New Developments: Biofilms in Surgical Wounds

Scott Cairns

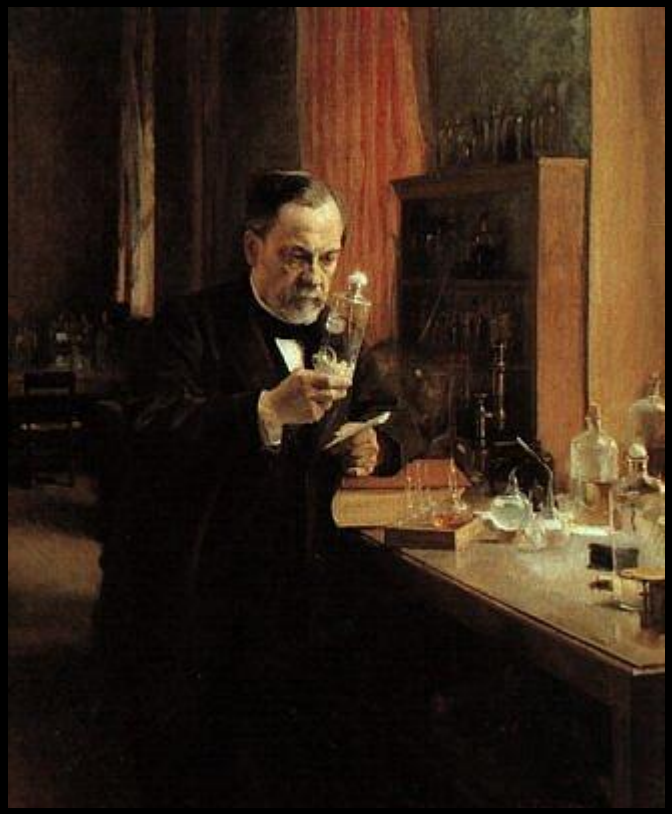
Research Fellow, Department of Wound Healing, Cardiff

Overview

- Introduction to biofilms
  - Formation
  - Dispersal
  - Resistance
- Biofilms on medical devices
- Biofilms in wounds

Introduction

- Pasteur
- Leeuwenhoek



Introduction

- Henrici 1933

  Presence of adherent species in rock pools.

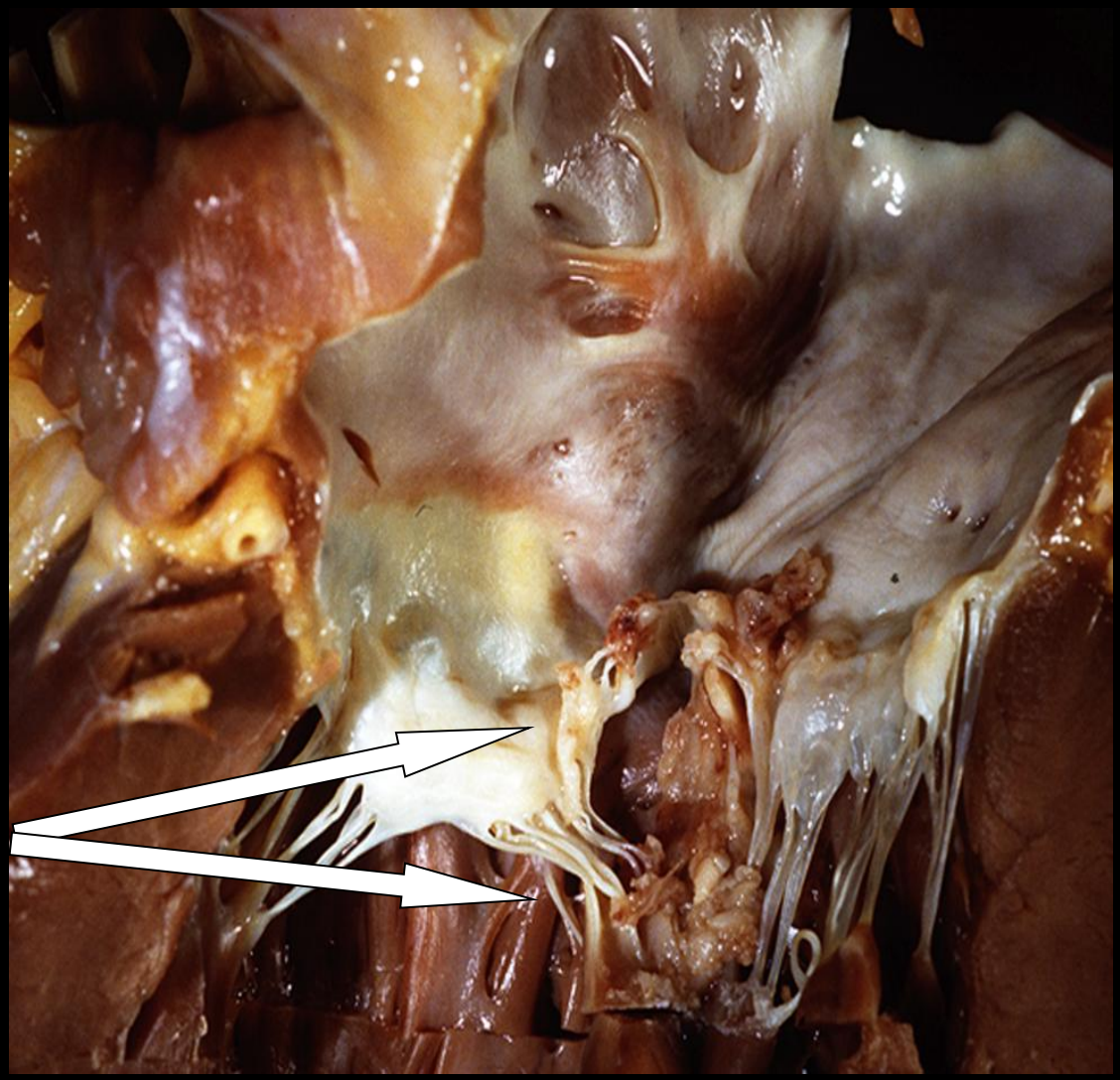
- Sobell 1943

  Increased numbers adherent compared with planktonic

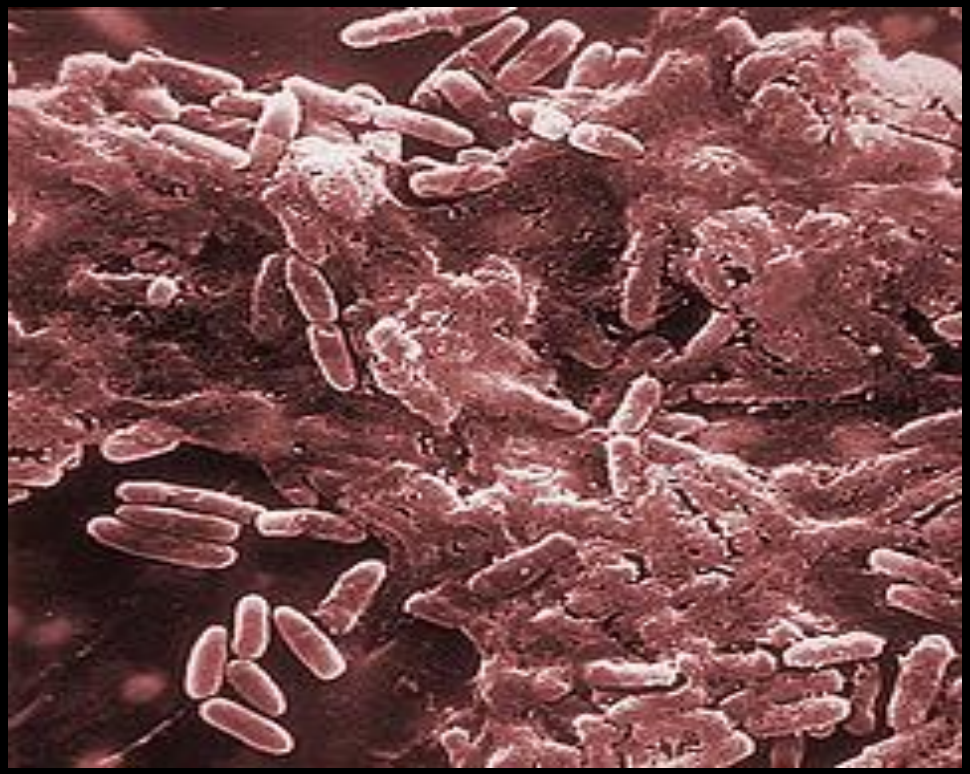
Introduction

Currently "adherent" infections thought to be the exception

- *Strep viridans*

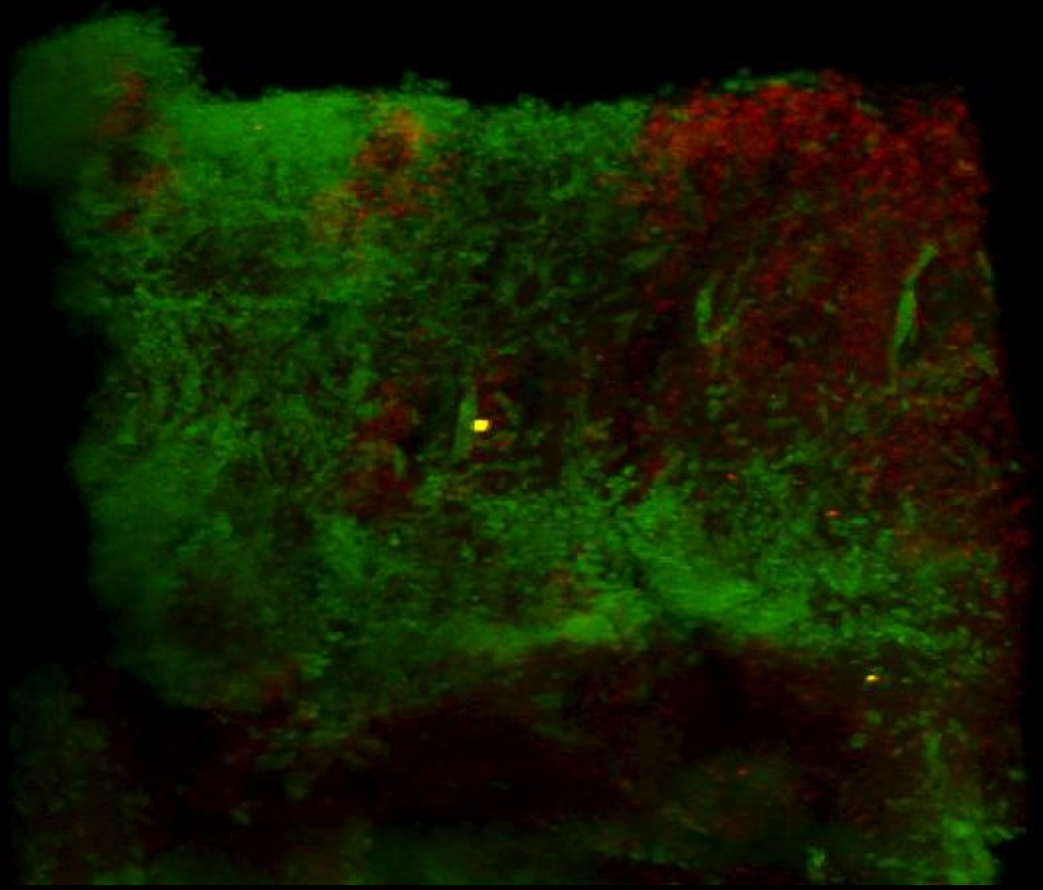


- SEM – allowed matrix visualisation



Lawrence, J. R. K., D.R. Hoyle, B.D. Costerton, J.W. Caldwell, D.E. (1991). "Optical sectioning of microbial biofilms." J. Bacteriol 173: 6558-67.

- Multiple species



- Definition

  “Polymicrobial community of adherent organisms with an extracellular polysaccharide matrix of their own making.”

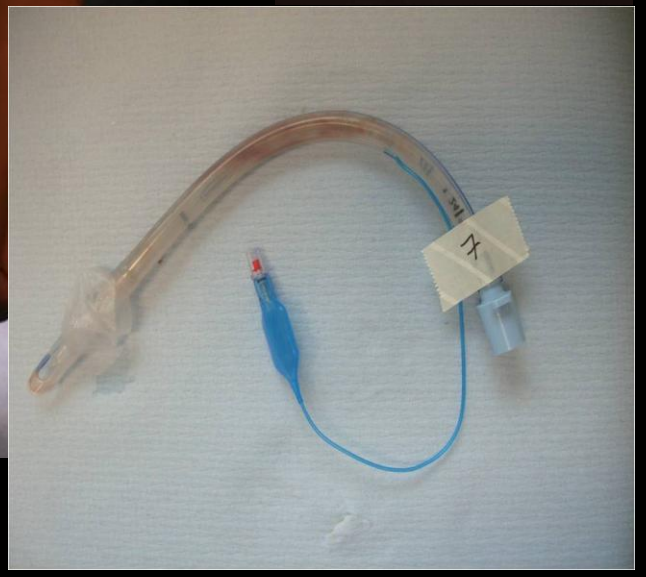
- Costerton, J. W., G. G. Geesey, et al. (1978). "How bacteria stick." Sci Am 238(1): 86-95.

Biofilms

- Dental
- Water treatment
- Ice-cream factories

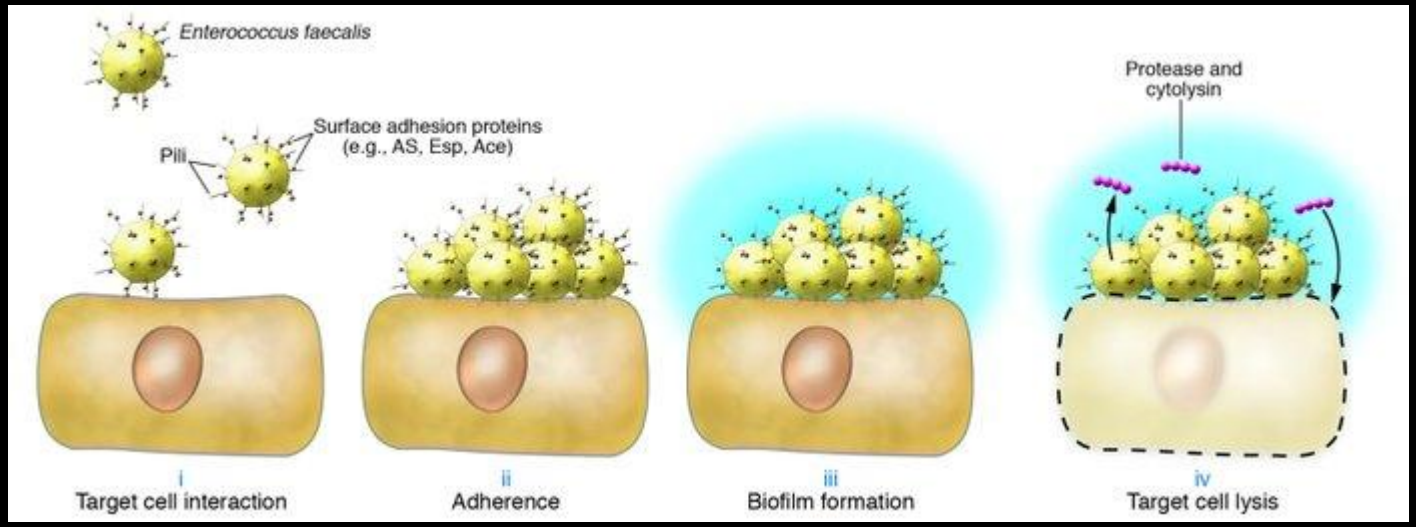
Biofilms

Present on:



Biofilm Formation

- Adherence is critical first step
- Contamination, Flow, Motility

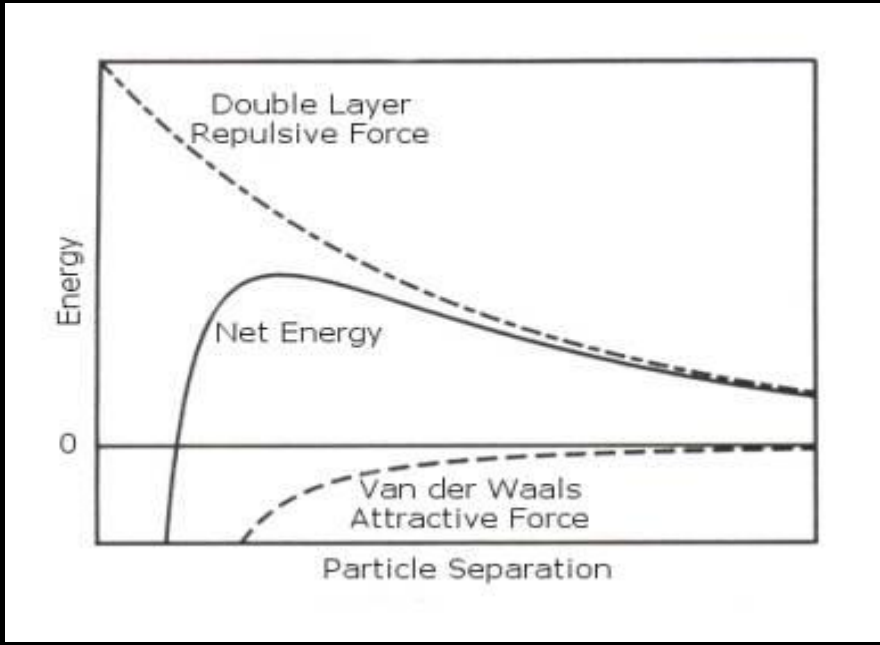


J. Clin. Invest. 116:10 doi:10.1172/JCI30088

Biofilm formation

Primary adhesion

- Balance of forces
  - Electrostatic
  - Hydrophobic interactions
  - Steric hindrance
  - Van der Waal's forces
  - Hydrodynamic forces



http://www.malvern.com/LabEng/industry/colloids/dlvo_theory.htm

An, Y. H. D., R.B Doyle, R.J. (2000). Mechanisms of bacterial adhesion and pathogenesis of implant and tissue infections. Towota N. J., Humana Press.

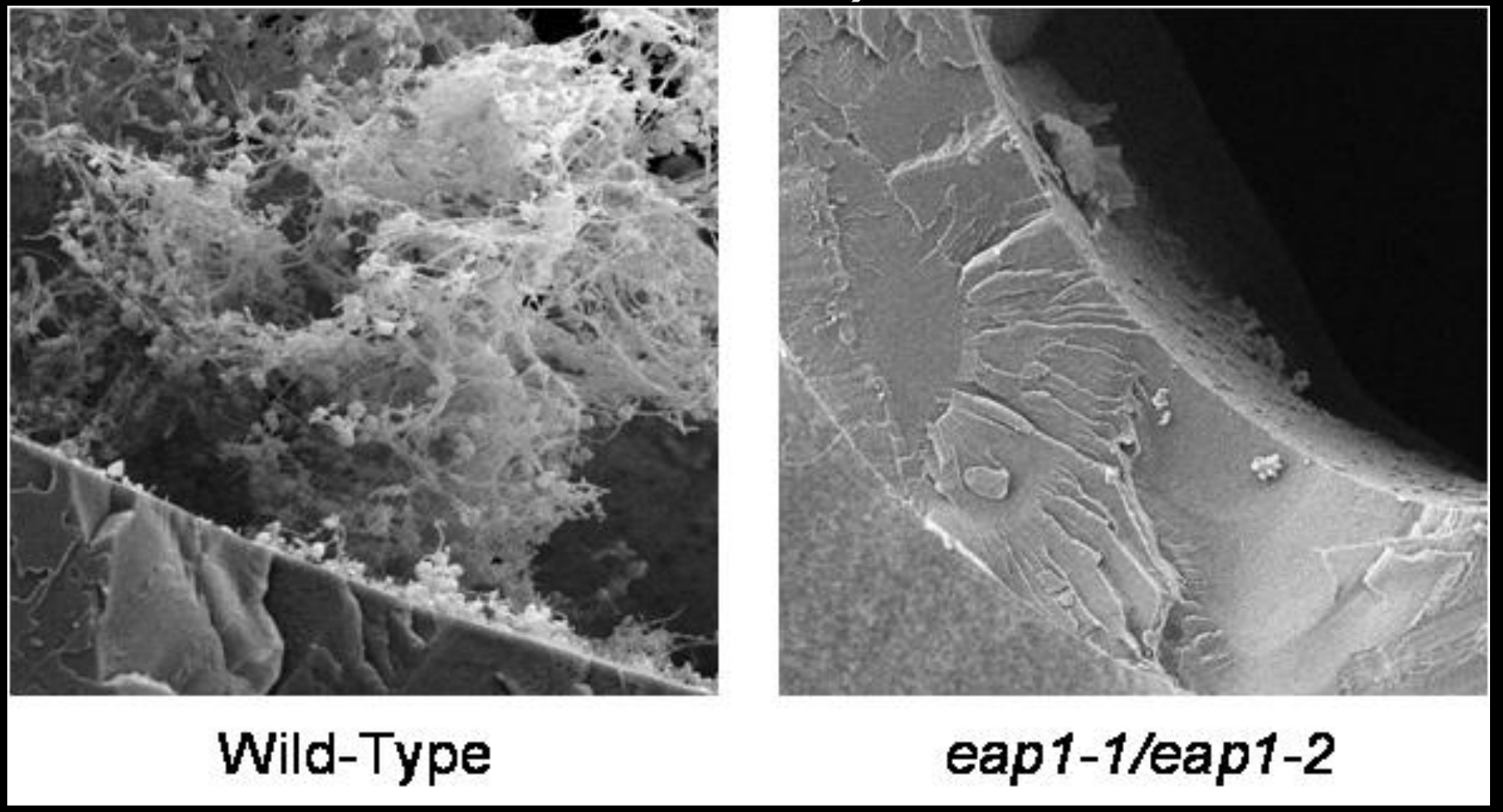
Biofilm formation

- Conditioning – salivary pellicle

<u>Secondary adhesion</u>

- Specific adhesins
  - *S. mutans* – bacterial adhesion molecule P1
  - *C. albicans* – enhanced adhesion to plastic (EAP1)
- Attachment
- Maintenance

Secondary adhesion (Enhanced adhesion to plastic EAP1)



Biofilms

- Dispersal
- Life cycle

http://www.erc.montana.edu/biofilmbook/MODULE_01/Mod01_Grn/Mod01_S01_Grn.htm

- Erosion or sloughing (Passive)

Kierek-Pearson, K. K., E. (2005). "Biofilm development in bacteria." Adv Appl Microbiol 57: 79-111.

Biofilms

<u>Active dispersal:</u>

- Shape biofilm architecture
- Necessary to move (environmental factors)

Disadvantages of Biofilm phenotype

- In Biofilm phenotype growth is restricted. Rate of DNA transcription is similar to that of stationary phase cells.

- Difficult to move whole biofilm

Waite, R.D. Papakonstantinopoulou, A. Littler, E. Curtis, M.A. (2005). “Transcriptome analysis of *Pseudomonas aeruginosa* growth: comparison of gene expression in planktonic cultures and developing mature biofilms. J. Bacteriol. 187:6571-6576.

Dispersal

Passive:

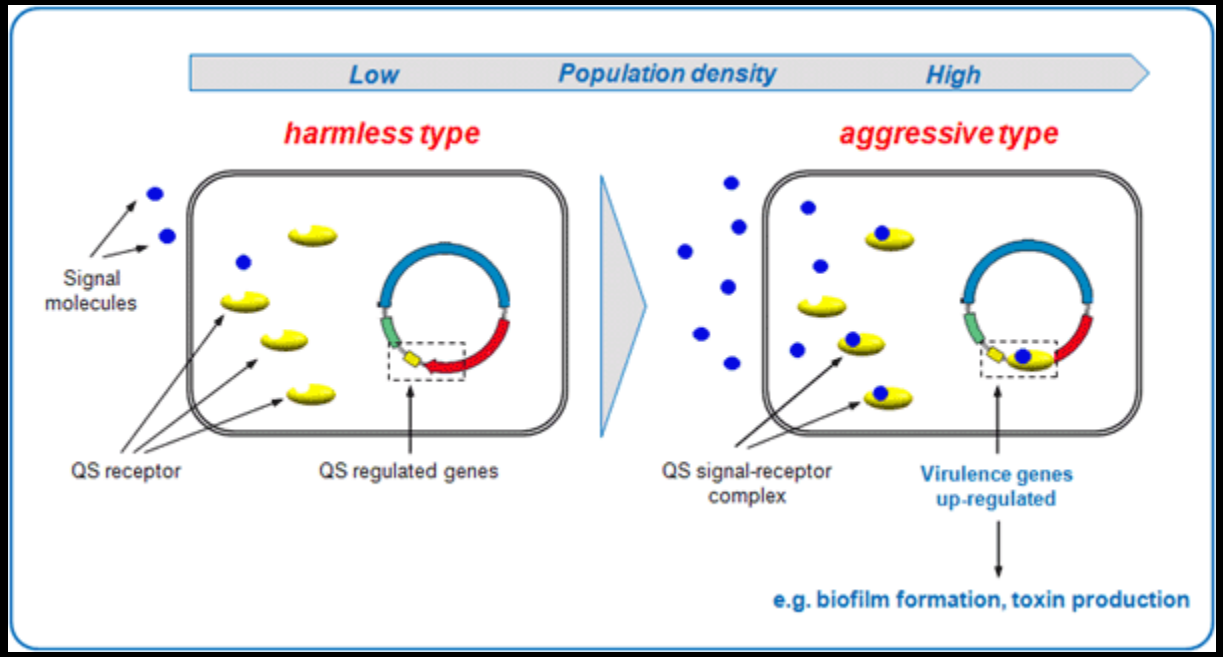
- Move requires decrease in adhesins and allow erosion to do the work

Active:

- Secrete hydrolytic enzymes to actively breakdown biofilm structure

Itoh, Y. Wang, X. Hinnebusch, J. Preston III, J.F. Romeo, T. (2005). “Depolymerization of β-1,6-N-acetyl-D-glucosamine disrupts the integrity of diverse bacterial biofilms.” J. Bacteriol. 187:382-387.

Quorum sensing



http://idaizon.files.wordpress.com

As the biofilm develops, sufficient numbers of bacteria produce the signalling molecules to generate a required response

De Lancey Pulcini, E. (2001). "Bacterial biofilms: a review of current research." Nephrologie 22(8): 439-441.

Quorum sensing

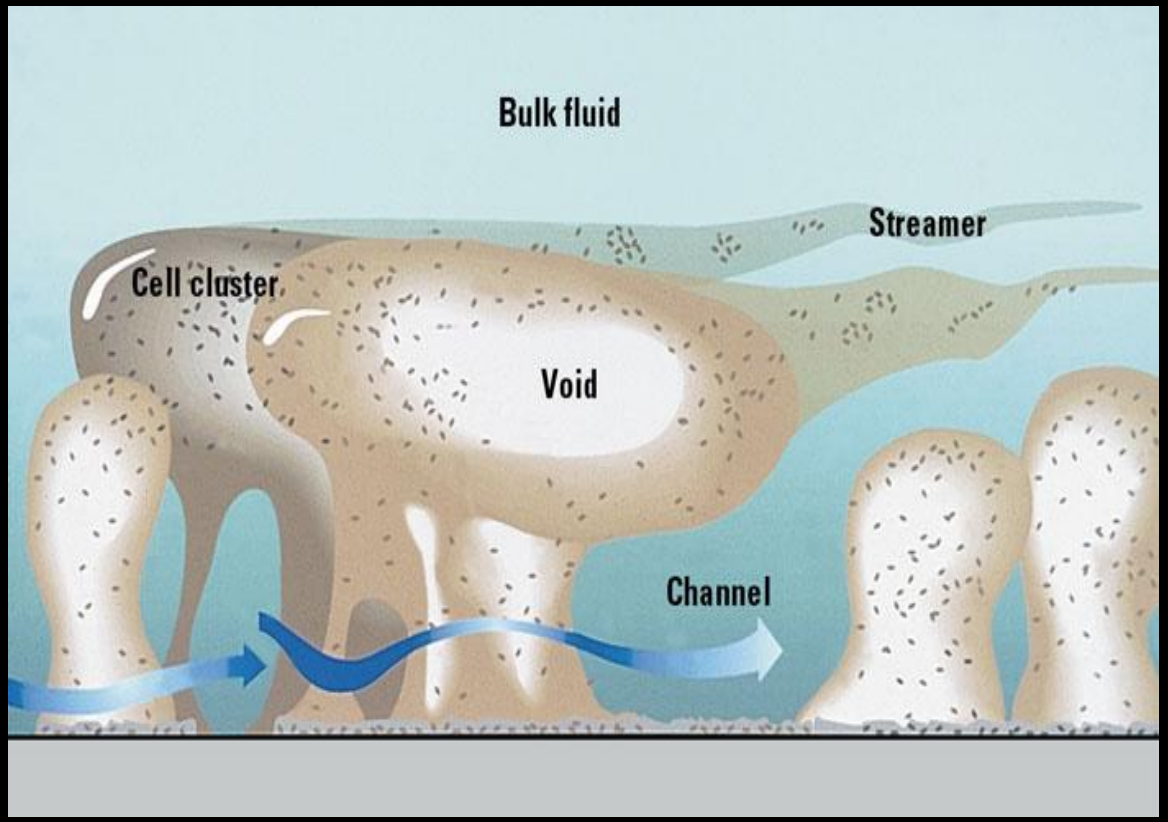
- Homoserine Lactones (HSLs)
  - Increase biofilm mass and density
- Nitric Oxide induces biofilm dispersal
  - Marker of anaerobic metabolism
  - Possibly seen as a sign of decreasing environmental fecundity

Extracellular polysaccharide matrix

- EPS makes up 85% of biofilm, with only 15% of the volume due to micro-organisms

<u>Resistance</u>

- Mechanical
- Environmental
- Antibiotics
- Predation



Extracellular polysaccharide matrix

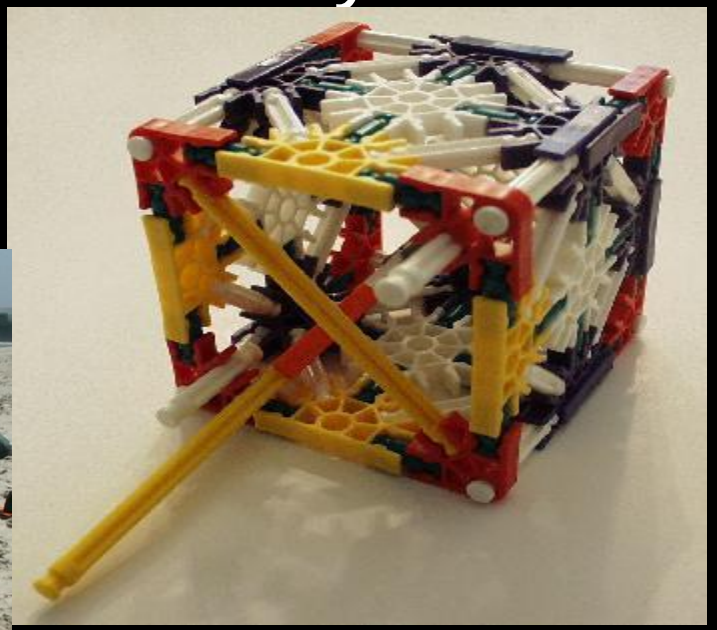
Mechanical Resistance:

- Flow – aerodynamic
- Wear and tear
- Debridement

Extracellular polysaccharide matrix

Environmental Resistance

- Cyanobacteria resistant to dehydration
  - internal struts
- UV protection



Extracellular polysaccharide matrix

Resistance to Predation

- WBC –neutrophils Antimicrobial peptides
- AB recognition
- Viral lysis
- Protozoal predation

Extracellular polysaccharide matrix

Antibiotic:

- Poor penetration
  - Esp. quinolones

Anderl JN, F. M., Stewart PS. (2000). "Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin." Antimicrob Agents Chemother. 44(7): 1818-24.

- Good glycopeptide penetrance

Dunne, W. M. M., E.O. Kaplan S.L. (1993). "Diffusion of rifampin and vancomycin through a Staphylococcus epidermidis biofilm." Antimicrob Agents Chemother 37: 2522-2526.

Antibiotic Resistance

- Complete saturation or small ab molecules still increased resistance

- Periplasmic sequestration (silver)
- Heterogeneity
- Persisters

Darouiche, R. O. D., A. Miller, A. Landon, G.C. Raad, I.I. Musher, D,M. (1994). "Vancomycin penetration into biofilm covering infected prostheses and effect on bacteria." J. Infect. Dis. 170: 720-723.

Antibiotic Resistance

Heterogeneity:

- Oxygen gradient within biofilm
- Metabolic variant within biofilm

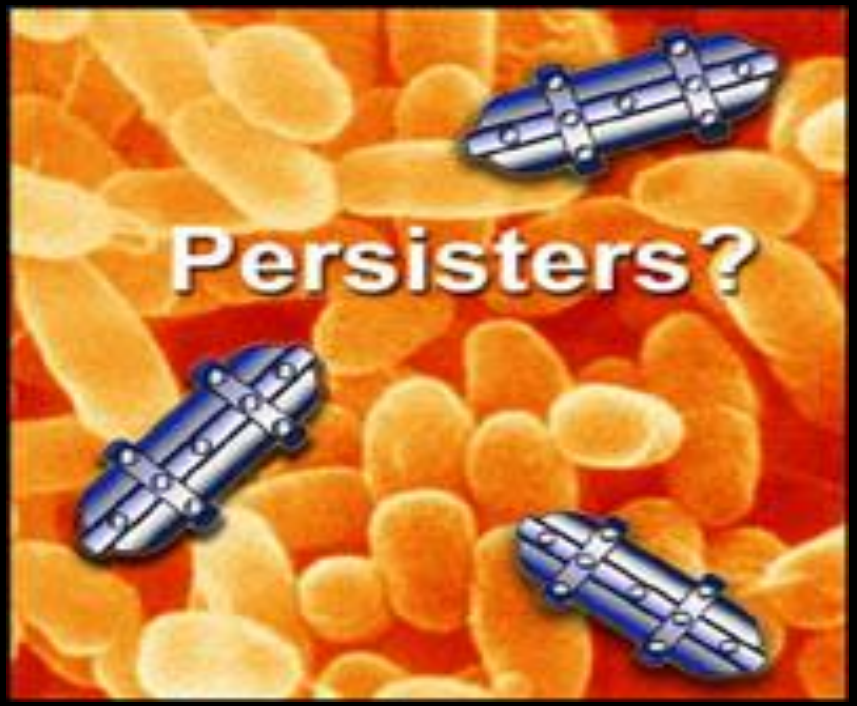
- These “microenvironments” mean that metabolism dependent antibiotics have varied efficacy

Stewart, P. S. (1994). "Biofilm accumulation model that predicts antibiotic resistance of Pseudomonas aeruginosa biofilms." Antimicrob Agents Chemother. 38(5): 1052-8.

Antibiotic Resistance

Persisters:

- 99% percent of bacteria killed at bacteriocidal doses
- 1% survive even in face of greatly increased concentrations

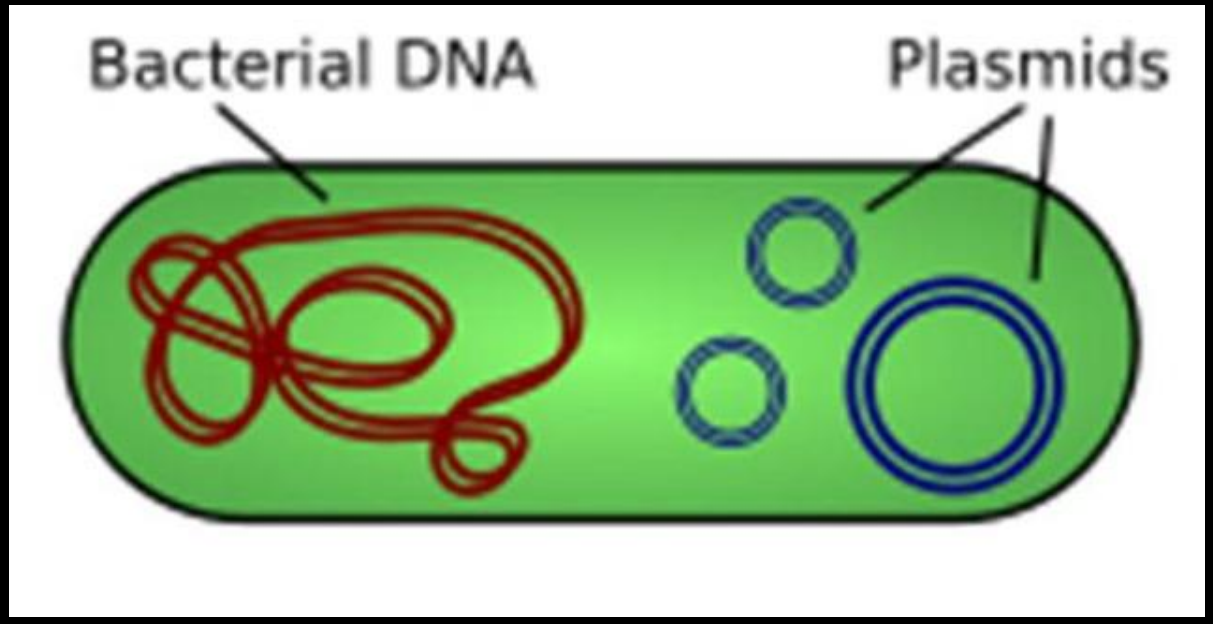


http://www.erc.montana.edu

Keren, I., D. Shah, et al. (2004). "Specialized persister cells and the mechanism of multidrug tolerance in Escherichia coli." J Bacteriol 186(24): 8172-80.

Antibiotic resistance

- Plasmid spread increased in biofilms



- Horizontal gene transfer
- Virulizing of apathogenic species

Biofilm Infections

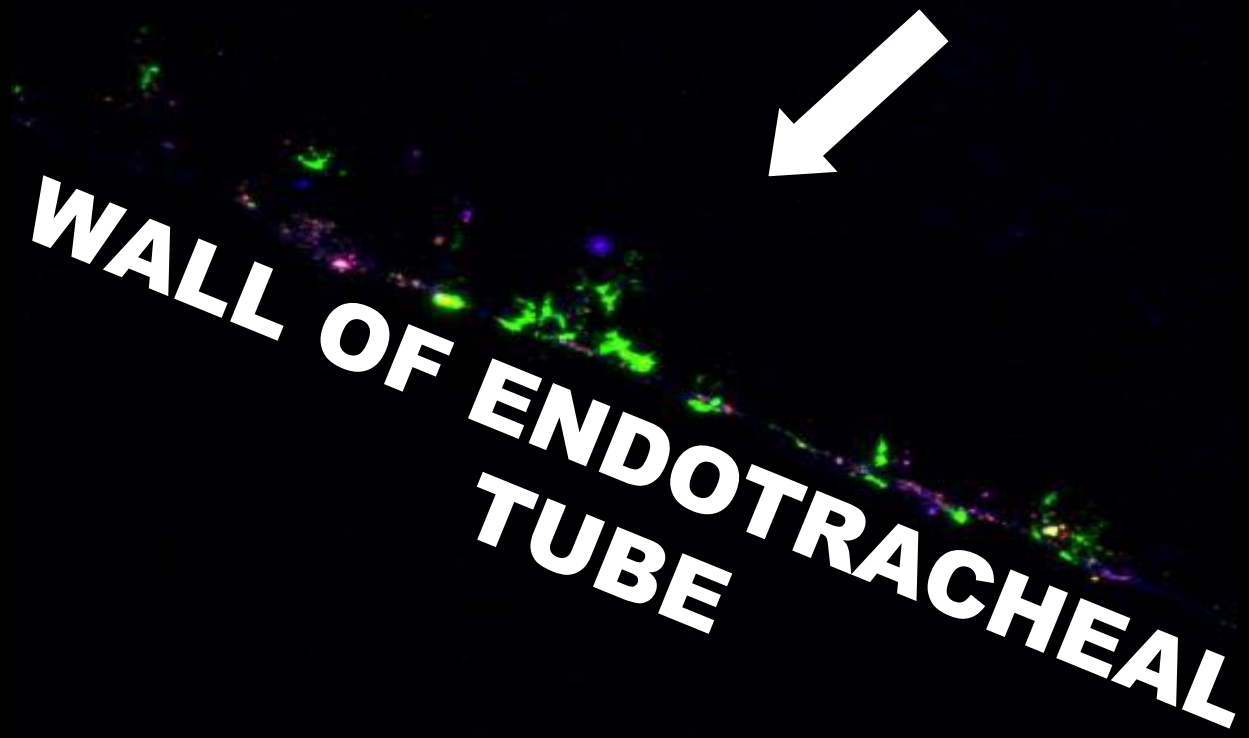
- Centre for Disease Control (USA) reports 80% of HCAI are biofilm related

- May be related to Medical devices (including sutures), Acute Wounds and Chronic Wounds.

Biofilms on medical devices

- Local Infection
- Reservoir
- Antibiotic Haven
- Device failure
- Recurrent Infection
- Wound Breakdown

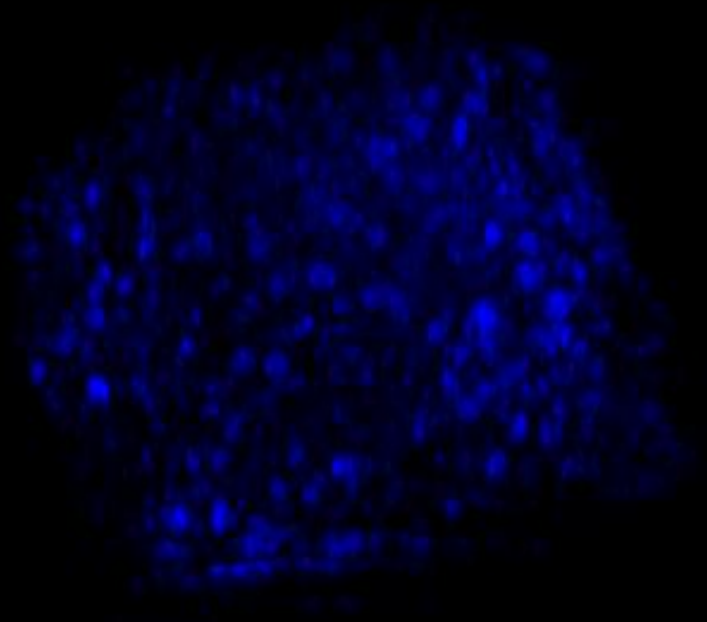
Biofilms on medical devices



- Septicaemia, urinary sepsis,
- Ventilator associated pneumonia

Biofilms on Medical devices

- Polyurethane
- Cobalt/Titanium
- Silicone
- Polygliactin/ Nylon/Polydiaxanone
- Inadine

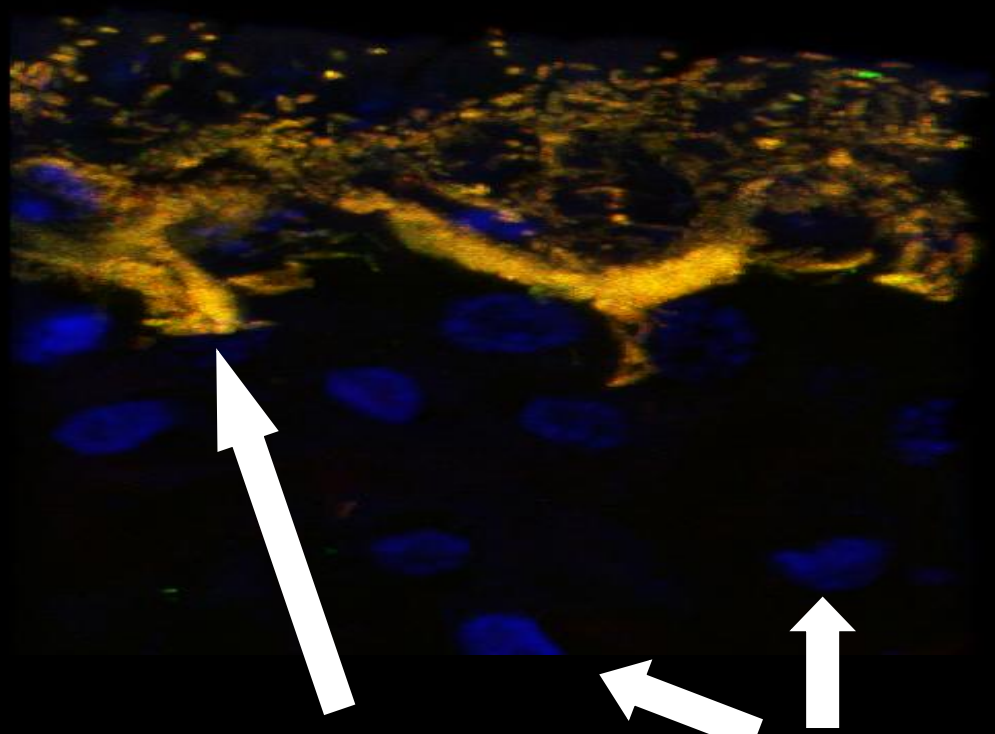


- Patient preparation
- Coatings : PTFE, antiseptics, Silver?
- Removal/Explantation

Biofilms in Acute wounds

Biofilms in Acute Wounds

- Seeding of bacteria into wounds allows for development of biofilm



Biofilms in Acute Wounds

Prevention techniques:

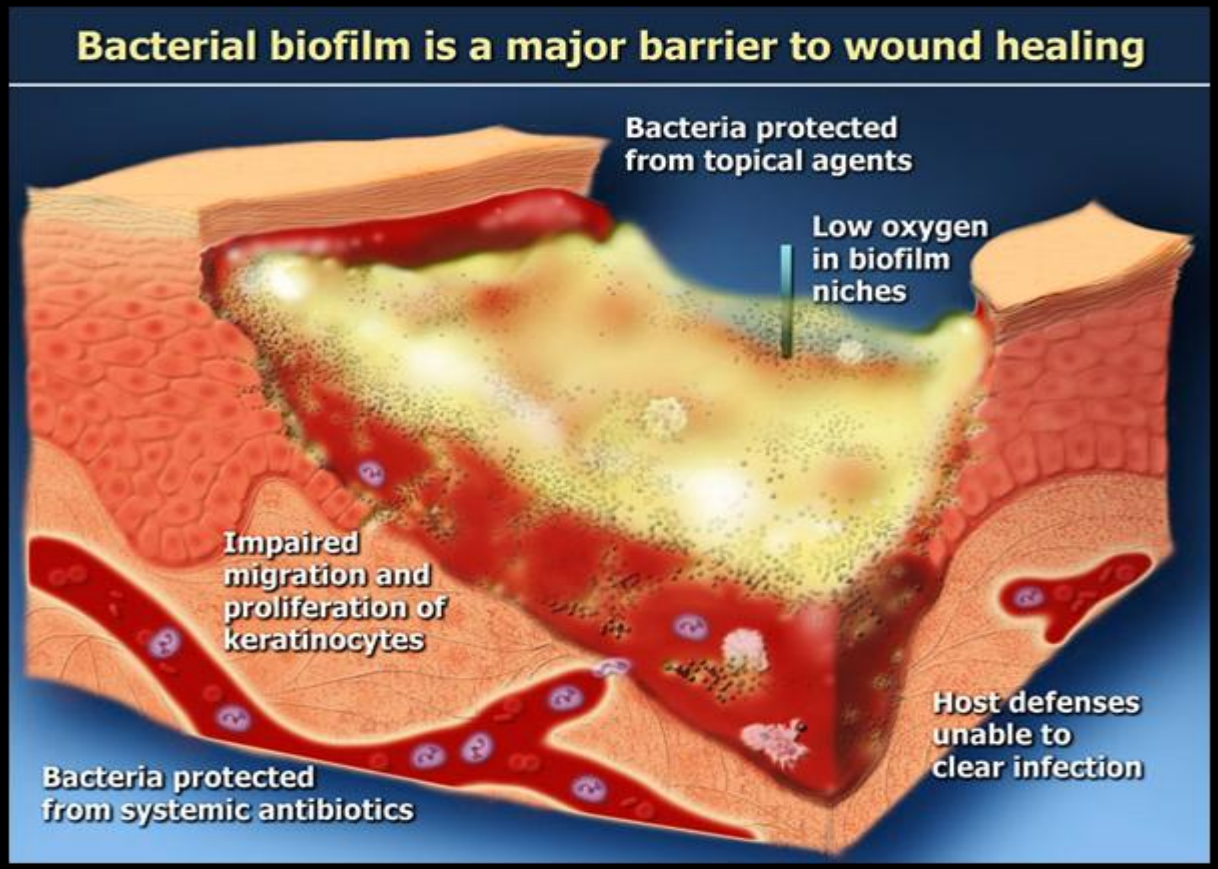
- Lower colony counts
- AB prophylaxis
- Patient selection
- Remove foreign materials

Biofilms in Chronic Wounds

Photo courtesy of Prof Keith Harding, WHRU, Cardiff University

Biofilms in Chronic Wounds

- Reservoir
- Antibiotic Haven
- Recurrent Infection
- Delayed Healing



Diagnosis of Biofilms

- Limitations of wound swabs
  - Sampling surface/aerobic organisms
- Limitations of blood cultures
  - Sampling planktonic cells
  - May provide limited information about original biofilm
- Clinical signs
- Molecular analysis

Treatment of Biofilms

- Antibiotics
- Silver
- Antiseptics
- Removal / Debridement
- Attack EPS matrix (surfactants)
- Attack QS (adhesins/NO)

Conclusions

- Biofilms have always been around
- Current techniques aren’t wrong, but need thought, and careful implementation
- Abs won’t be around forever