Biofilms

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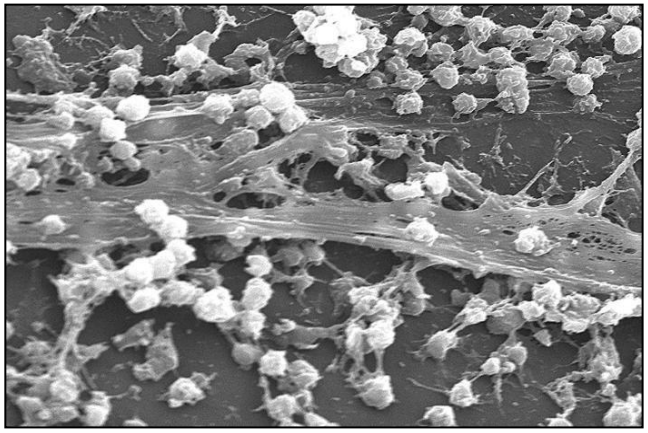
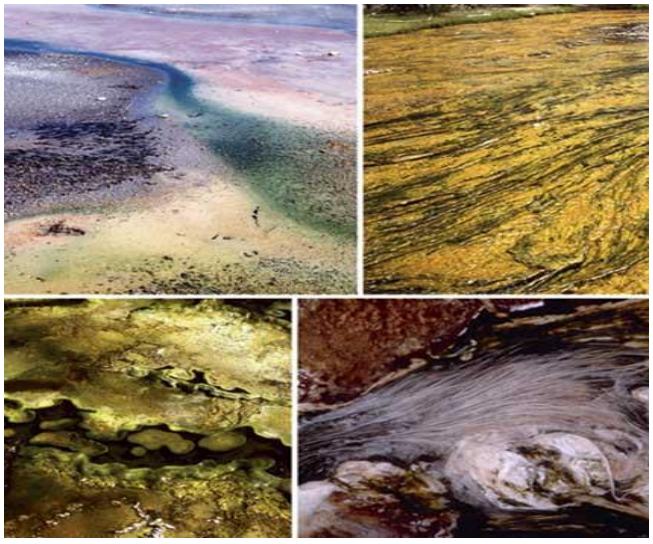
Agenda

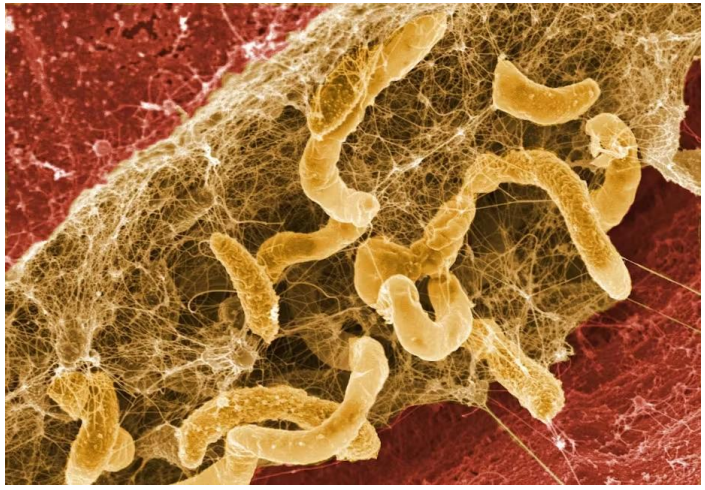
- Biofilm Basics
- Studying Biofilms
- Hospital Biofilms
- Controlling Biofilms
- Summary

Biofilm = Surface-Associated Life forms

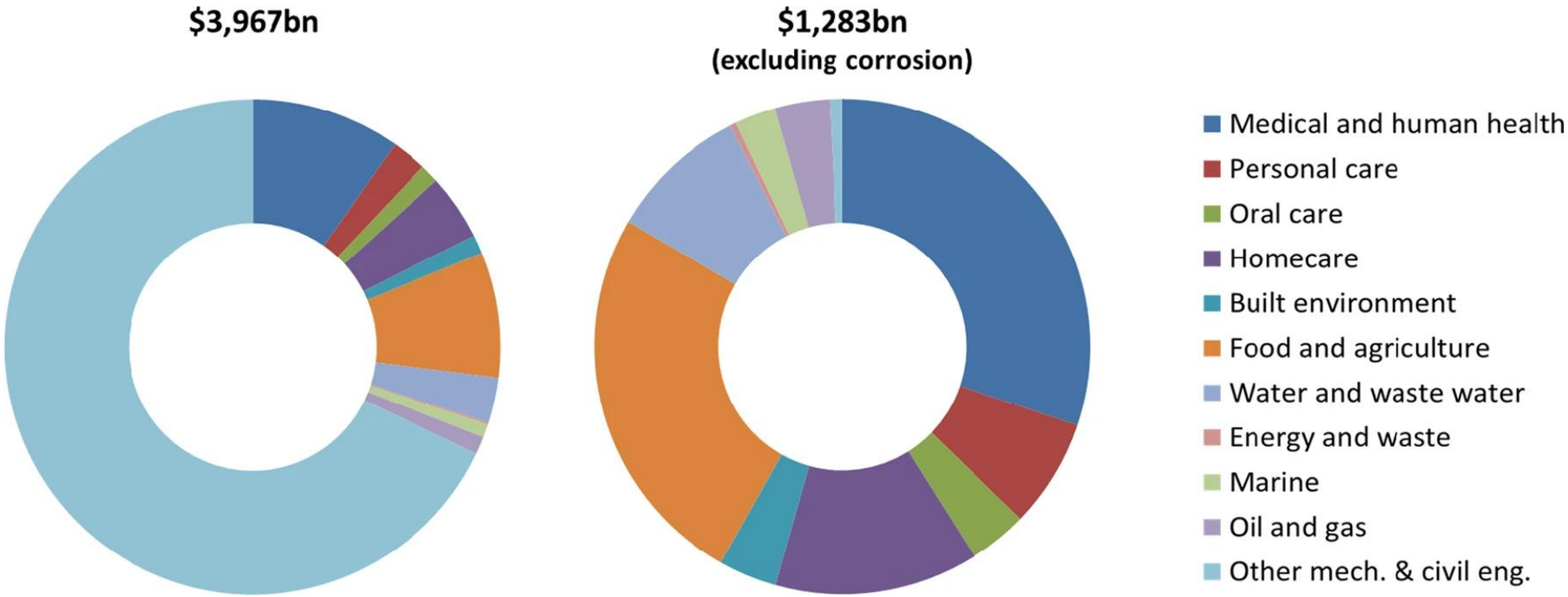
Bacteria, Fungi, Protozoa, Algae, Crustacean







Biofilm Economic Impact



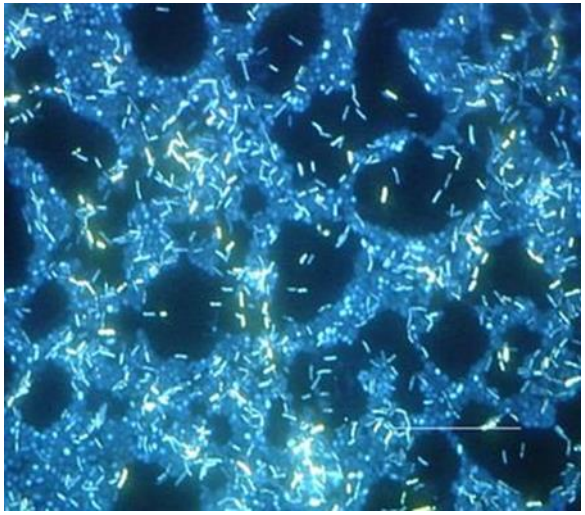
The US National Institutes of Health reported that over 80% of microbial infections in the body are due to biofilms

Cámara, M., Green, W., MacPhee, C.E. et al. Economic significance of biofilms: a multidisciplinary and cross-sectoral challenge. npj Biofilms Microbiomes 8, 42 (2022).

Biofilm Basics

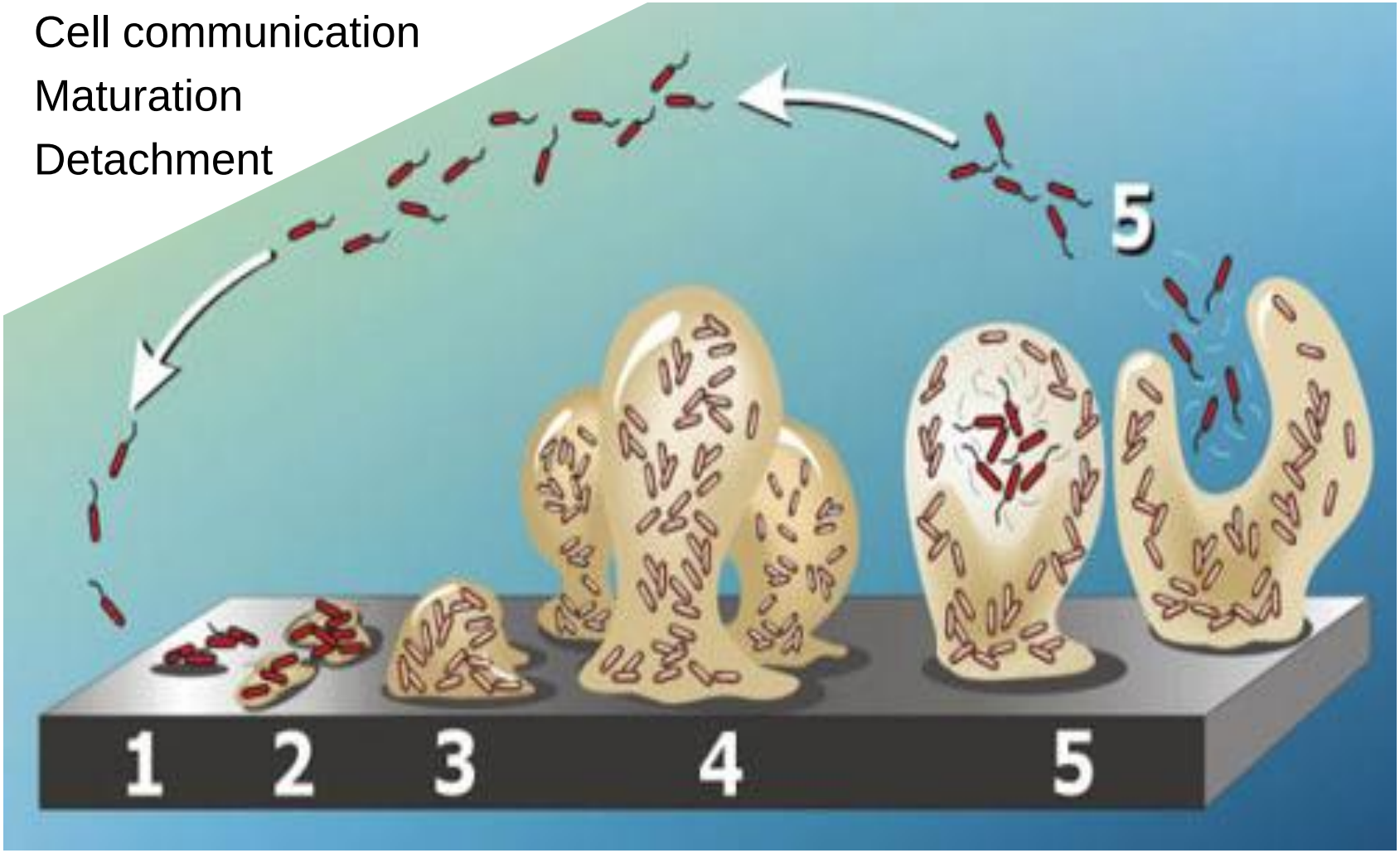
Biofilms

- A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance (EPS) matrix.
- Biofilms can form on living tissues, indwelling medical devices, water system piping or natural aquatic systems.
- Slower growth rate than free-floating cells.
- Can be 1000 times more resistant to antibiotics than planktonic counterparts.



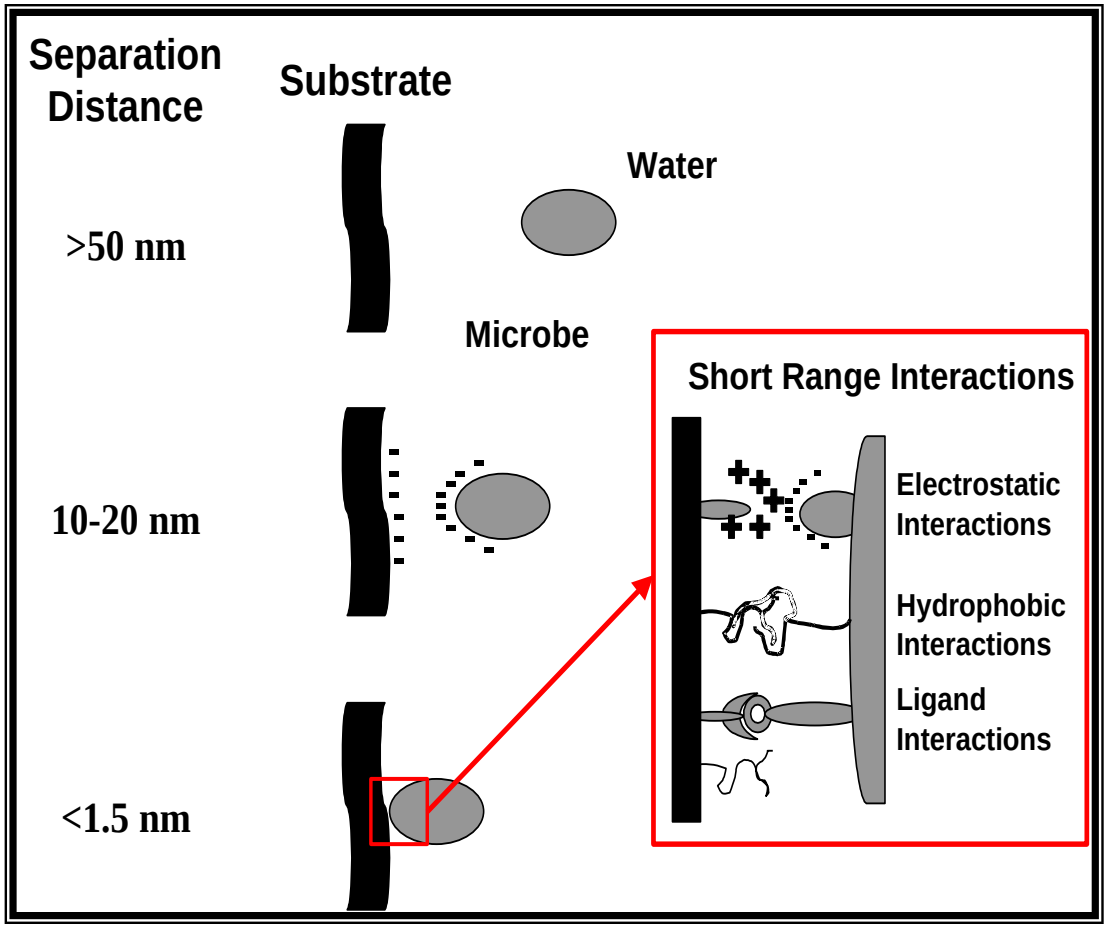
Biofilm Development

1. Transport to a surface (reversible)
2. Irreversible attachment
3. Cell communication
4. Maturation
5. Detachment



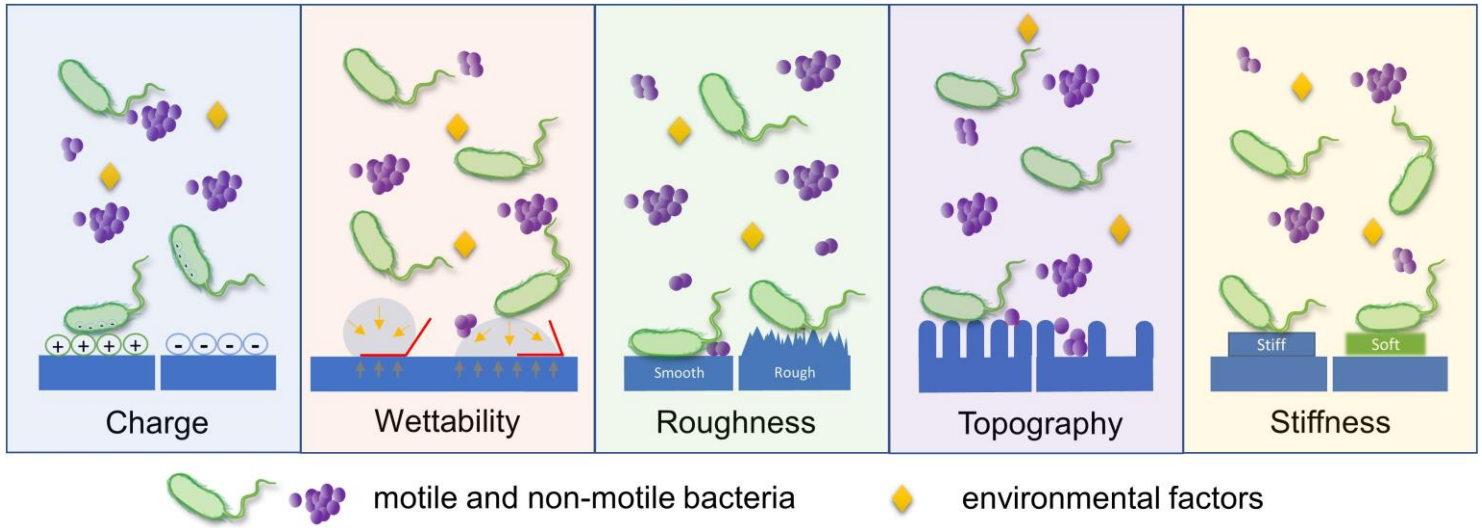
Adhesion

- Non-specific Binding
  - Hydrophobic
  - Electrostatic
  - Biofilm polymers
- Specific Binding
  - Adhesins
    - *S. aureus*
      → Cholesterol
    - *S. epidermidis*
      → Gycolipids
    - *C. albicans*
      → Glycoprotiens

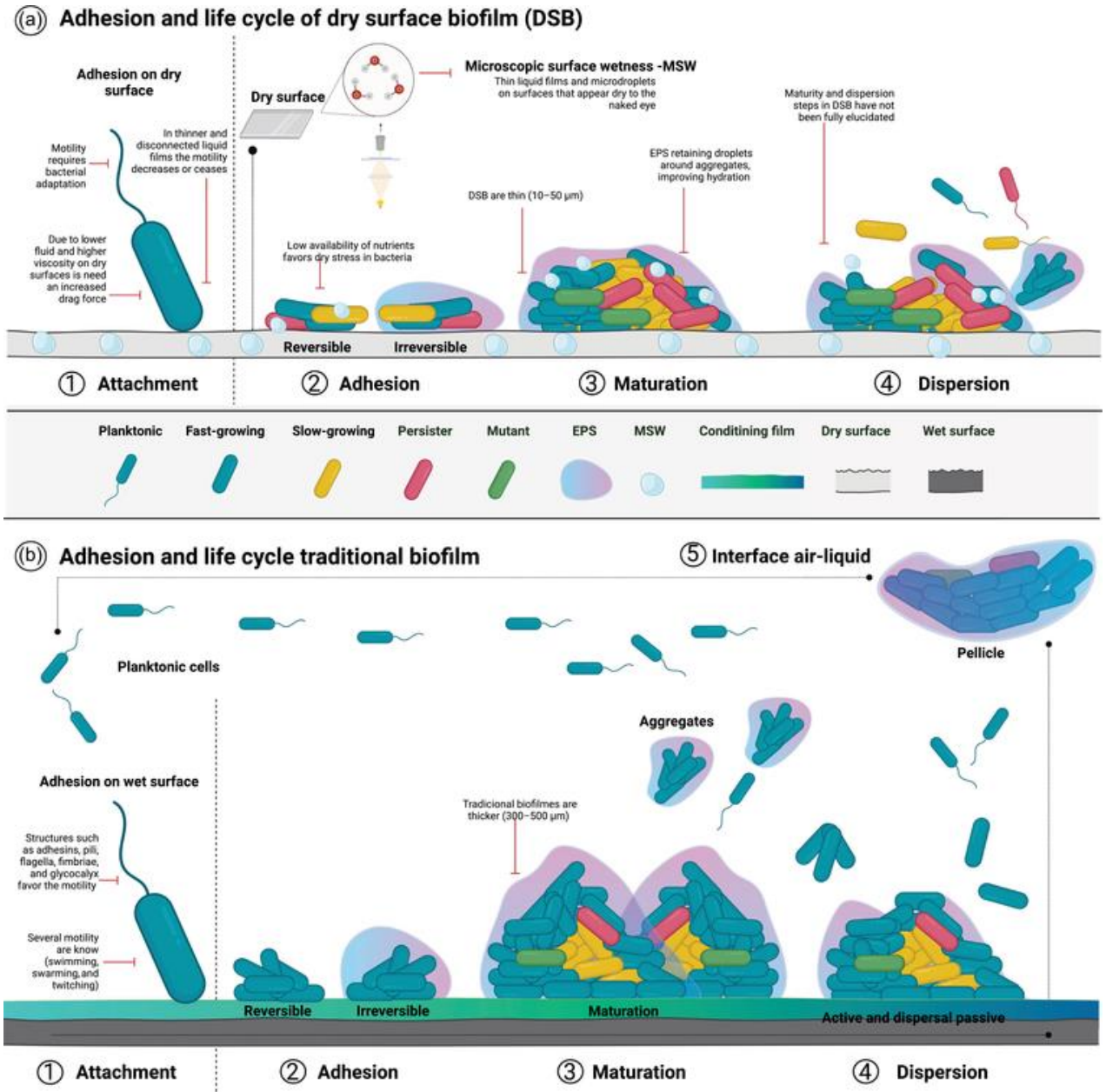


Attachment

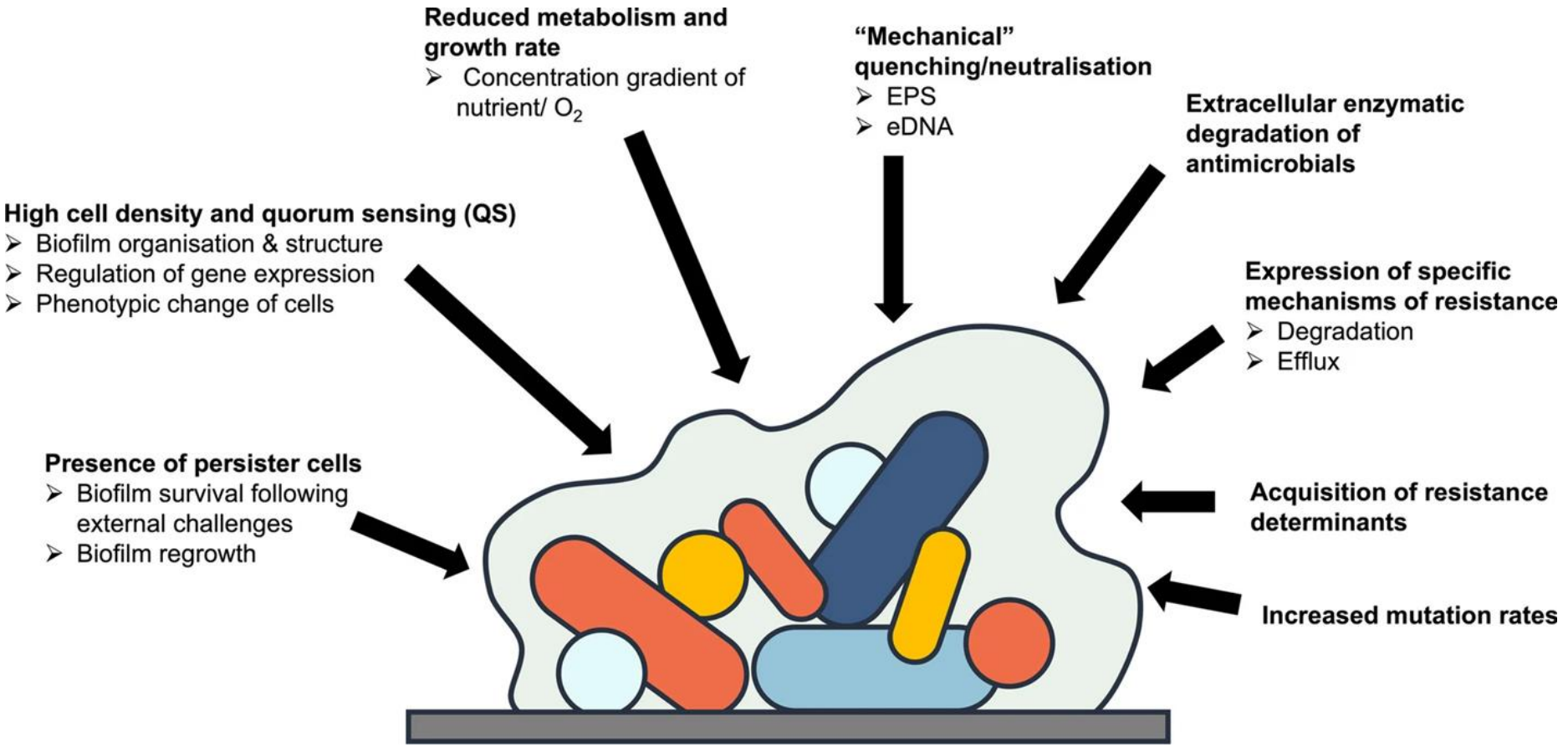
- Surface texture - rough increases attachment rate because sheer forces are diminished and surface area higher.
- Hydrophobicity – attachment is generally quicker to hydrophobic, nonpolar surfaces (plastics).
- Hydrodynamics – linear velocity determines how quickly a cell associates with a surface.
- Medium – pH, nutrients, ionic strength, temp.
- Cell Motility – fimbriae, flagella, pilli



Dry vs Traditional Biofilm



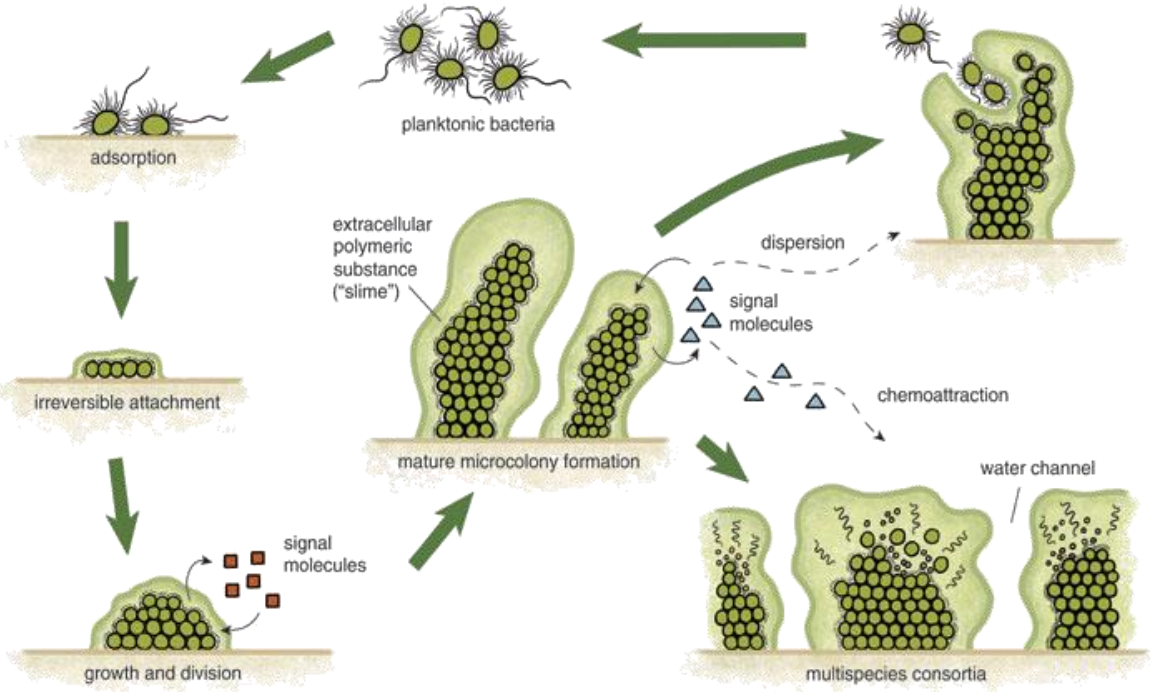
Biofilms and Resistance



Maillard, JY., Centeleghe, I. How biofilm changes our understanding of cleaning and disinfection. Antimicrob Resist Infect Control 12, 95 (2023). https://doi.org/10.1186/s13756-023-01290-4

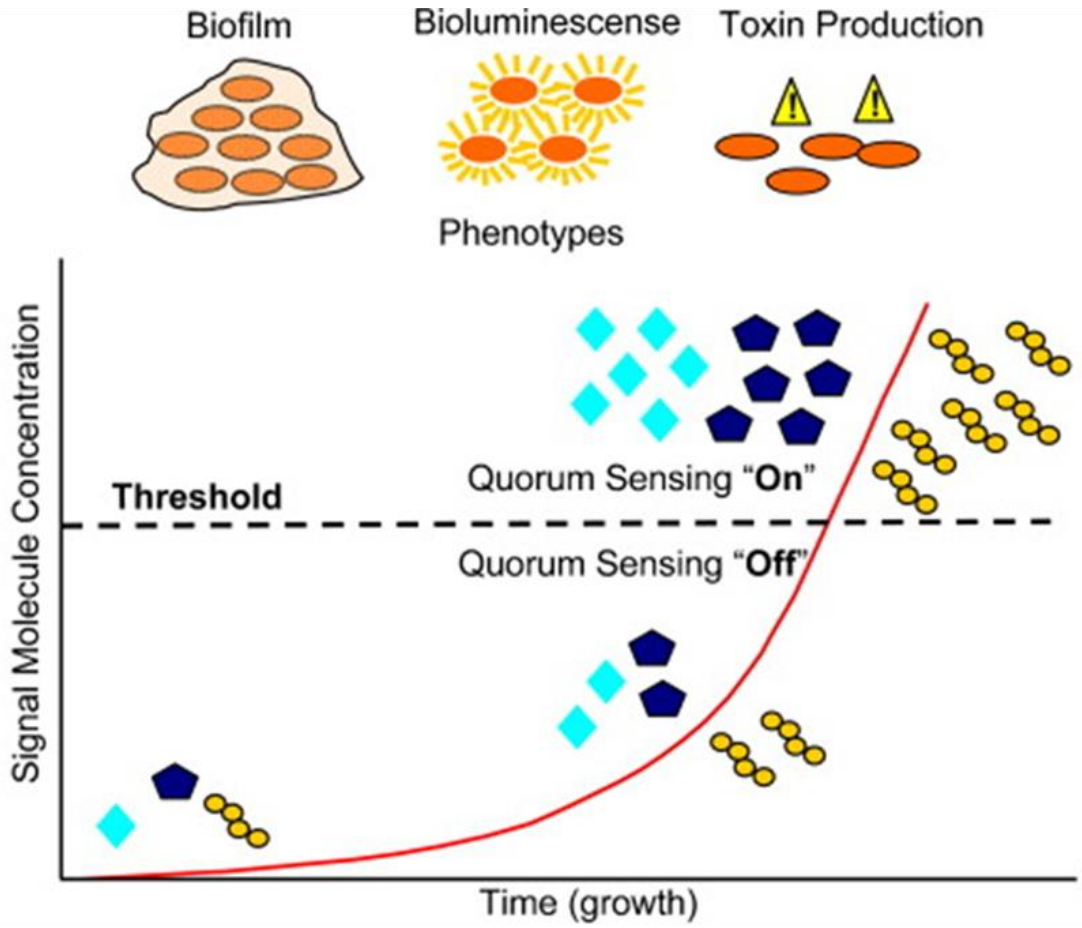
Quorum Sensing

- Microbial Communication - Team Play
  - Control gene expression in response to population density
- Small molecules
  - Gram-negative bacteria - N-acyl homoserine lactones
- Critical numbers of cells trigger response
  - *P. aeruginosa*
    - Protease regulation by lasR and lasI
  - *C. albicans*
    - Farensol regulation of attachment



Cell-Cell Communication

- Key role in biofilm maturation
- Controls which genes are triggered, and population densities are sensed.
- Production and release of signaling molecule
- Activation of virulence, biofilm, sporulation, toxins, bioluminescence….

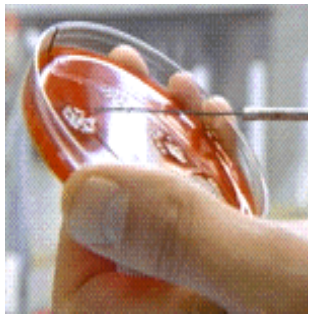


www2.hawaii.edu/~mcooney/oest740/Quorum_sensing_ppt.pdf

Studying Biofilms

Biofilm Bench Assays

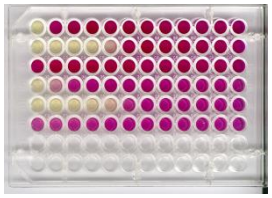
Microbe



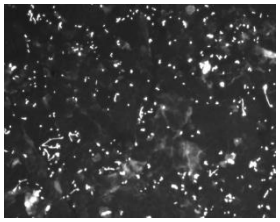
+

Inert objects
Skin
Body tissues

96 Well Plate Assay

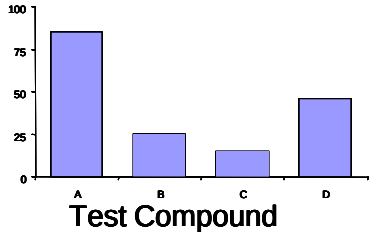


Automated Microscopy
Image Analysis

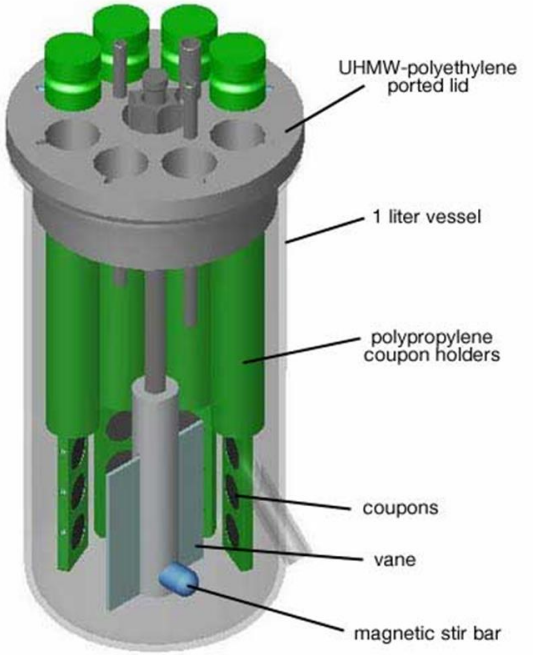


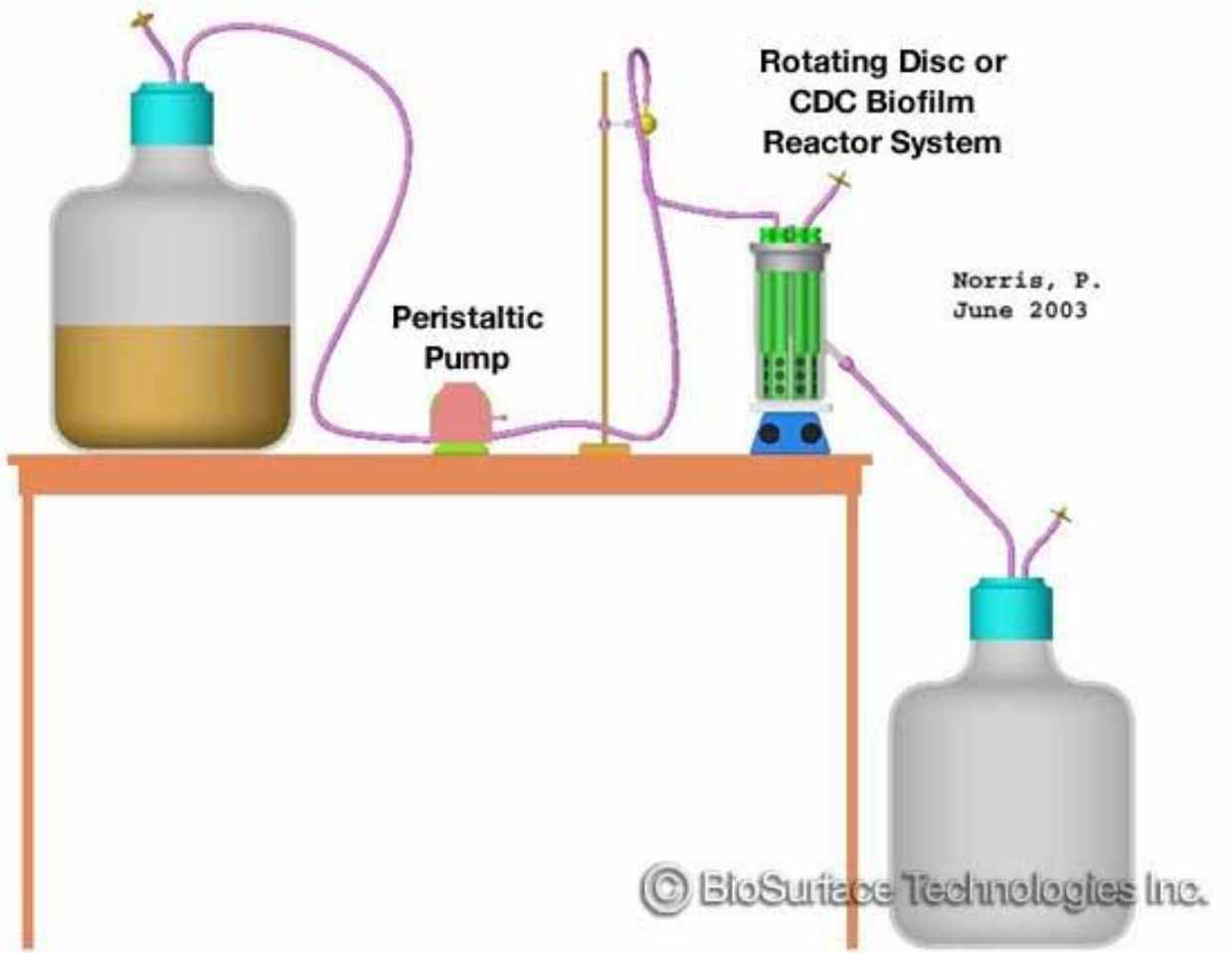
Surface Extraction
Plate Count

Adherence



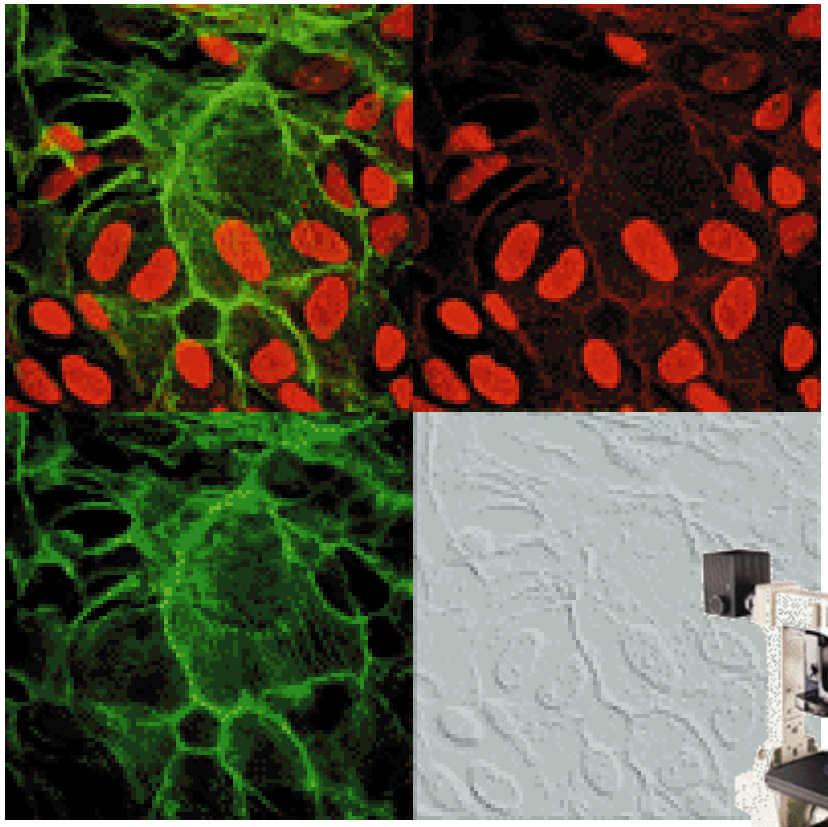
CDC Biofilm Reactor

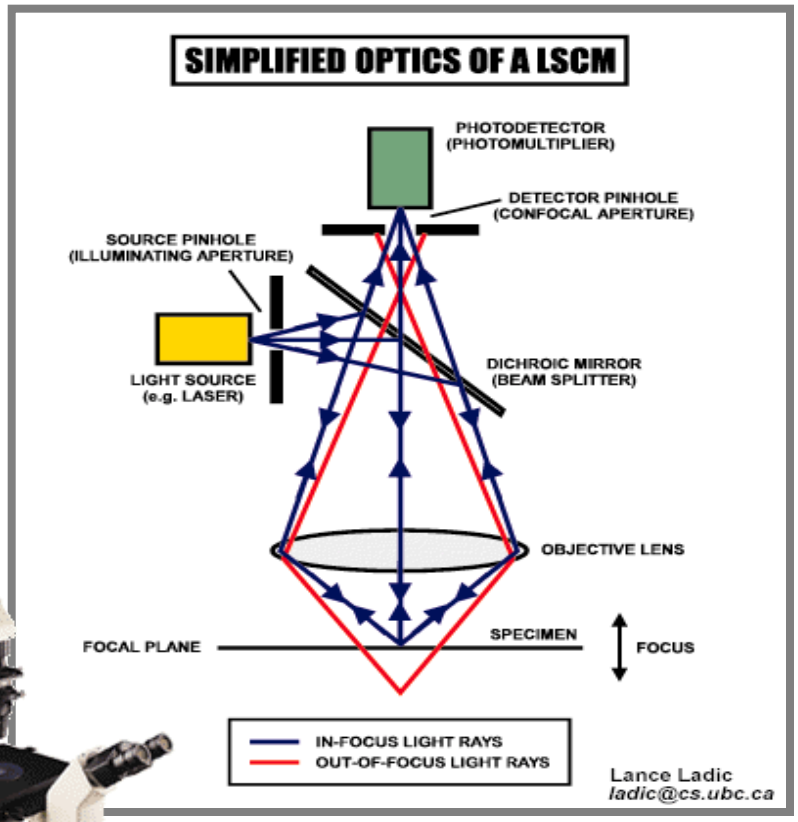




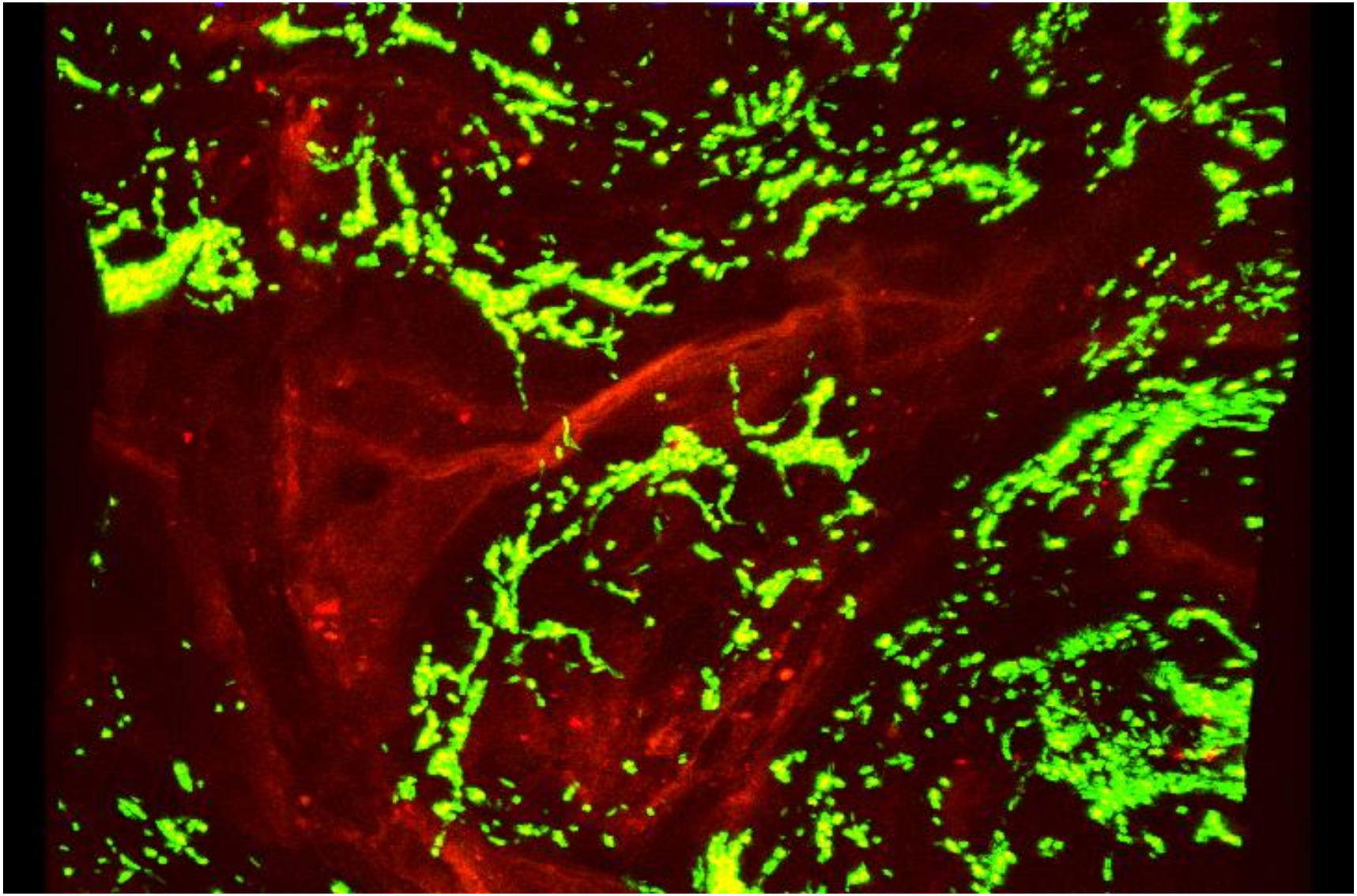
Used in the EPA Disinfectant Biofilm Claim

Confocal Microscopy





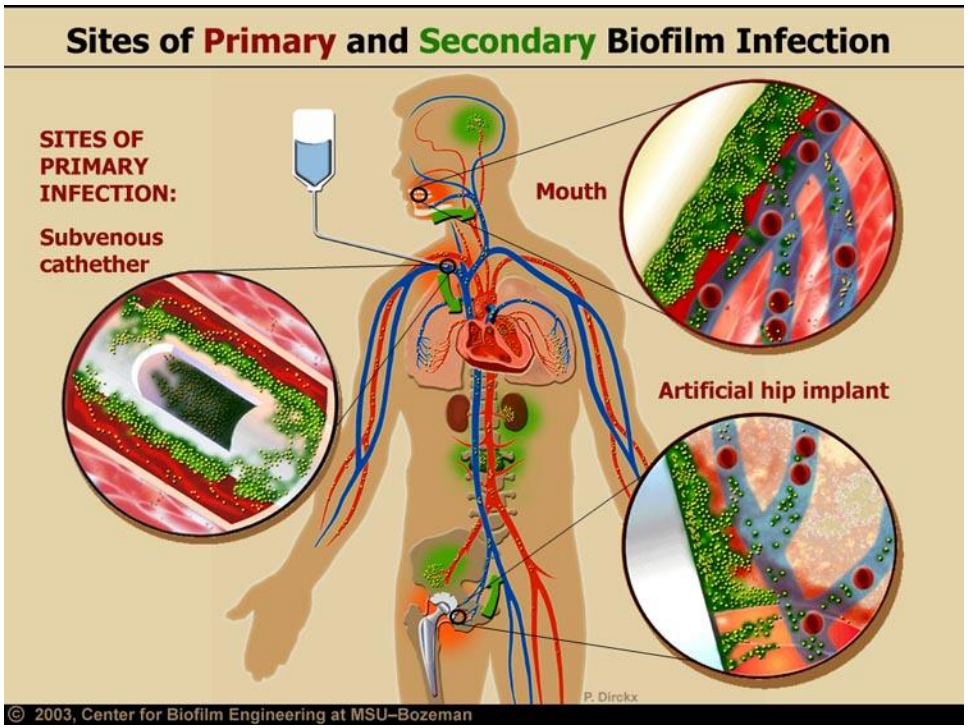
Bacterial Biofilm on Skin



Hospital Biofilms

Biofilm Infections

- 80% of microbial infections in the body are biofilms
- Dental plaque
- Urinary tract infections
- Cystic fibrosis
- Otitis media
- Infective endocarditis
- Tonsillitis
- Periodontitis
- Necrotizing fasciitis
- Osteomyelitis
- Infectious kidney stones
- Chronic inflammatory diseases

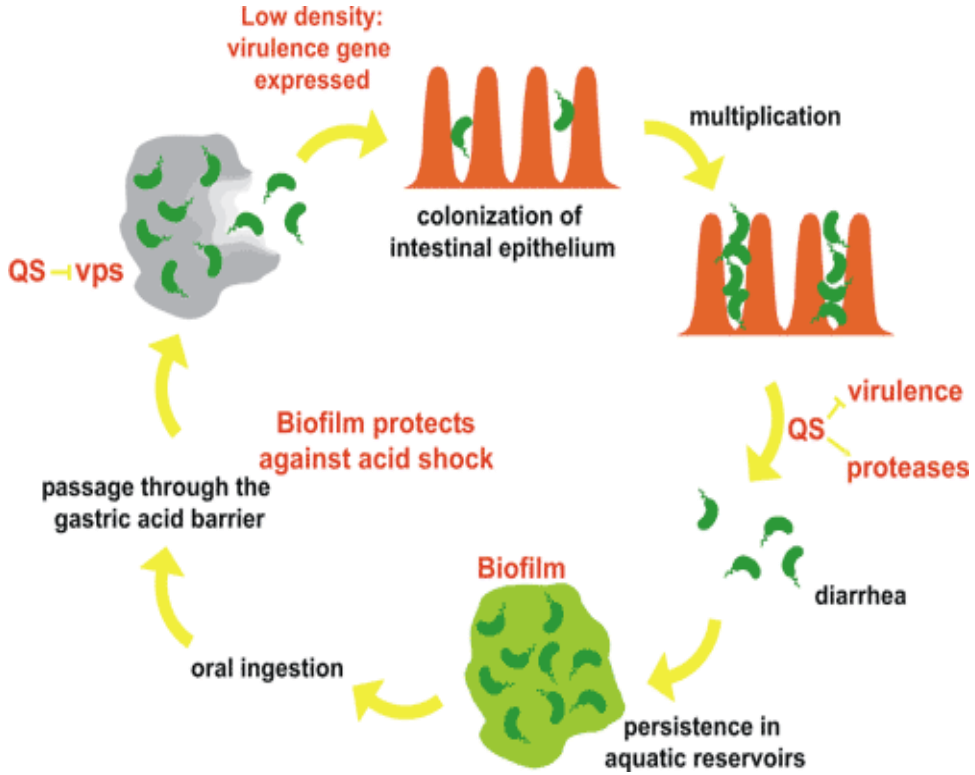


Indwelling Devices and Implants

Biofilms Impact Infections and Wounds

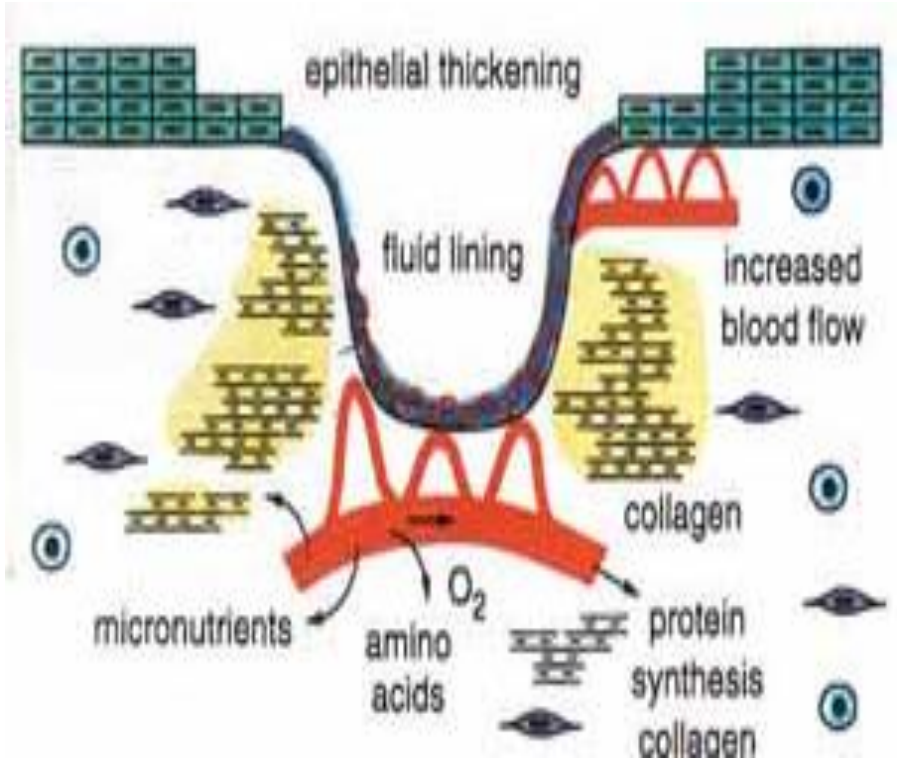
Biofilms and Infection Control

- Persistence in host
- Persistence in environment



Biofilms and Wound Healing

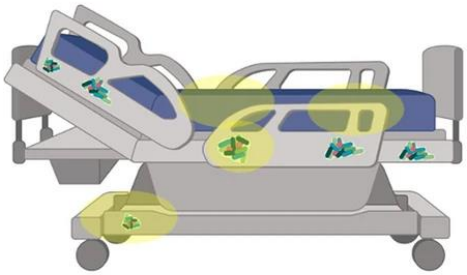
- Persistence in host
- Triggering of host immune system
- Prolonging initial phase of healing



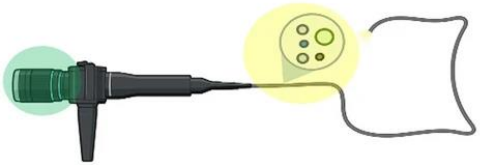
Hospital Biofilms

Drain Biofilms

Dry Surface Biofilms



Medical device biofilms



PARAMETERS TO CONSIDER IN INFECTION PREVENTION AND CONTROL

- Microbicidal effect – reducing microbial burden specifically MDRO
- Dec reassign biofilm biomass
- Regrowth

- Microbicidal effect – reducing microbial burden specifically MDRO
- Preventing transfer – ensuring the surface is safe
- Detection

- Elimination of all microorganisms in all the medical device parts following mechanical, enzymatic, chemical processes

CHALLENGES

- Sub-optimal microbicidal activity
- Limited window for interventions
- Sink location and usage
- Rapid regrowth of identical biofilm
- No standard efficacy test protocol in Europe
- Limited number of standard tests in the USA

- Detection
- Effective combination of removal/microbicidal efficacy
- No standard efficacy test protocol

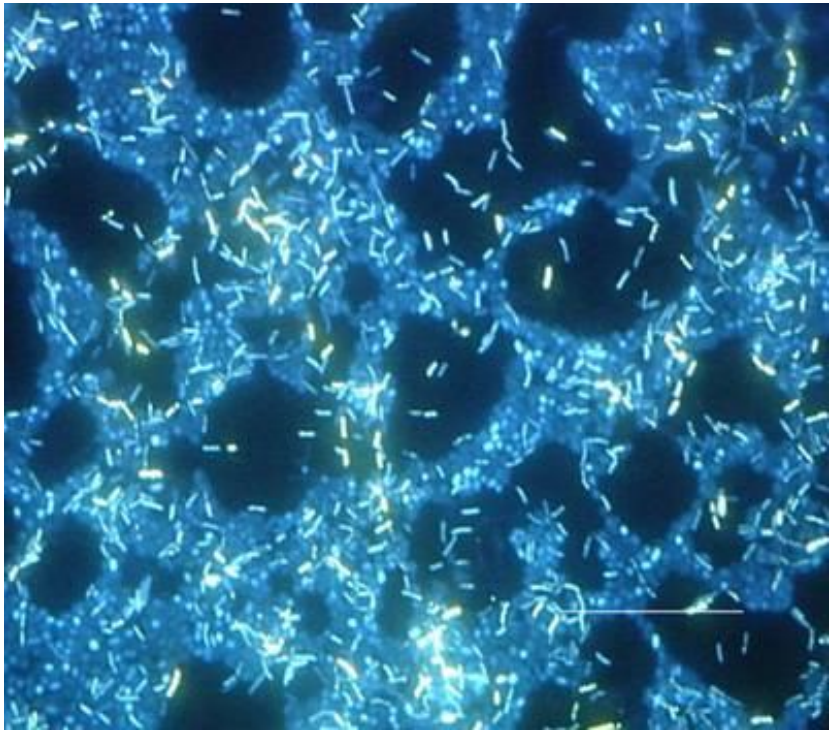
- Detection
- Bacterial regrowth during storage
- Education
- No standard efficacy test protocol

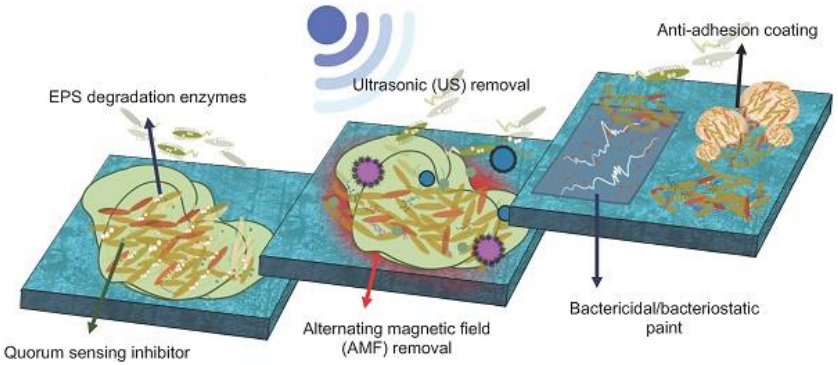
Maillard, JY., Centeleghe, I. How biofilm changes our understanding of cleaning and disinfection. Antimicrob Resist Infect Control 12, 95 (2023). https://doi.org/10.1186/s13756-023-01290-4

Biofilm Control

Stopping Biofilms

- **Energy/Mechanical**
  - Microfiber wiping
  - Ultrasonic & Bio-acoustics
- **Disinfection**
  - Oxidizing biocides
- **Polymer coatings**
  - Surfaces that block adhesion
  - Controlled release of antibiotics
  - Anti-infectives that interfere with bacterial metabolism
- **Surface patterning**
  - Nano-topography to limit microbial adhesion and growth





Using Biofilms for a Health Advantage

Pre- and Pro- Biotics

Biofilm Control

Biosurfactants

- Lipopeptide
- Glycolipids

Chemical treatment

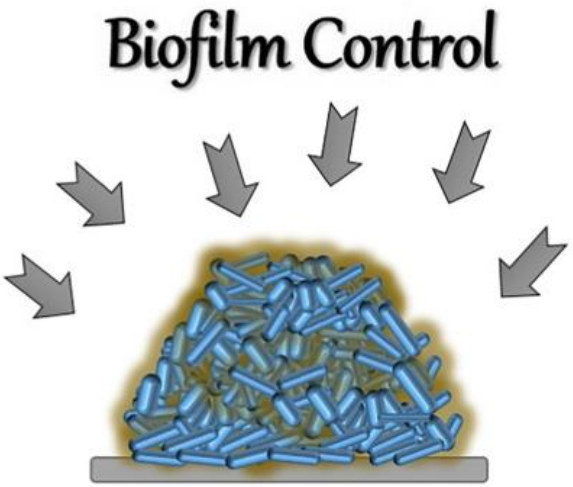
- Detergents
- Biocides
- Surfactants

QS inhibition

- Inhibition of signal molecules production
- Degradation of signal molecules
- Binding to receptors

Mechanical removal

- Freezing
- Thawing
- Scraping
- Sonication



Bacteriocins

- Nisin
- Sakacin 1
- Sonorensin
- Plantaricin

Other bactericidal technics

- High Hydrostatic Pressure
- Non-thermal Plasma
- Photocatalysis (generation of ROS)

Nanotechnological method

- Steel coatings

Bacteriophages

- Phages
- Phage-derived proteins

Enzymatic disruption

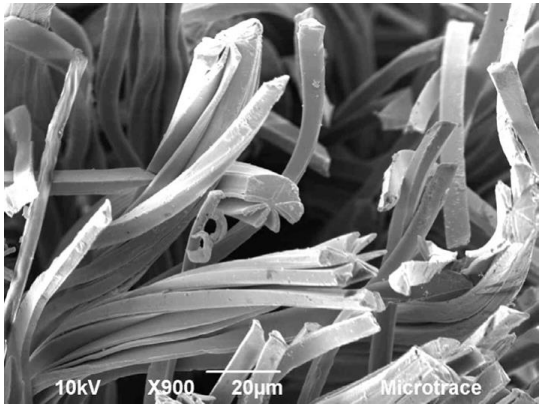
- Proteolytic enzymes
- Polysaccharide-degrading enzymes
- Oxidative enzymes
- Anti-QS enzymes

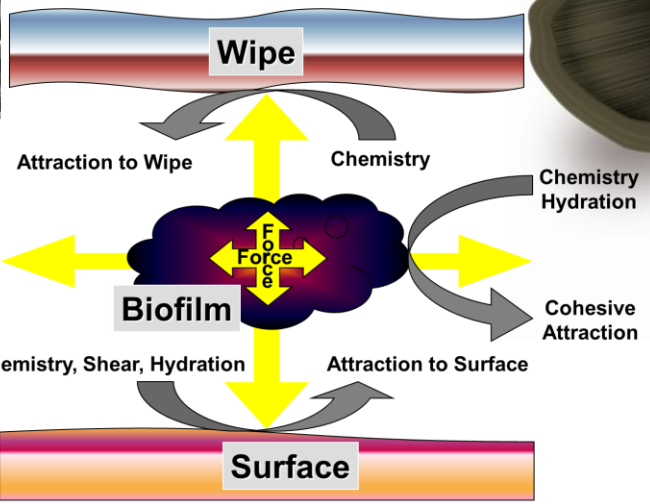
Natural compounds derived from plants

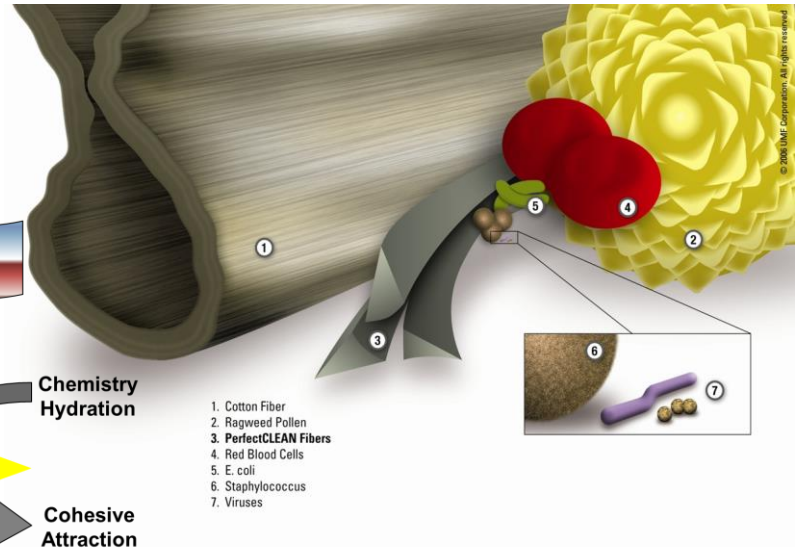
- Essential oils
- Components of essential oils

Mechanical Removal

- Wiping - Microfiber cloths
  - Detergents/Biosurfactants
  - Disinfectants
- Shear driven performance

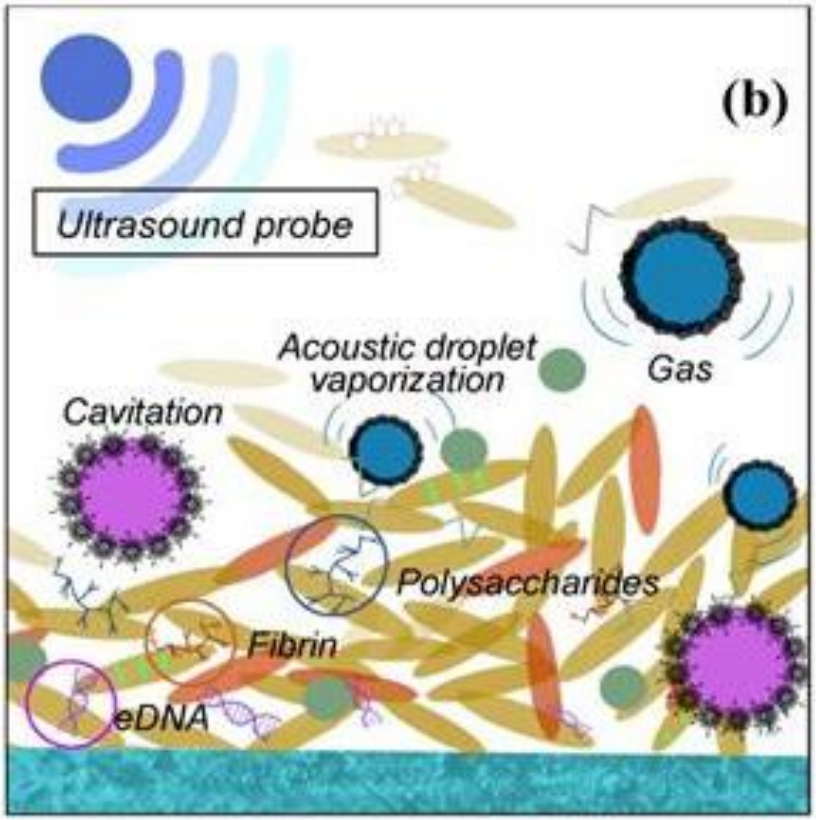






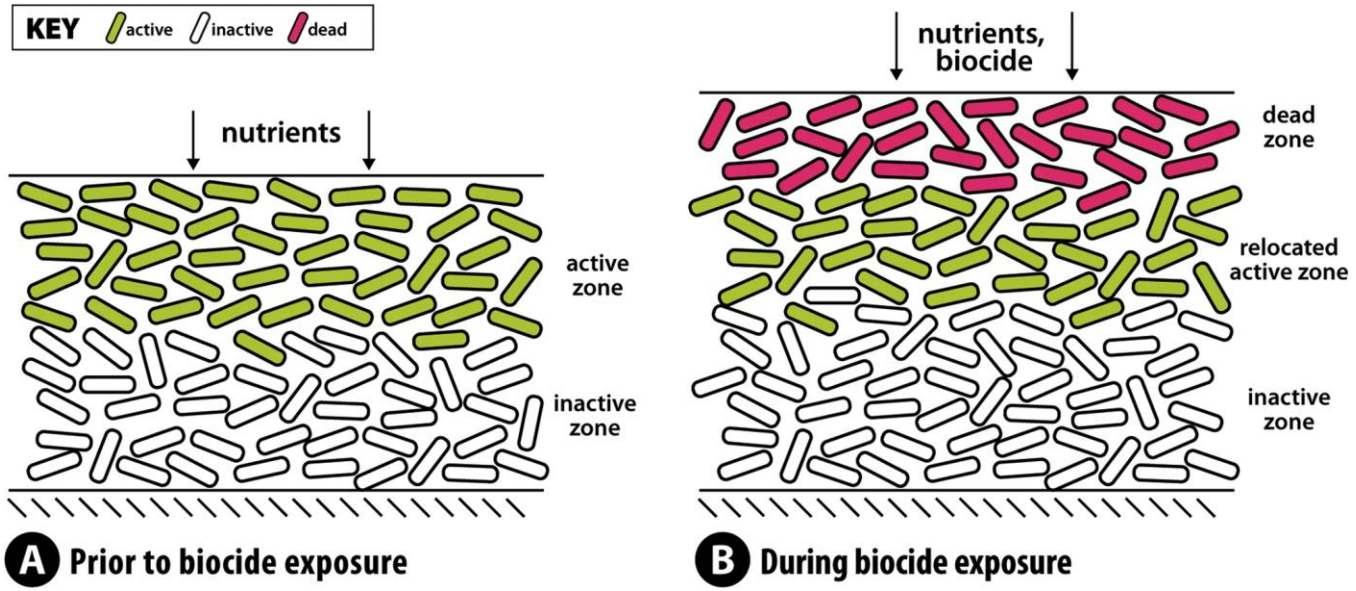
Ultrasound

- Augment effects of anti-microbial treatment
- Surfaces that focus ultrasound energy
- Surfaces that enhance local cavitation effects



Disinfectants

- Oxidizing
  - Bleach
  - HOCl
  - Dichloroisocyanuric acid*
  - Peracid mixture*
  - Peroxide
  - Ozone
  - Iodine
- Non-Oxidizing
  - Metals (Cu, Ag)
  - QUATS
  - Alcohol
- Mixed
  - Quat & Peroxide*

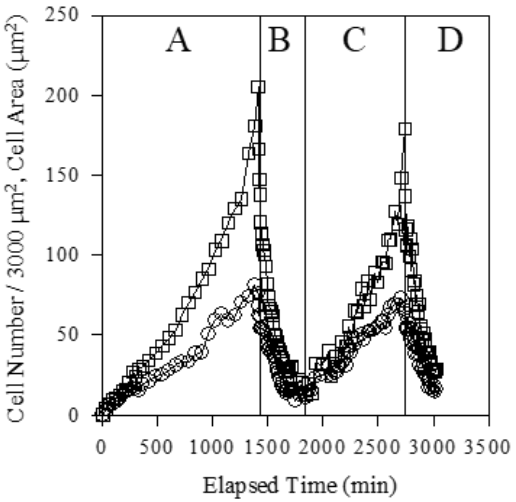


*EPA Biofilm Claim

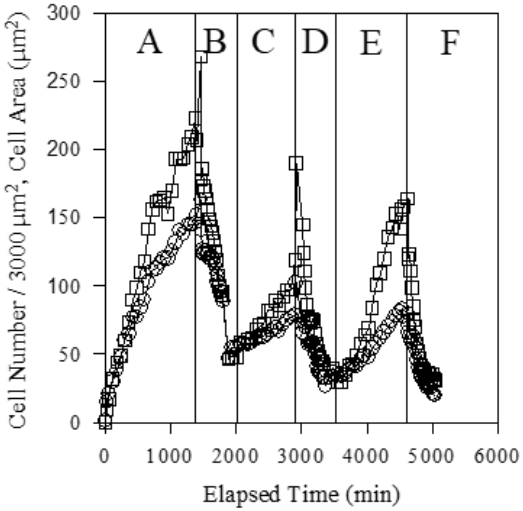
Stewart, P.S., Owkes, M. Simulation of catalase-dependent tolerance of microbial biofilm to hydrogen peroxide with a biofilm computer model. npj Biofilms Microbiomes 9, 60 (2023). https://doi.org/10.1038/s41522-023-00426-z

Biofilm Removal and Return

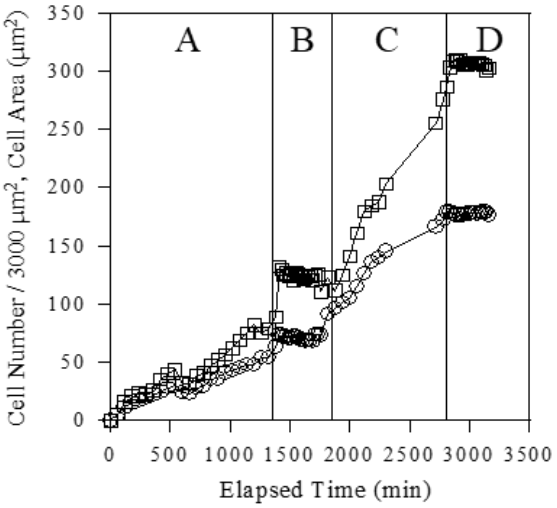
Ozone Treatment



Chlorine Treatment



Iodine Treatment



US 6,498,862

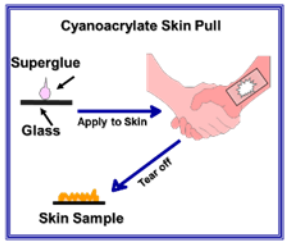
Controlling *Candida* Biofilms

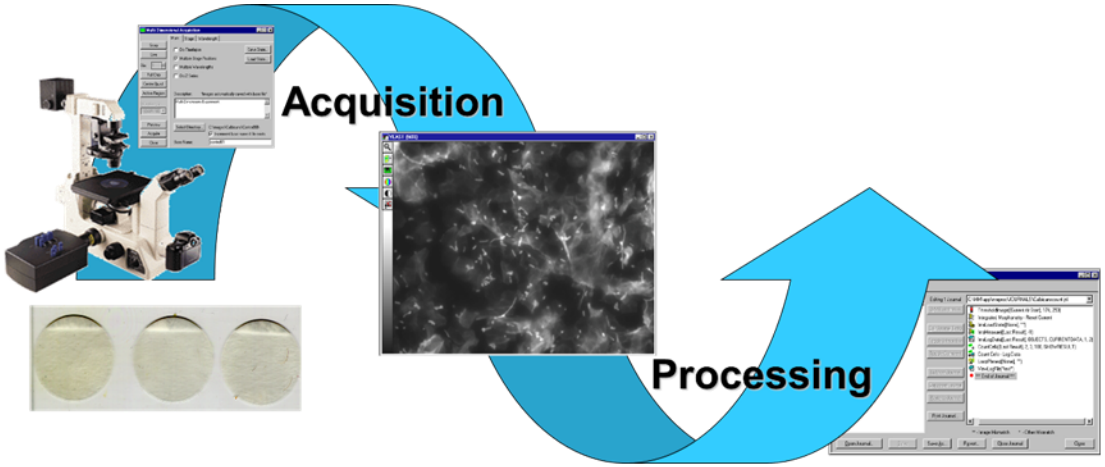
SKIN SAMPLING METHODS

Excised Skin
- Acquired from adult palm with razor blade

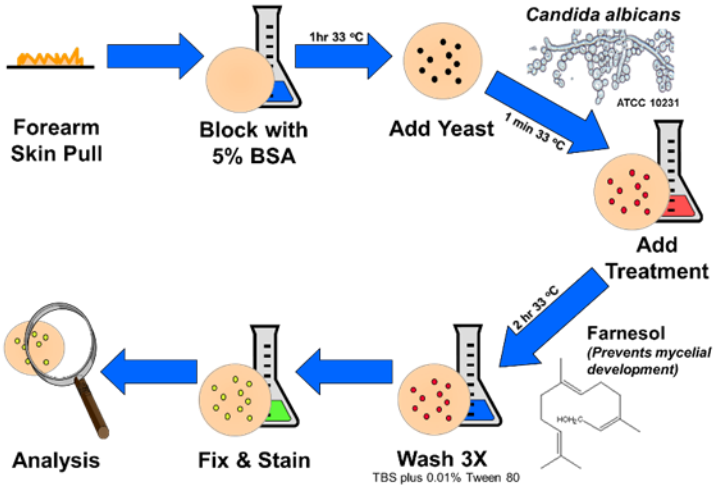
Tape Strip Skin Pull
- Four pulls of D-Squame® disc (CuDerm Corporation, Dallas, TX)
- Samples from adjacent adult volar forearm sites

Cyanoacrylate Skin Pull
- Cyanoacrylate glue (Pacer Technology, Rancho Cucamonga, CA) smeared on glass slide
- Placed on adult volar forearm





SKIN BIOFILM FORMATION

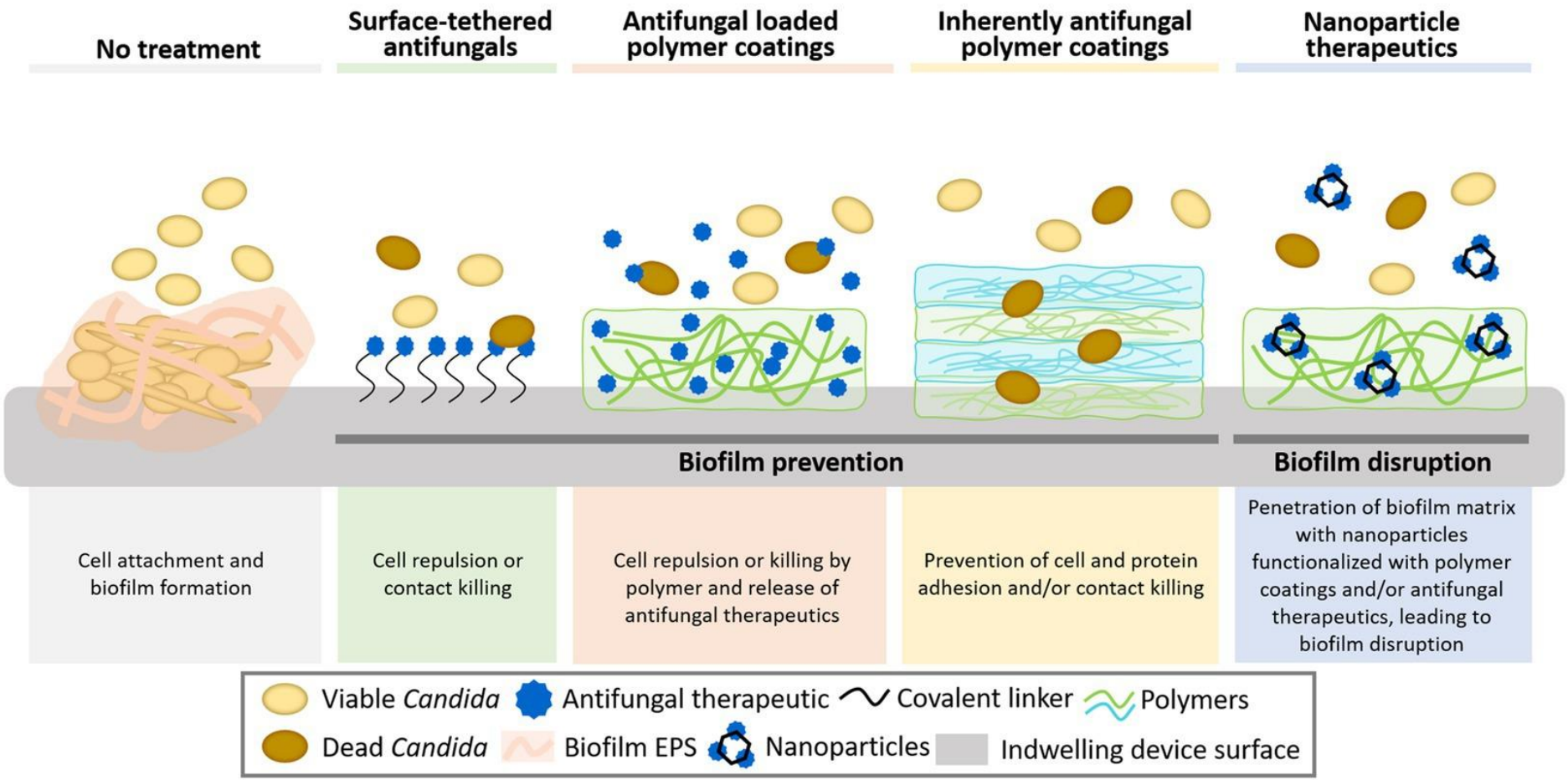


BIOFILM FORMATION AND CONTROL

	D-Squame® Tape Skin Pull		Cyanoacrylate Skin Pull	
	Control	Farnesol	Control	Farnesol
Total Yeast Count (90 Views)	19251	6963	15322	955
% Inhibition of Attachment		64		94

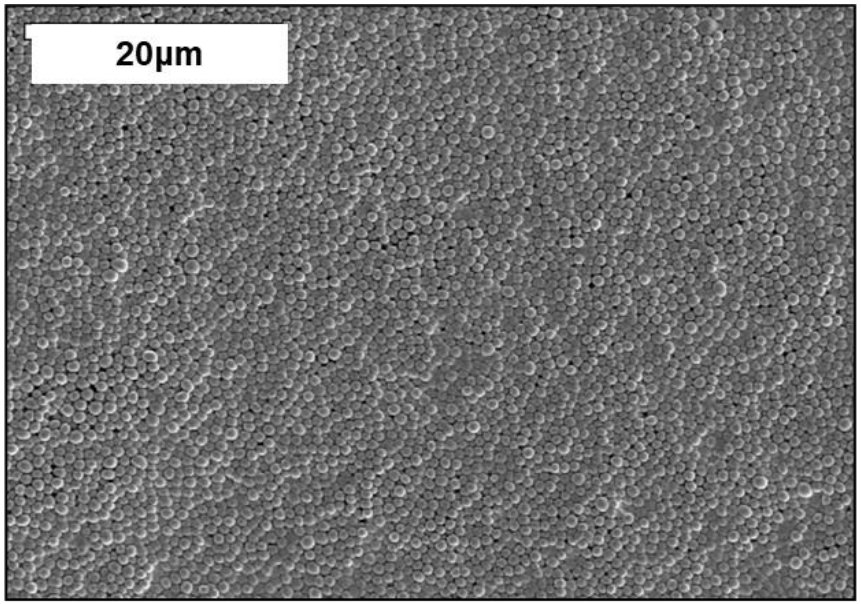
Skin Sample and Treatment	Statistical Grouping (p<0.01)			
D-Squame® Tape - No Treatment	A			
Cyanoacrylate Pull - No Treatment		B		
D-Squame® Tape - Farnesol Treatment			C	
Cyanoacrylate Pull - Farnesol Treatment				D

Anti-Adherent & Anti-Microbial Coatings

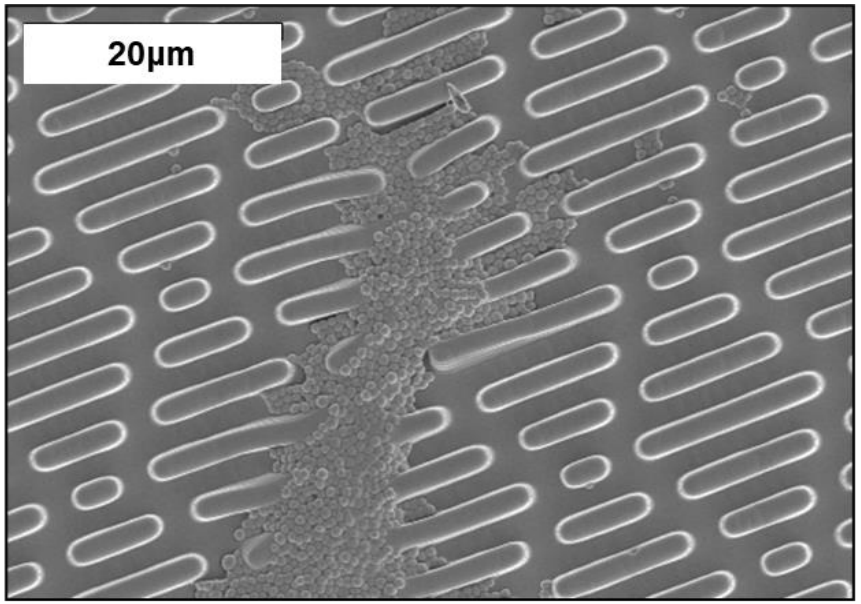


Vera-González, N. and Shukla, A., 2020. Advances in biomaterials for the prevention and disruption of Candida biofilms. Frontiers in microbiology, 11, p.538602.

Surface Patterning



DAY 21, Smooth (2000x)



DAY 21, Sharklet AF™ (2000x)

- Mature biofilm colonies dispersed over surface

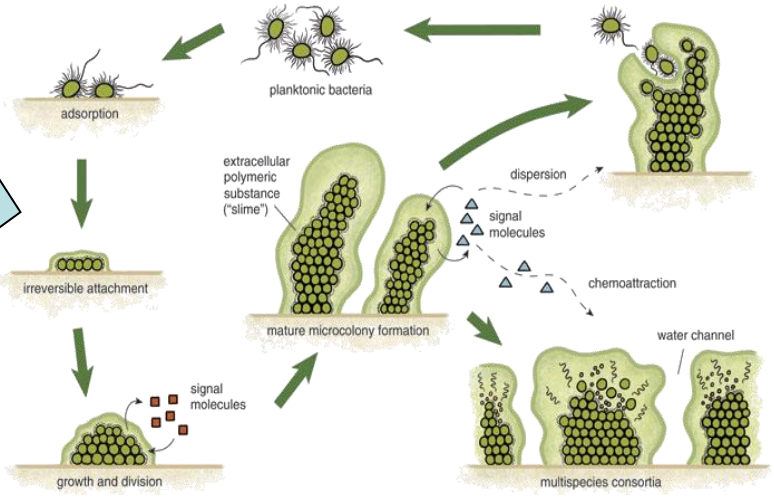
- Areas surrounding colonies are virtually devoid of bacterial adhesion.

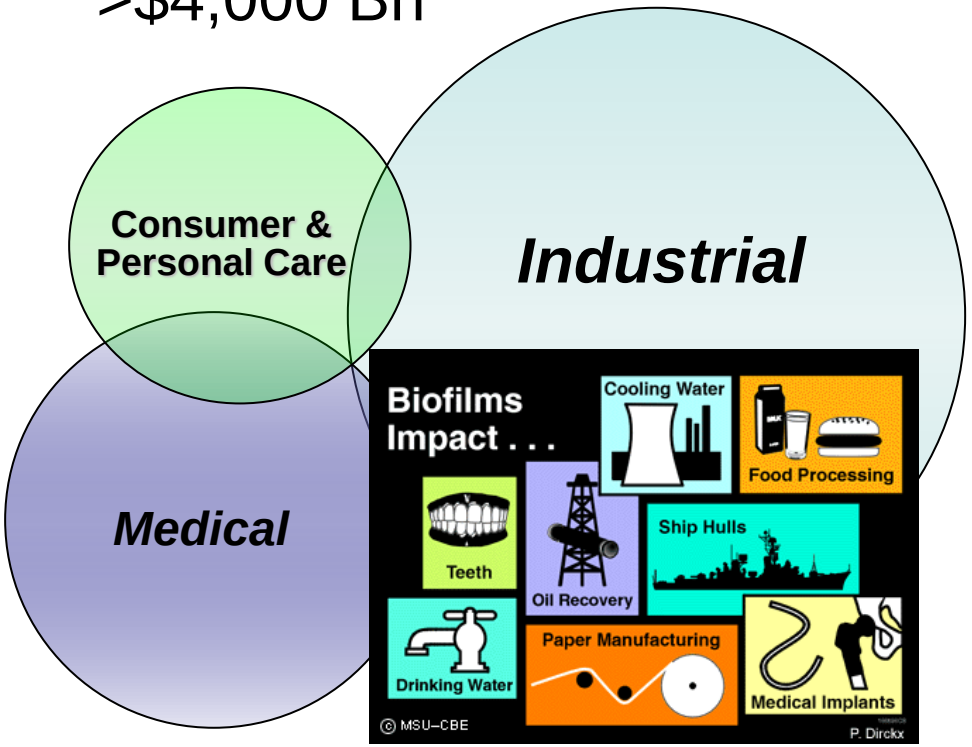
Technology: Micron-scale (Sharklet) patterns on plastic surfaces have demonstrated ability to block attachment of marine organisms and bacteria

Summary

Biofilms

- Virtually all microbes grow on any “wet” or “dry” surface
- Biofilms are highly resistant to eradication
- Global impact of Biofilms >$4,000 Bn





Biofilm Control Strategy

Mechanical/Energy

- Microfiber wiping
- Ultrasonic & Bio-acoustics to alter surface state
- Biosurfactants

Disinfection

- Oxidizing biocides (Look for EPA Claim)

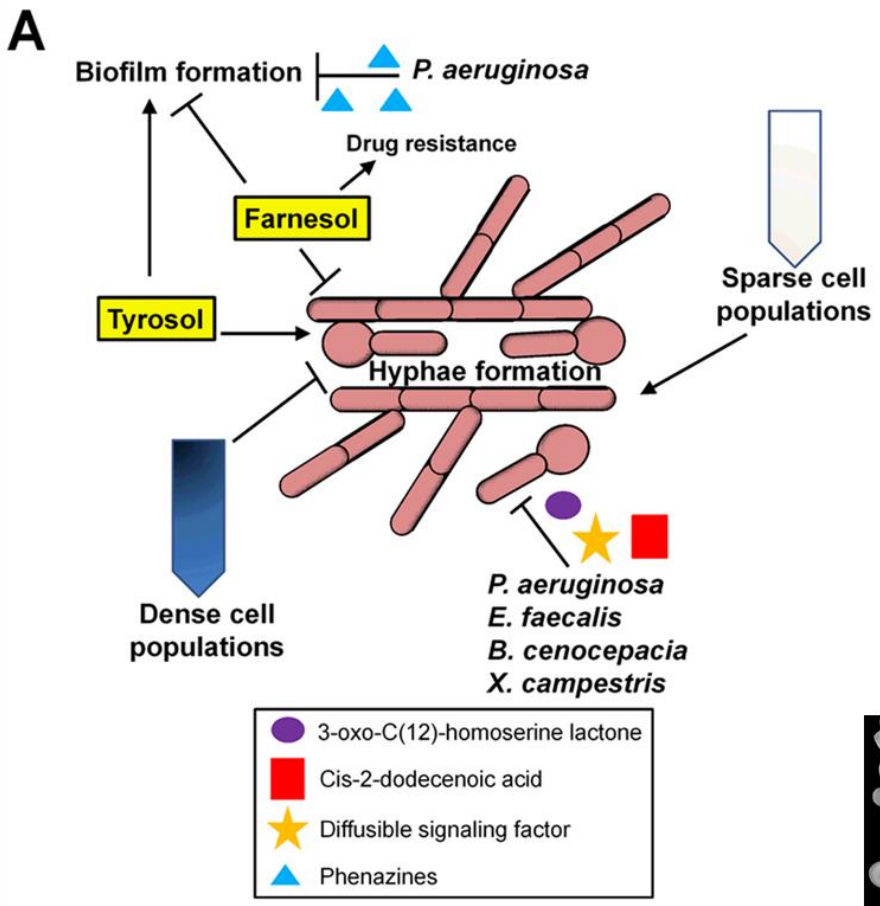
Polymer Coatings

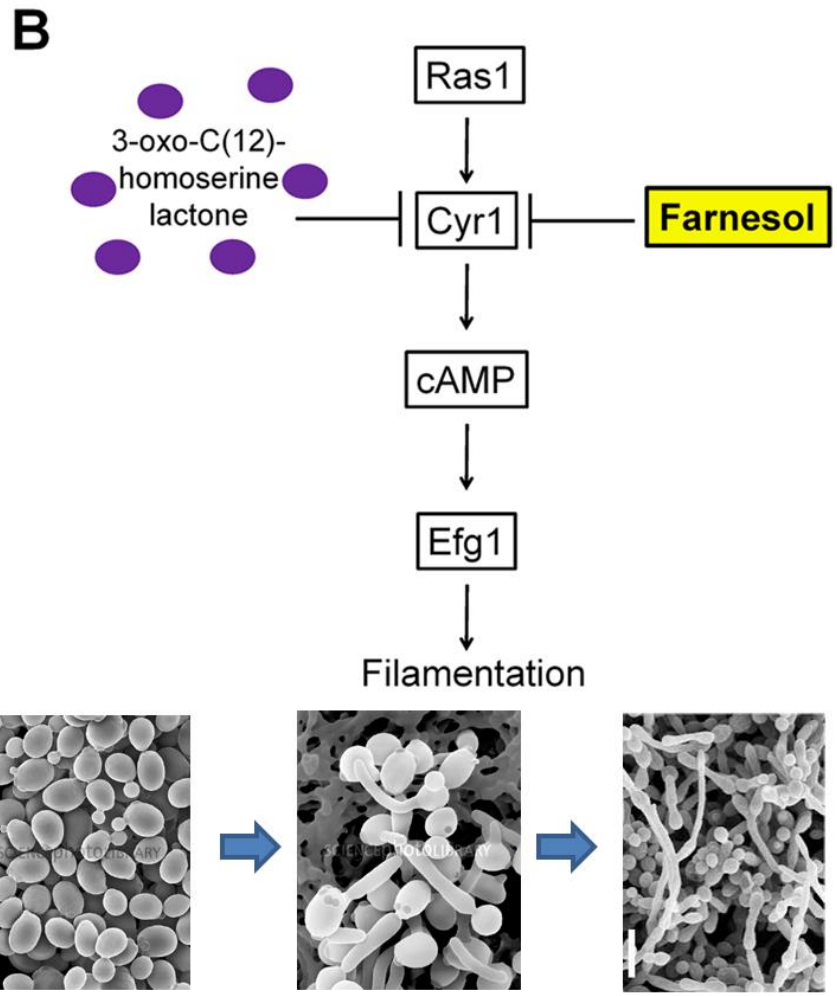
- Biocompatible surfaces that block adhesion
- Controlled release of antibiotics/biocides
- Anti-infectives that interfere with bacterial metabolism (QS inhibitors)

Surface Patterning

- Nano-topography to limit bacterial adhesion and growth

Candida Quorum Sensing





Mallick EM, Bennett RJ (2013) Sensing of the Microbial Neighborhood by *Candida albicans*. PLoS Pathog 9(10): e1003661.

http://www.sciencephoto.com/