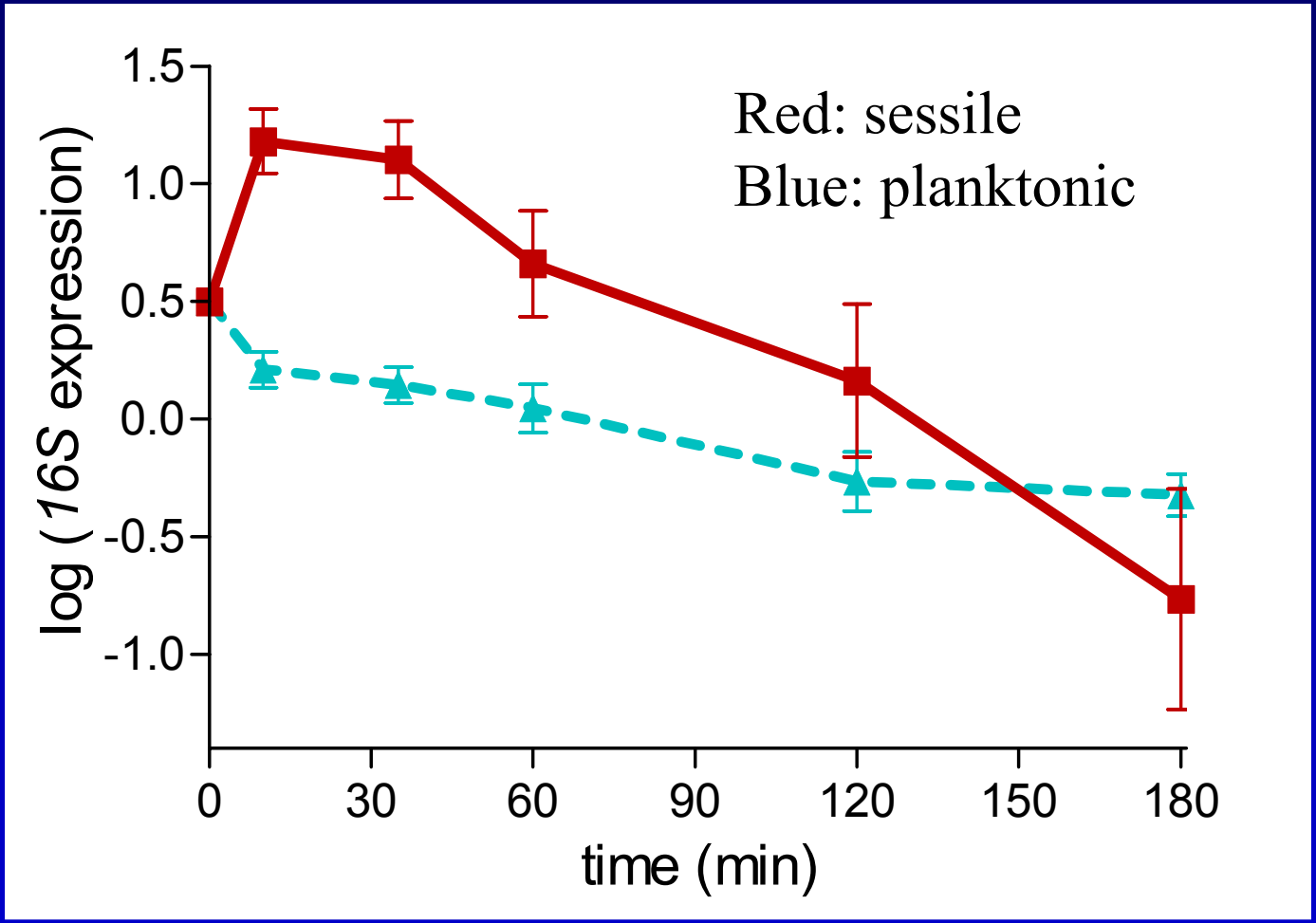
persistence



Characteristics of a biofilm : persistence



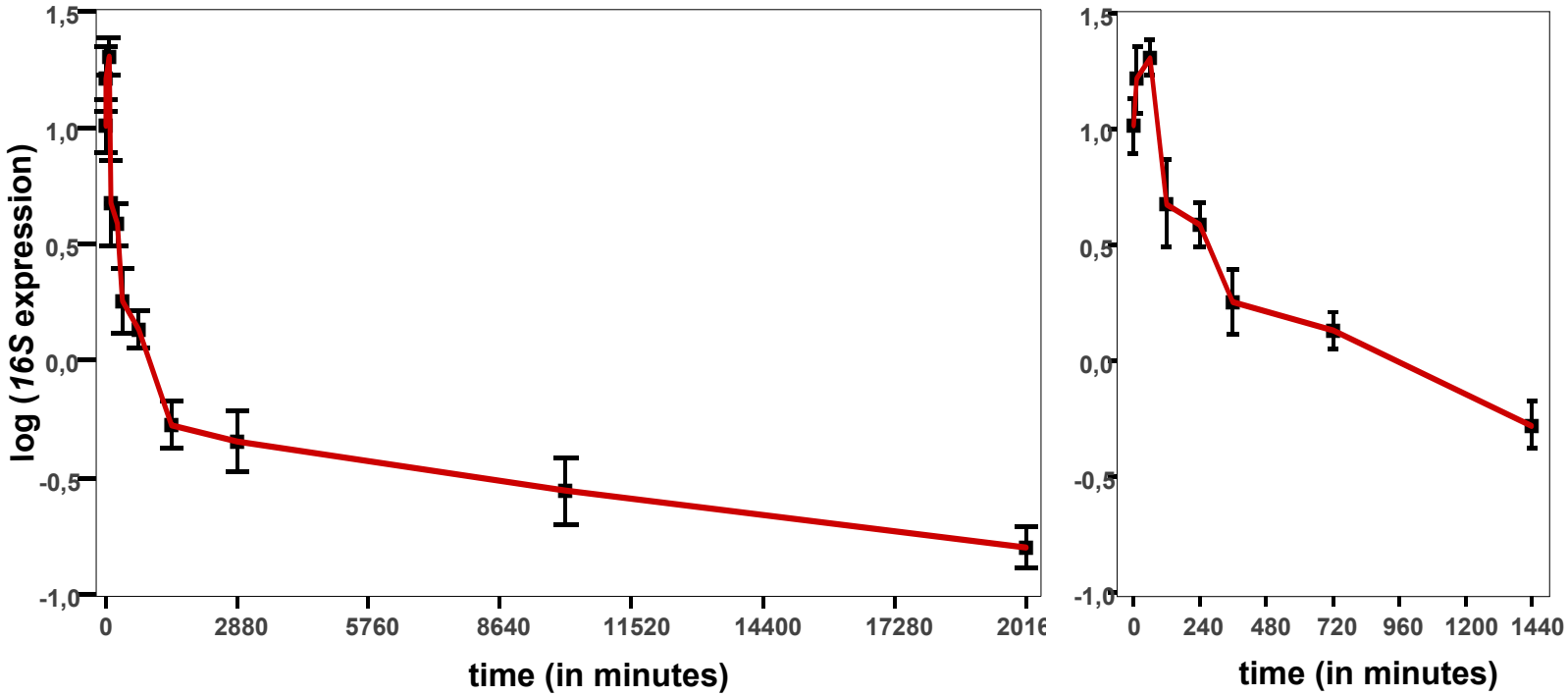
Characteristics of a biofilm : persistence



16S gene expression in FBI-associated *S. epidermidis* *in vitro*

Vandecasteele, submitted

Characteristics of a biofilm : persistence



16S gene expression in FBI-associated *S. epidermidis in vivo*

Vandecasteele, submitted

Summary

- biofilms are the result of colonisation of surfaces by bacteria
- Biofilms are complex, structured bacterial communities with special characteristics
- Persistence of biofilms is due to altered characteristics of bacteria in the biofilm and a local immune deficiency
- Many (chronic) infections contain biofilms