Diabetic foot & infection biofilm: what you can't see is finally what you can get

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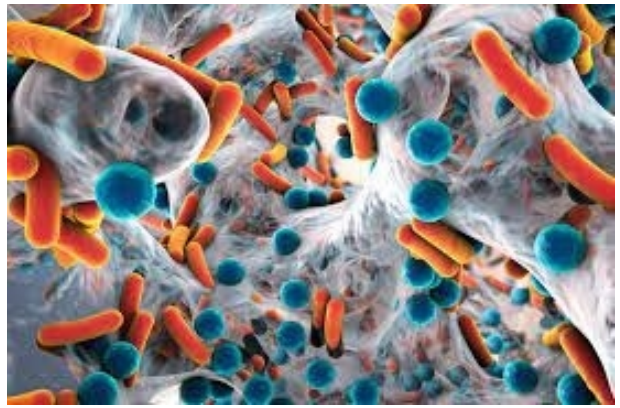
I have no conflicts of interest to declare

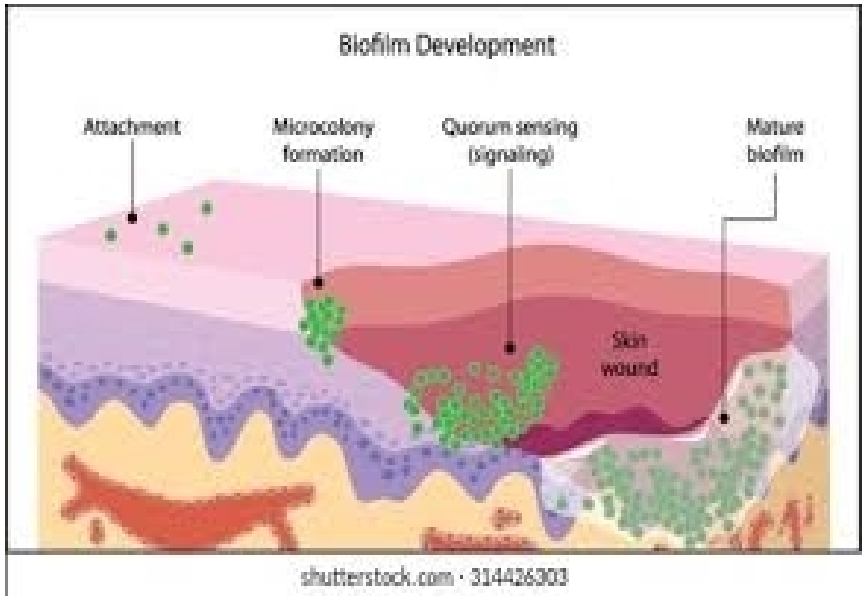
Introduction

Biofilm formation is an important physiopathology step in diabetic foot ulcers (DFU)—it plays a main role in the disease progression and chronicity of the lesion, the development of antibiotic resistance, and makes wound healing difficult to treat



Definition

- Biofilm refers to the complex, sessile communities of microbes found either attached to a surface or buried firmly in an extracellular matrix as aggregates. The biofilm matrix surrounding bacteria makes them tolerant to harsh conditions and resistant to antibacterial treatments
- The antibiotics available till date are ineffective for treating these biofilm.



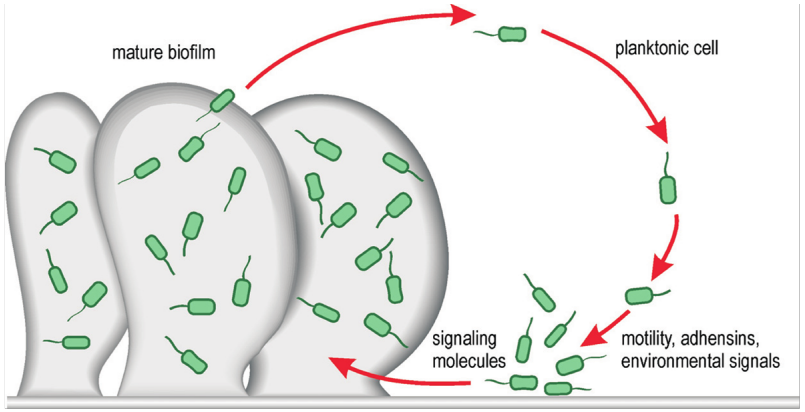
- Only 6% of acute wounds contained biofilms as opposed to 60–80% for chronic wounds (James *et al*. 2008; Dowd *et al*. 2009)
- Epidemiological studies have shown that biofilm formation is associated with more than 60% of all human infections

(Virulence.2018 Jan 1;9(1):522-554)

CAUSES

- Existing literature showed that both genetic and environmental factors contribute towards the microbial biofilm formation (Maric and Vranes 2007)
- Bacteria can adapt to different environmental conditions by modulating their biofilm structure (Maric and Vranes 2007)
- Different signals from environment, such as availability of certain nutrients, presence of oxygen, temperature and pH, take part in regulation of a biofilm formation
- In addition to microorganisms, components from the host, such as fibrin, platelets or immunoglobulins, may be integrated into the biofilm matrix
- Non-optimal dosing of antibiotics and risks of underdosing

- **Period biol, Vol 109, No 2, 2007.**



Biofilm formation.

Diabetic and lack of healing

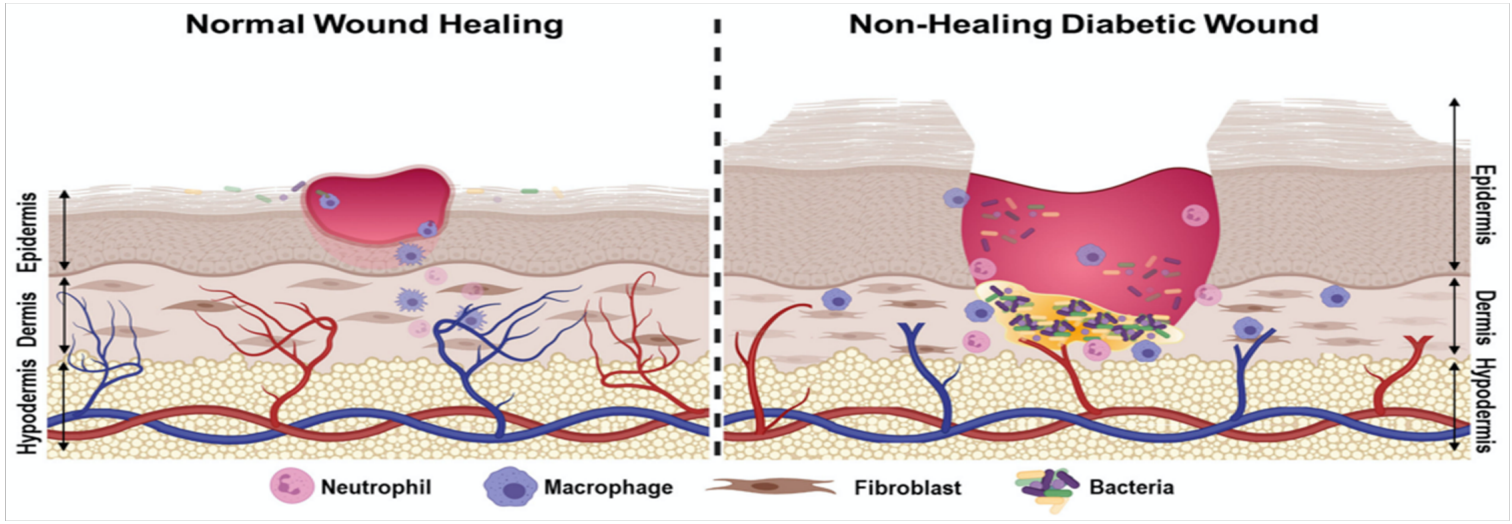
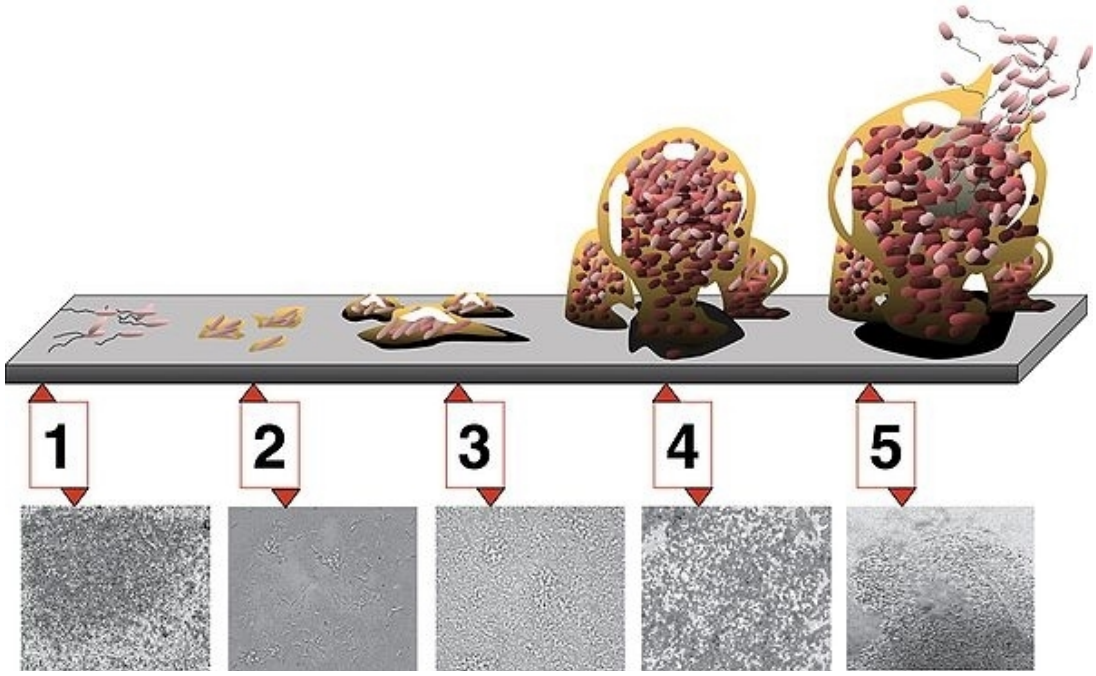


Figure 1. Pathophysiology of diabetic wounds. Diabetic wounds exhibit deregulated angiogenesis, chronically sustained sub-optimal inflammatory response, increased levels of reactive oxygen species, and persistent bacterial colonization that often develops into a hard-to-treat biofilm. Created with BioRender.com, 29 July 2021.

5 steps biofilm development in vitro



Step 1 : Initial attachment ;

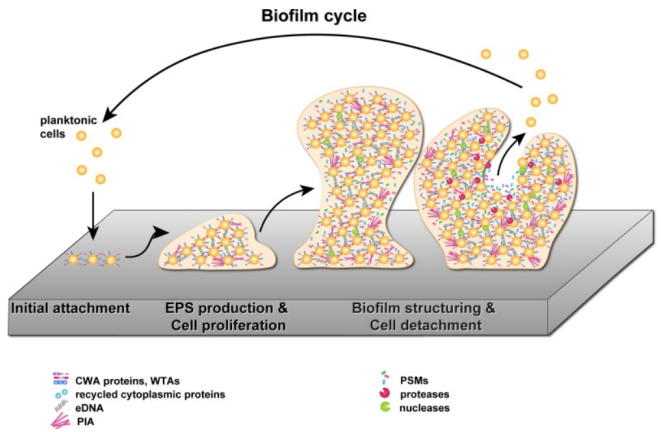
Step 2 : Production of the extracellular matrix and irreversible attachement;

Step 3 : Cell proliferation and biofilm structuring;

Step 4 : Maturation ;

Step 5 : Erosion and cell detachment

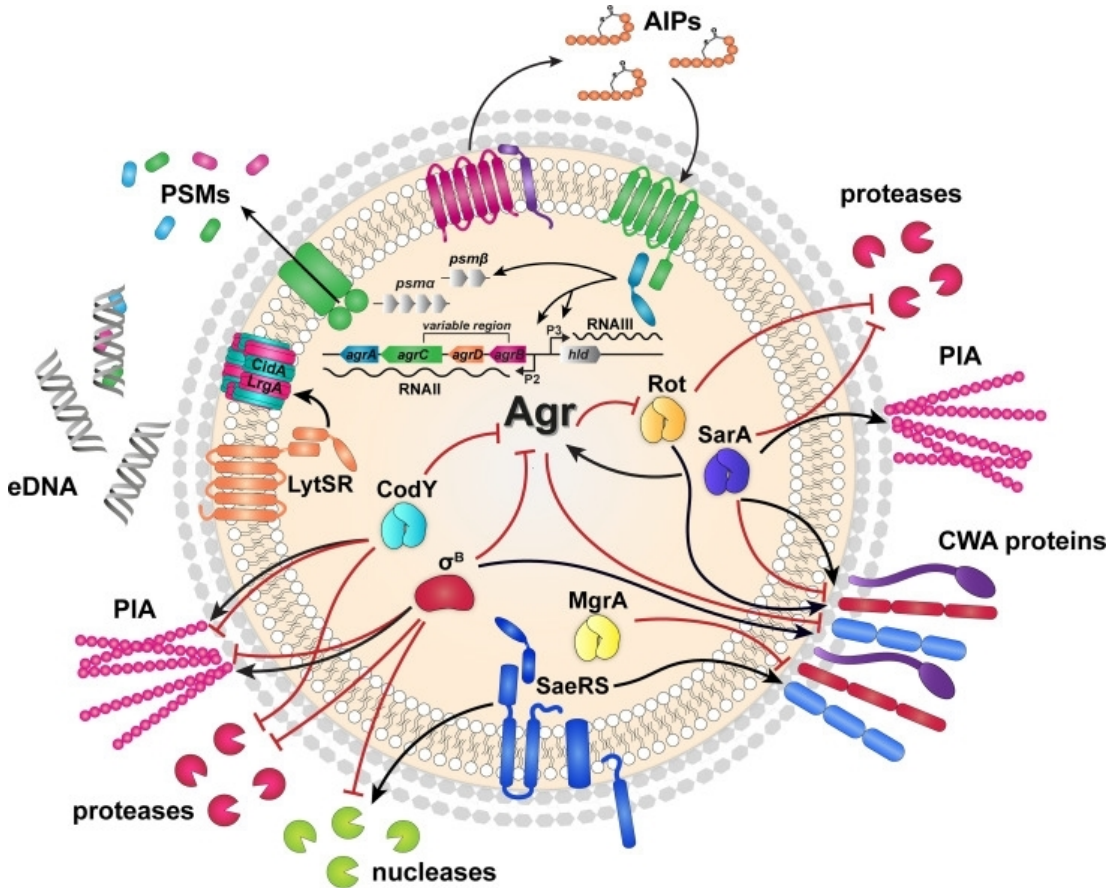
Photomicrographies represent of the *Pseudomonas aeruginosa* biofilm development



[Microbiol Mol Biol Rev.](#) 2020 Sep; 84(3)


CHU
de Liège

Composition



Regulatory network of staphylococcal biofilm formation. Shown is a simplified overview of the Agr quorum sensing system, its regulatory interaction with the most important biofilm regulators (LytSR, SigB, CodY, SaeRS, MgrA, SarA, and Rot) and their influence on the molecular determinants of biofilm formation. Black arrows indicate stimulation, and red blunted arrows indicate repression.

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Matrixome

Biofilm is componed of **microbial cells** firmly attached to a surface and a **matrix** composed of extracellular polymeric substances **(EPS) :**

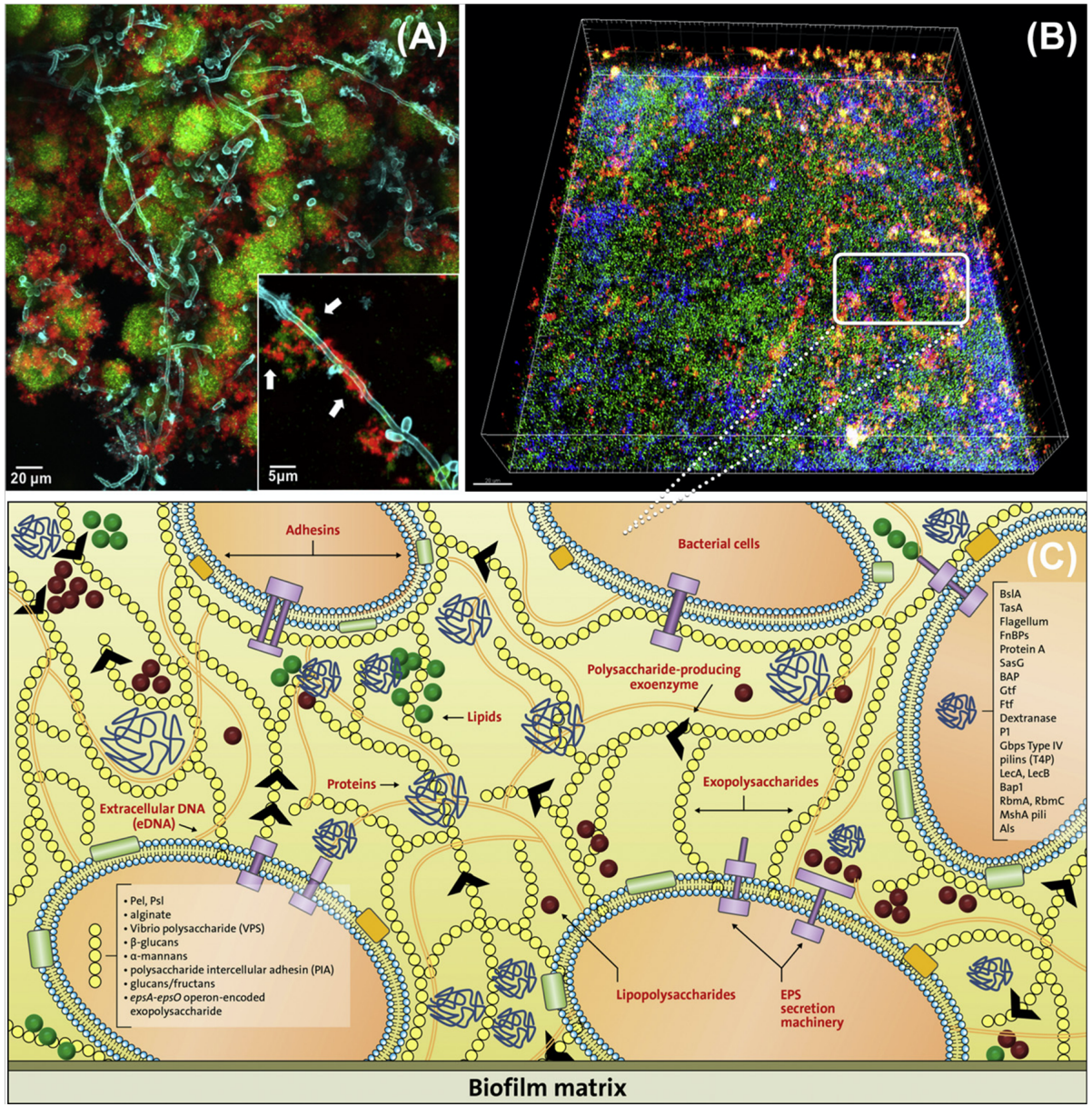
Exopolysaccharides

Nucleics acids (eDNA-eRNA)

Proteins

Lipids

Others biomolecules

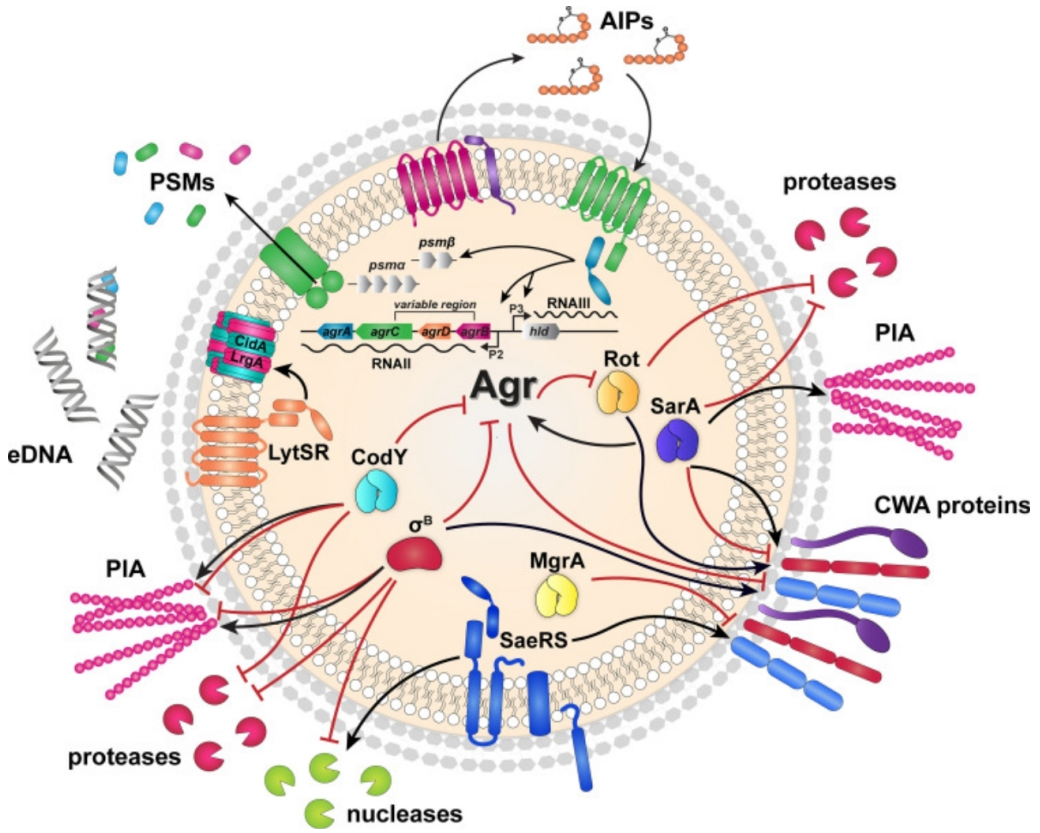


Trends in Microbiology, Month 2020, Vol. xx, No. xx

Composition

EPS contribute to the unique attributes of biofilm lifestyle and virulence by promoting :

- Microbial adhesion
- Cements cells together
- Allows intercellular interactions
- Enhance biofilm tolerance to antimicrobials and immune cells



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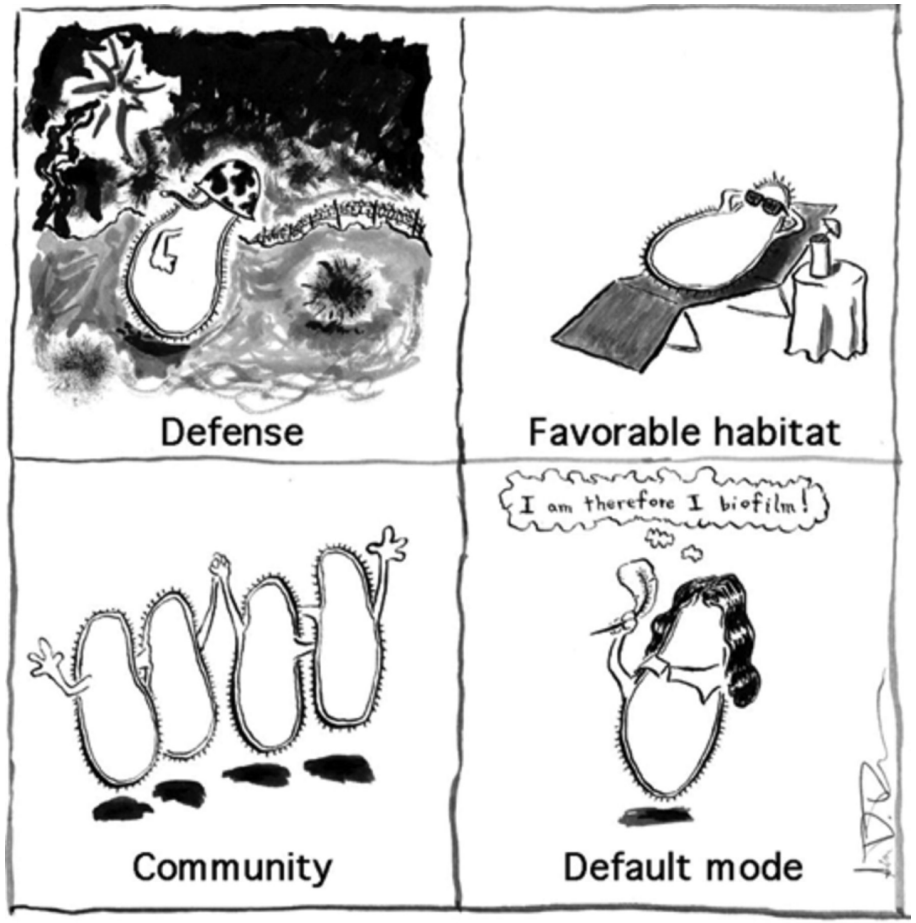
Consequence

Biofims can delay wound healing and increase the risk of infection for the patient

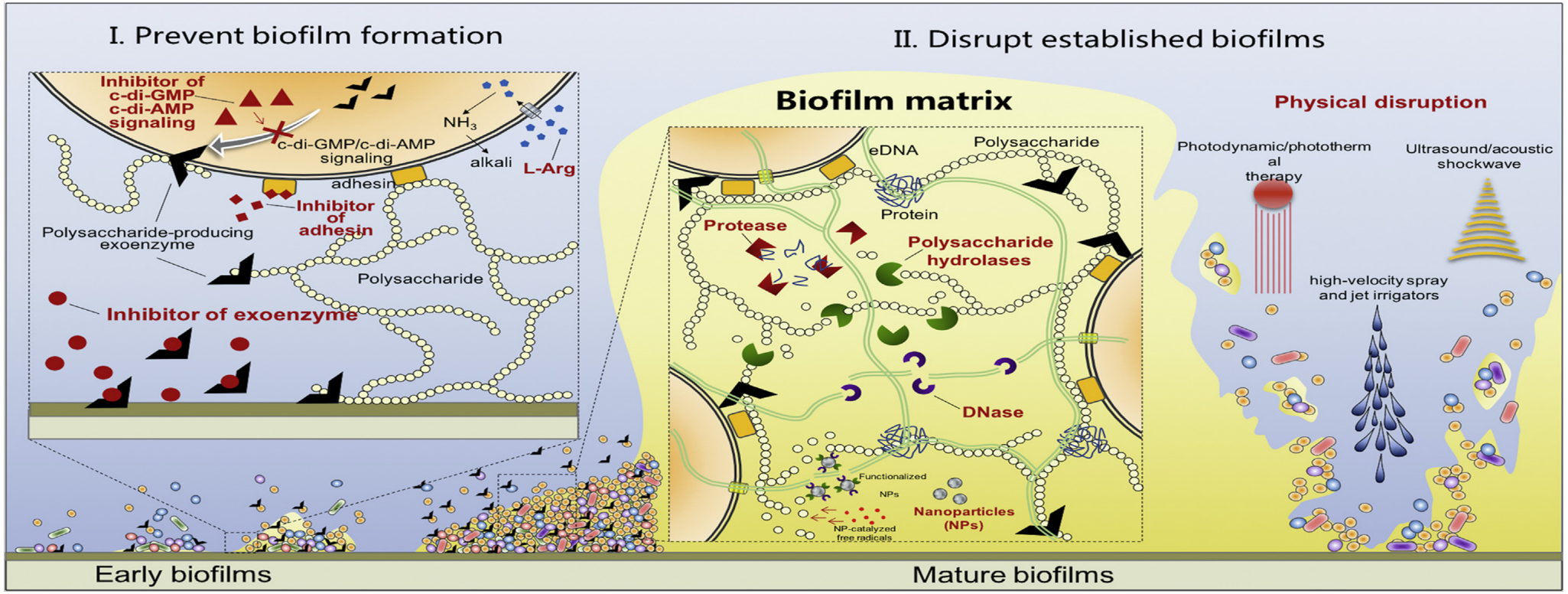


Dr. Sean D. Taverna's artistic interpretation of the four driving forces behind bacterial biofilm formation ...

FEMS Microbiol Lett, Volume 236, Issue 2, July 2004, Pages 163–173,

Treatment

When bacteria exist as a biofilm, they are significantly less susceptible to antibiotics; this is a result of metabolic changes to cells within the biofilm and structural features influencing drug permeability (Jolivet-Gougeon and Bonnaure-Mallet, 2014)



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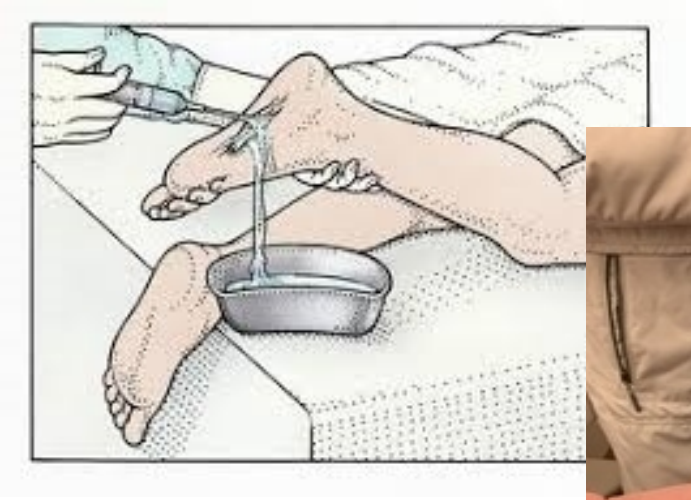
Treatment



PREVENTION by

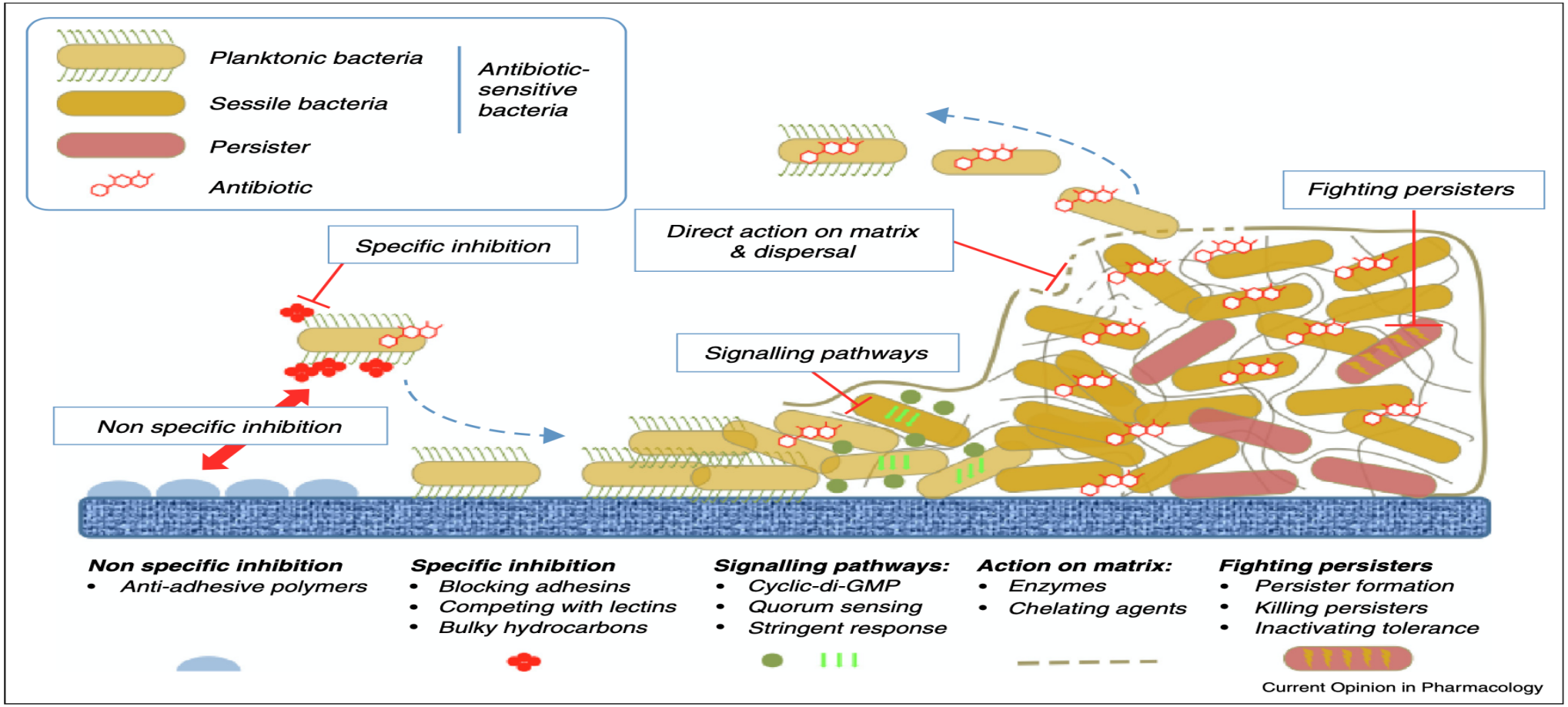
- Regular debridment is recommanded as a « window of opportunity » to promote topical antiseptic
- Wash thoroughly

Treatment

- **Weak organic acid (WOA),** as hydrochloric or sulphuric acid have a stronger antimicrobial effect to rely on their relatively higher hydrophobicity and lipid permeability allowing them to diffuse into the bacterial cell cytoplasm before dissociation occurs (Hirshfield et al., 2003)
- **Povidone-iodine** (PVP-I) demonstrates potent efficacy against biofilms formed by a variety of microbes. The broader spectrum of antimicrobial activity of PVP-I should be advantageous vs the more limited spectrum of antimicrobial activity shown by **polyhexanide (PHMB)** and **silver-containing products - International Wound Journal Volume 18, Issue 3**

- **Chlorhexidine** was tested first at a minimum inhibitory concentration and then at higher concentrations and it was not able to destroy the biofilm - Am J Infect Control 2013 Dec;41(12)

Biofilms: bacterial phenotypes and therapeutic targets.



Biofilms: bacterial phenotypes and therapeutic targets. Schematic drawing of the successive steps of biofilm formation and maturation highlighting the different bacterial phenotypes encountered and their susceptibility to antibiotics. The five major approaches to combat biofilms are represented with their impact on biofilm formation or integrity and their possible combination with antibiotics.

- **Bacteriophage** are viruses, which target bacteria and can cause cell death
- Phage have been used clinically to treat infections caused by E. coli, Klebsiella pneumoniae, Staphylococcus aureus and P. aeruginosae

  (Salmond and Fineran, 2015)
- **Photo inactivation** where light (as blue light-wavelengths between 400 and 470 nm) is used to either directly damage bacteria by exciting intracellular porphyrins, which release reactive oxygen species, or to activate an inert photo-sensitive dye that releases toxic reactive oxygen species (Sperandio et al., 2013).
- **Negative pressure** wound therapy with irrigation or instillation may lower the bacterial burden in chronic wounds and prevent biofilm formation.

Conclusion

- Enhanced understanding of the multifaceted nature of biofilm matrix can also lead to more efficacious approches to contrôle biofilm-related diseases.

- Prevention the biofilm development and bacterial proliferation is the first treatment.
- Promote regular mecanical debridment
- Target the good antibiotics and the good doses to reduce bacterial resistance and so the risk to develop this biofilm