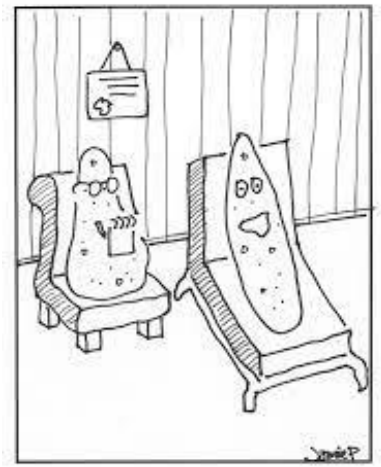
Biofilms

- **NACMID Annual Conference**
  - September 23, 2024
  - James T. Griffith Ph.D., CLS (NCA)
    - Chancellor Professor Emeritus
  - University of Massachusetts
    - Managing Partner
  - Forensic DNA Associates, LLC

Learning Objectives

- 1) Describe the conditions and process for a typical biofilm formation
- 2) Discuss the current and future importance of biofilms in clinical medicine
- 3) Describe how medical biofilms are typically identified
- 4) Describe a current approach to biofilm treatment



I just can't go with the flow anymore.
I've been thinking about joining a biofilm.

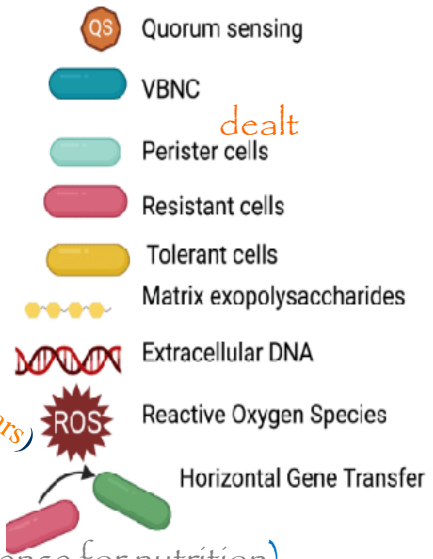
Laboratory Profession

FDA

Forensic DNA Associates

Protection Exposed

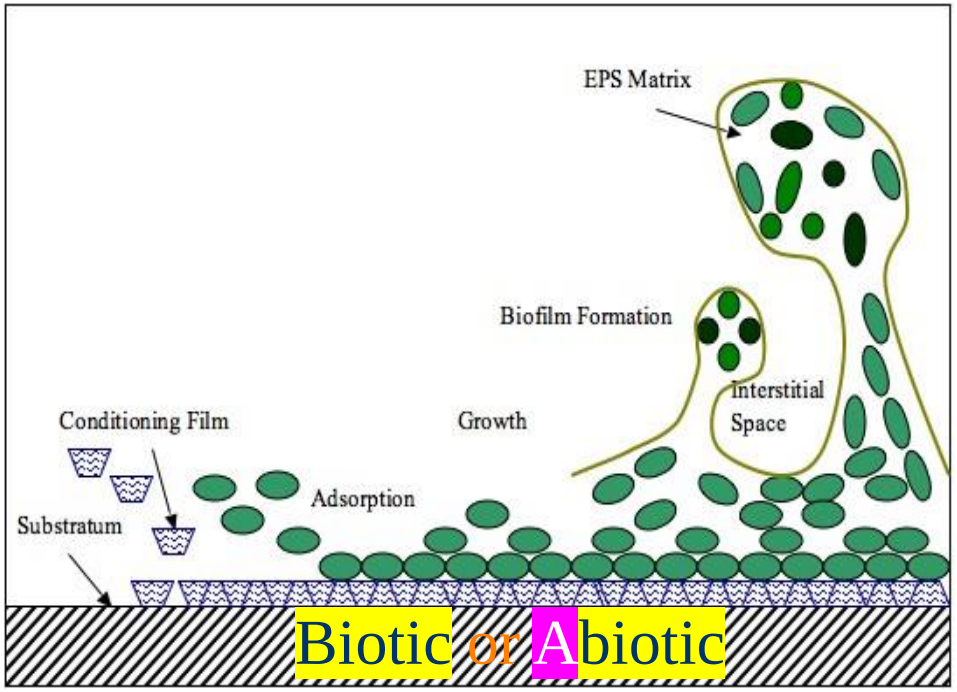
- Definition = Structured communities of microbial species embedded in a biopolymer matrix on a biotic or abiotic substrata
  - May be one or several organisms
  - Sessile organisms:
    - attached
  - Planktonic organisms:
    - free living -- most of what we have dealt with traditionally
- First observed by Anton van Leeuwenhoek
  - 1684 (Animacules in "scurf" on teeth)
  - Sticky layer resisted vinegar cleaning
- First scientific study = 1943 (Zobell) (300 Years)
- Term "Biofilm" from Bill Costerton (1978)
- Benefits of Biofilm Formation to Bacteria
  - Adherence to hospitable locale
  - Syntrophic metabolism (Mutual dependence for nutrition)
  - Horizontal gene transfer
  - Disease reservoir



Source: Rita Chandki, Priyank Banthia, and Ruchi Banthia; Biofilms: A microbial home; J Indian Soc Periodontol. 2011 Apr-Jun; 15(2): 111–11

Protection Exposed

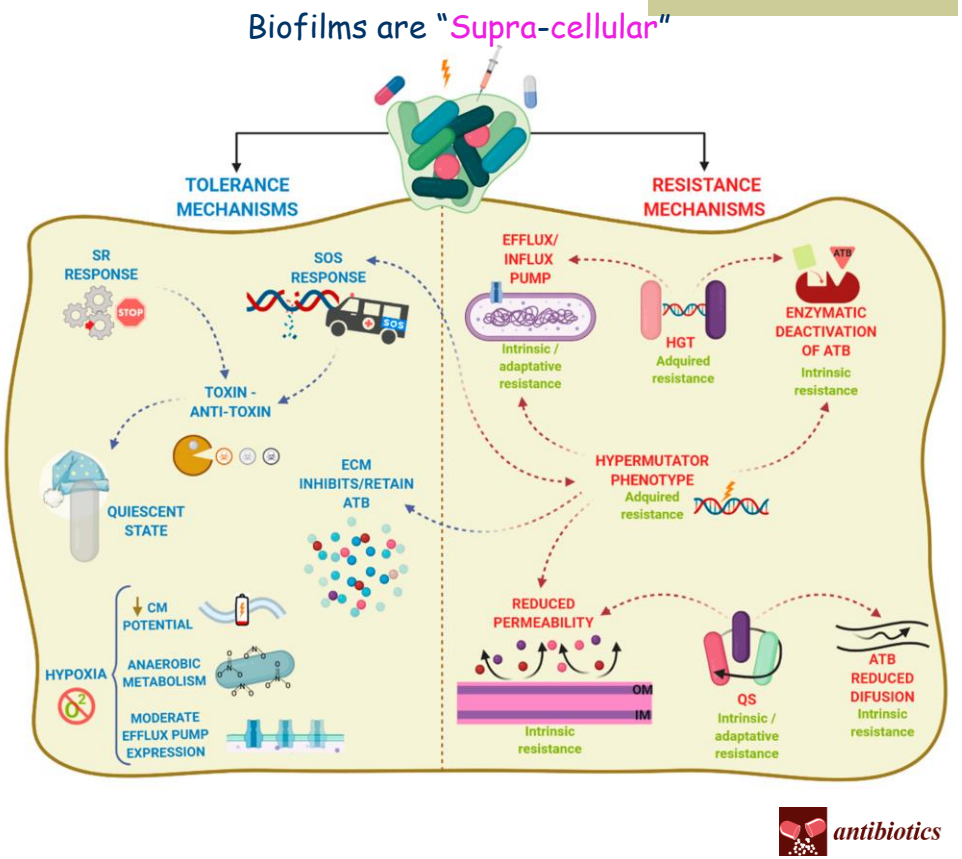
- Biotic substrata
  - Humans (any tissue) can serve this role
- Abiotic substrata
  - Any "implanted' material can be a biofilm host
- Either way, biofilms are generally resistant to;
  - Biocides
  - Antimicrobial chemotherapy
  - Humoral defenses
    - Antibodies, etc.
  - Cellular defenses
    - Phagocytosis, etc.



Source: Zeuko'O Menkem,E.; **The Mechanisms of Bacterial Biofilm Inhibition and Eradication: The Search for Alternative Antibiofilm Agents;** 5/24/22 In "Focus on Bacterial Biofilms"

Protection Exposed

- Planktonic bacterial cells are susceptible to antibiotics, antibodies, and phagocytes
- Biofilm (sessile) cells are highly protected from all antibacterial agents, not foolproof, but quite good
- Multidrug tolerance
- Resistance to both opsonization and phagocytosis
- If the 'attached surface' is 'immuno-inert', or (in a living organism) immunocompromised, a biofilm will survive even better
- Biofilms exhibit altruism and cooperation
  - So do Ants
  - Do humans ?



antibiotics

Source: Uruen,C., Chopo-Escuin,G., Tommassen, J., et.al.; **Biofilms as Promoters of Bacterial Antibiotic Resistance and Tolerance,** *Antibiotics* **2021**, *10*(1), 3

Protection Exposed

- Common and famous biofilms
  - This way of being a microorganism, did NOT evolve to cause us problems, or mess us up
  - It likely was a response to a hostile environment
  - Probably pretty old
    - 3+ Billion y.o.

Source: Rather,M., Gupta,K., and Mandal, M.; ***Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies***; Braz J Microbiol. 2021 Dec; 52(4): 1701–1718

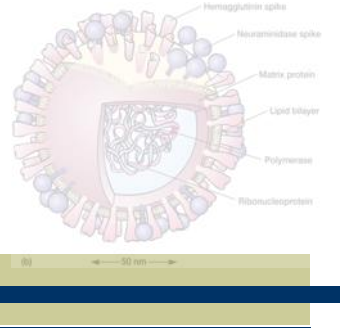
Protection Exposed

- Ubiquitous in nature
  - Occur anywhere sufficient moisture and a surface come together
    - solid/liquid interfaces
    - moist/soft
    - tissue/air
    - liquid/air
    - Yellowstone N.P.
    - Lemonade Creek

Rhodophyta spp. (Red Algae) plus giant viruses (1,000s of species)

Source: Pennisi, E; **Giant viruses played a key role in early life, study in Yellowstone hot spring suggests DNA analyses reveal viruses have infected red algae—and spurred evolution—for at least 1.5 billion years;** 9 Apr 2024, ***Science***

Protection Exposed



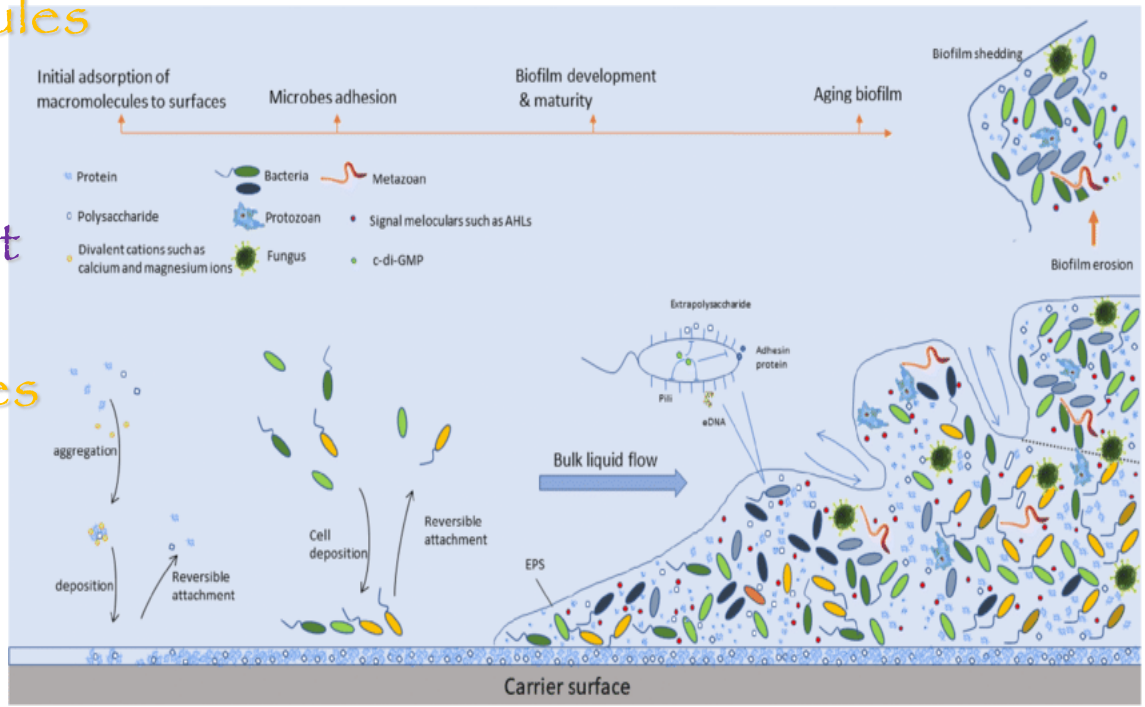
- <1% of the world microorganisms are cultible
- We think we have a handle on how many microorganisms there are, but what we know is only a guess

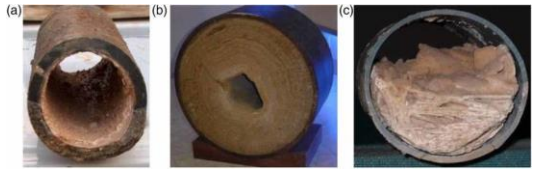
Habitat	Cultured (%)
Seawater	0.001-0.1
Freshwater	0.25
Mesotrophic Lakes	0.1-1.0
Estuarine Waters	0.1-3.0
Activated Sludge	1-15
Sediments	0.25
Soil	0.3
Univ. of Vienna	# based on direct cell counts

Source: World Health Organization; Threats of Biofilms (3/29/23)

Environmental Biofilms

- Legume root nodules
- Termite, ruminant digestion
- Sewage treatment bioreactors
- Contact lens cases
- Dental units
- Water Pipes





Source: World Health Organization; Threats of Biofilms (3/29/23)

ID Biofilms

- Dental plaque
- Endocarditis
- Urinary catheter Implants

Cystic Fibrosis

Otitis media

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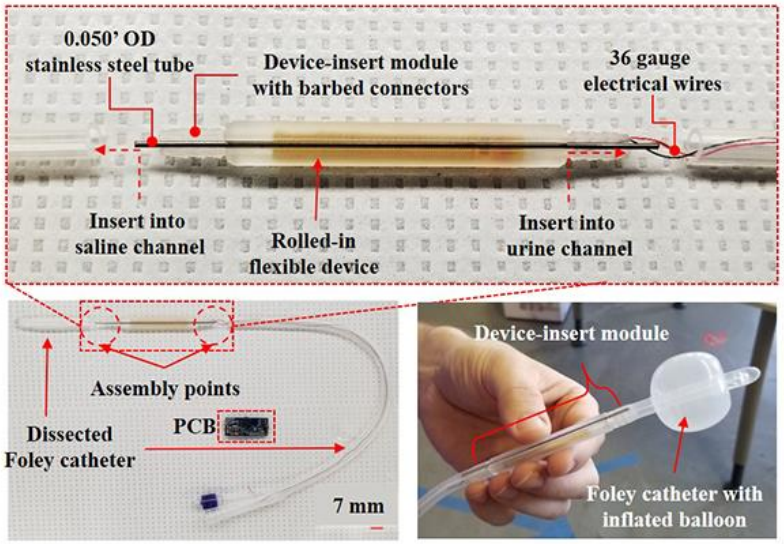
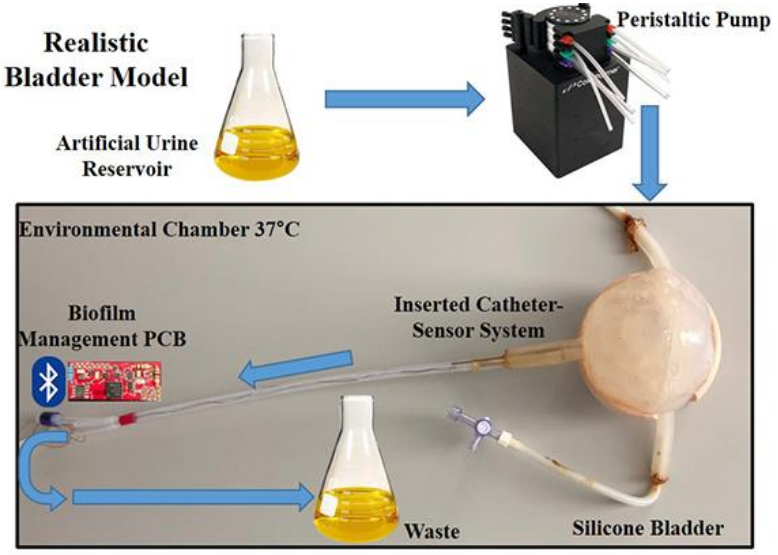
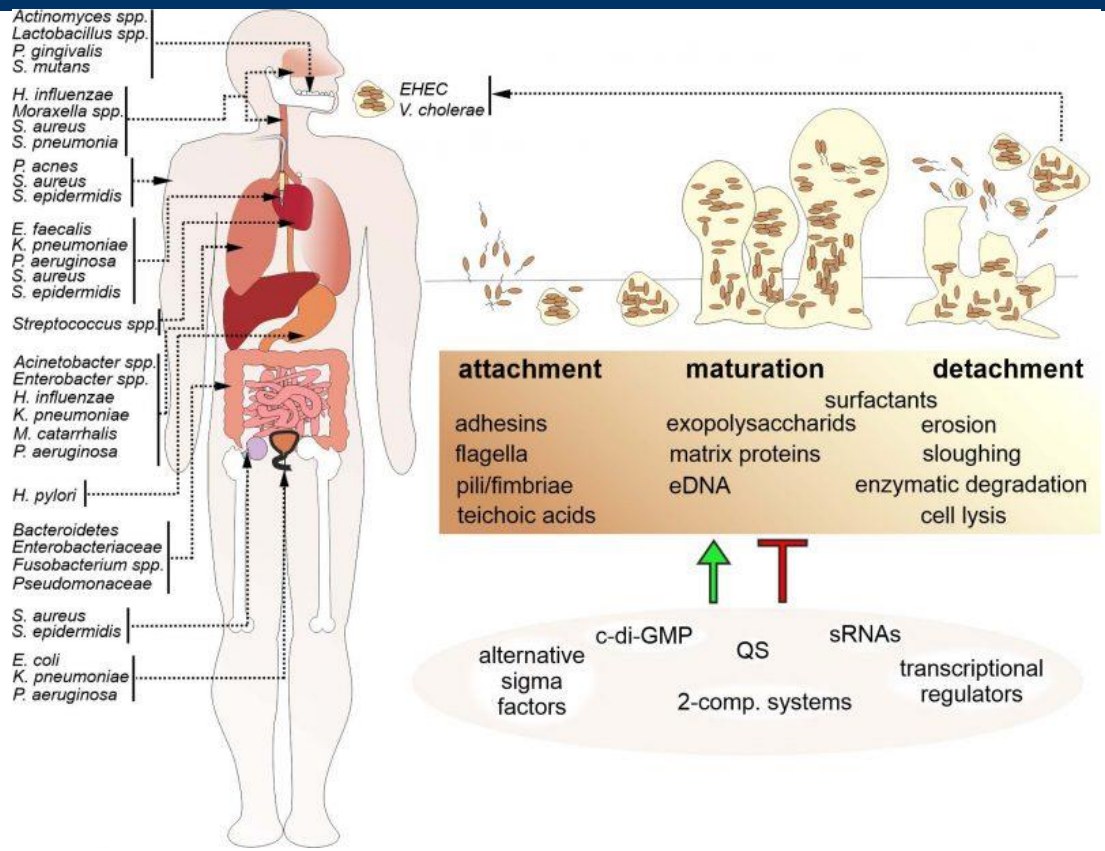




Source: University of Maryland; **Biofilm-fighting system for urinary catheters proves effective in simulated environment; April 2, 2021**

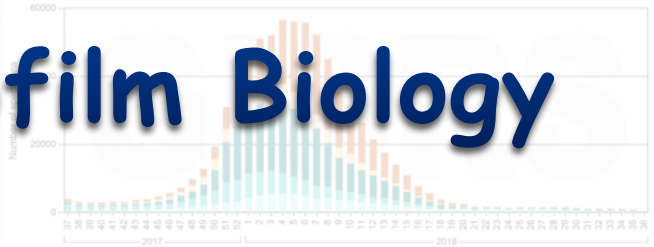
Range of Microorganisms

- Typical microorganisms associated with HUMAN biofilms
- *Sth. aureus*
- *Sth. epidermidis*
- *Ps. aeruginosa*



Source: Adina Schulze, Fabian Mitterer, Joao P. Pombo, et.al ; Biofilms by bacterial human pathogens: Clinical relevance – development, composition and regulation – therapeutical strategies; Microbial Cell, Vol. 8, No. 2, pp. 28 - 56; doi: 10.15698/mic2021.02.741

Biofilm Biology

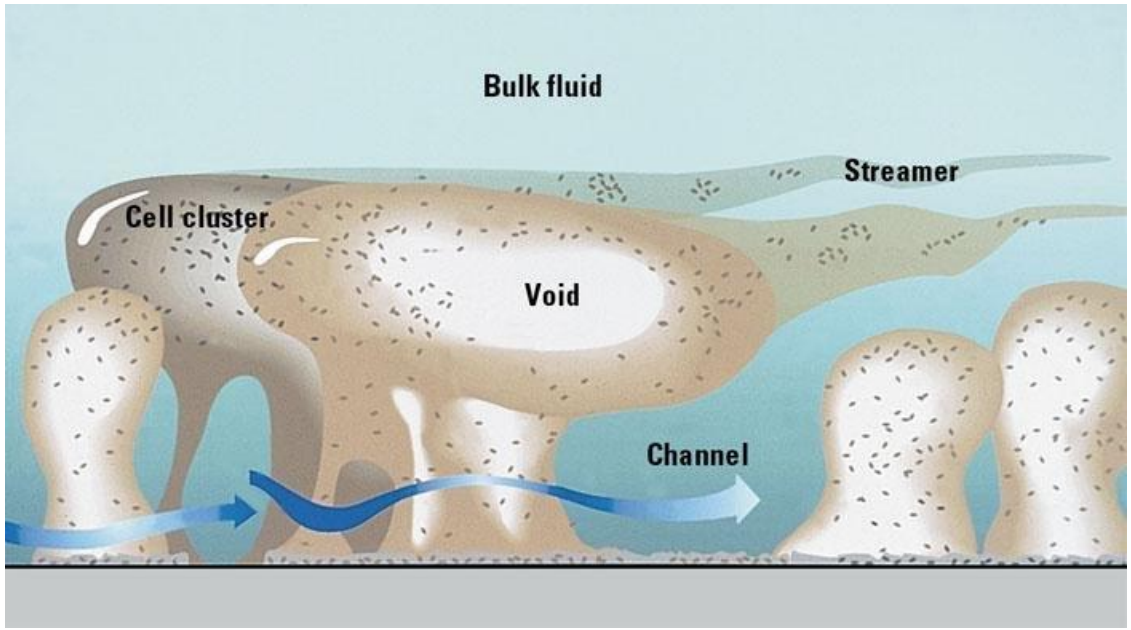


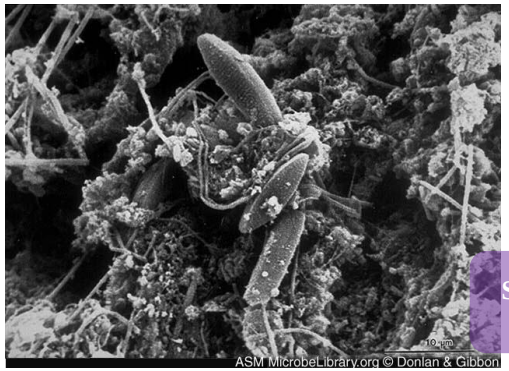
Bacterial strain	Gram stain	Types of infections
Staphylococcus aureus	Gram-positive	Chronic biofilm infections: chronic wound infection, right valve endocarditis, lung infections in patients with cystic fibrosis
Staphylococcus epidermidis	Gram-positive	Endocarditis: catheter-related infection, joint prosthesis infection
Streptococcus pneumoniae	Gram-positive	Lung infections, bacterial meningitis, acute or chronic otitis
Listeria monocytogenes	Gram-positive	Co-culture interactions with *Pseudomonas*, *Vibrio* strains, listeriosis, contamination of food products
Burkholderia cepacia	Gram-negative	Opportunistic infections in patients with blood cancer
Escherichia coli	Gram-negative	Hemolytic uremic syndrome, acute diarrheic syndrome, urinary tract infections
Klebsiella pneumoniae	Gram-negative	Bacteremia, liver abscess, urinary tract infections
Pseudomonas putida	Gram-negative	Urinary tract infection
Pseudomonas aeruginosa	Gram-negative	Osteomyelitis, ventilator-associated pneumonia, lung infections in patients with cystic fibrosis, opportunistic infections in neutropenic patients, nosocomial infections.
Pseudomonas fluorescens	Gram-negative	Bioremediation, biocontrol- *Pythium*, *Fusarium*, antimicrobial properties –
Rhizobium leguminosarum	Gram-negative	Biocontrol properties – *Pythium*
Lactobacillus plantarum	Gram-positive	Salmonella infection
Lactococcus lactis	Gram-positive	Gastrointestinal tract infections

Source: Zeuko'O Menkem,E.; **The Mechanisms of Bacterial Biofilm Inhibition and Eradication: The Search for Alternative Antibiofilm Agents;** 5/24/22 In "**Focus on Bacterial Biofilms**"

Biofilm Architecture

- The overall architecture has protective layers
  - Dilution
  - Seeding
  - Anchoring





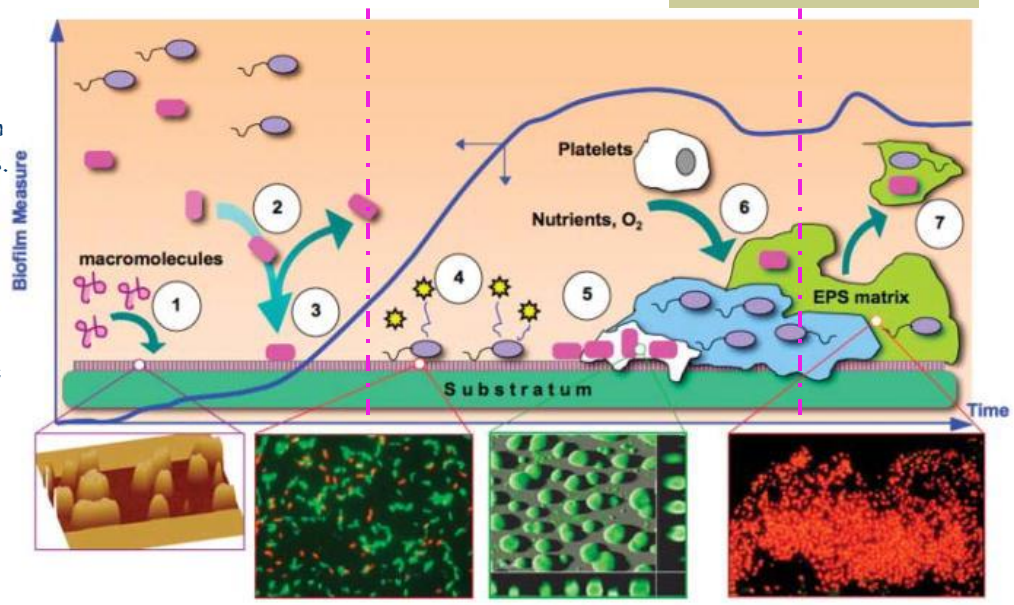
SEM native biofilm, mild steel surface @ 8 weeks, Industrial water system

SpringerLink

Source: De Beer ,D., Stoodley,P.; ***Microbial Biofilms***, in; **The Prokaryotes** pp 904–937, 2006, Springer-Link

Biofilm Formation

- **Steps;**
- ① Substratum
- ② Macromolecule deposition at the substratum by a combination of many transport mechanisms. Cells may leave the surface or be permanently attached
- ③ Then, they cause quorum signals
- ④ Results in up-regulation of various genes (many related to virulence) on a community-wide basis
- ⑤ Attached cells secrete copious polymers, biofilm continues to accumulate
- ⑥ Accumulating biofilm consumes ambient nutrients, electron donors and acceptors, and attracts other bacterial species or mammalian cells.
- ⑦ Shear stress, cell signaling = detachment, sloughing off (Planktonic), to move downstream (in CVS = thromboembolism)



Processes governing biofilm formation;
Blue line shows time course of net accumulation of biofilm on an initially clean substratum

Source: courtesy G. McFeters and ASM.org image library

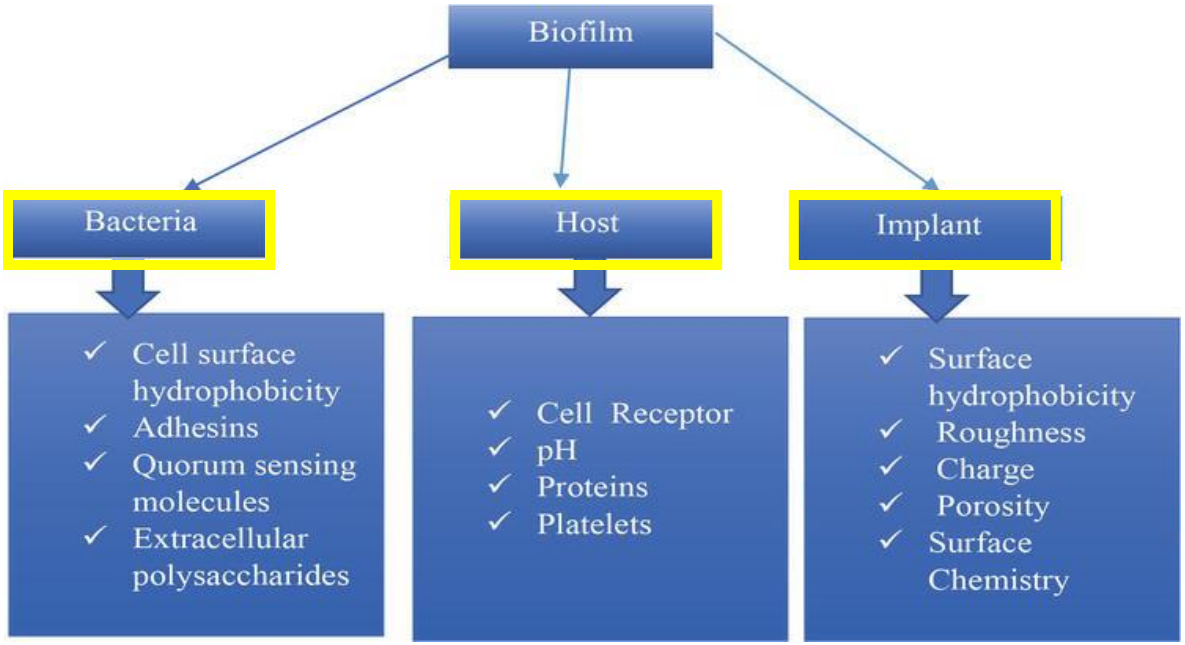
Biofilm Biology

- Formative process is a "recruiting" event much like the construction of a coral reef
- Quite developmental over time
- LOTS of holes

Source: Coral Reef Ecosystems (2/1/2019)

Biofilm Biology

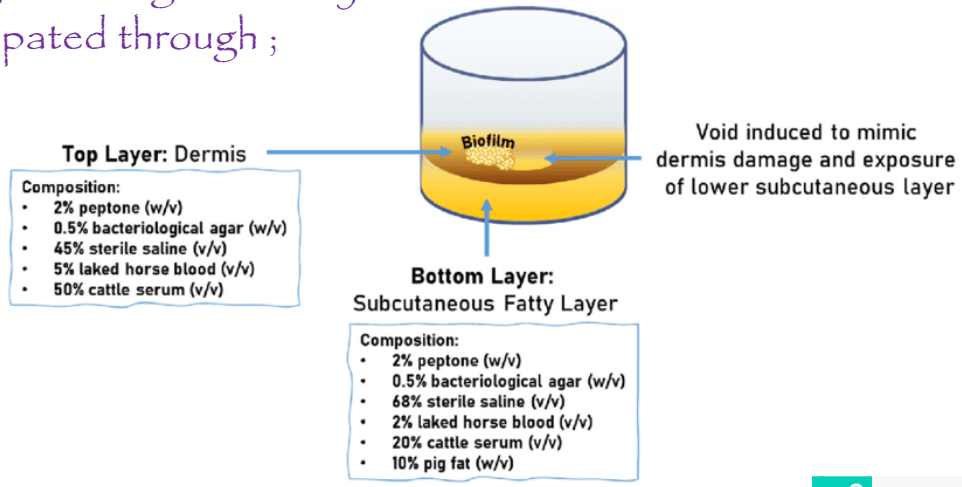
- Each player contributes specific enablers in medical biofilms



Source: Threats of Biofilms, WHO; 2/17/2020

Biofilm Biology

- Biofilms:
  - Act as a Hydrogel (Extremely hydrated polymer gel)
  - Exhibit Viscoelastic properties
    - Elastic (solid) and viscus (liquid-like)
  - With these consequences
    - Seconds - absorbs shear by behaving elastically
    - Hours / days - shear is dissipated through ;
      - Viscus flow
        - No detachment
      - Streamlined
        - Reduces drag



Source: Vyas,H., Xia,B., and Mai-Prochnow,A.; ***Clinically relevant in vitro biofilm models: A need to mimic and recapitulate the host environment***, Jan. 2022

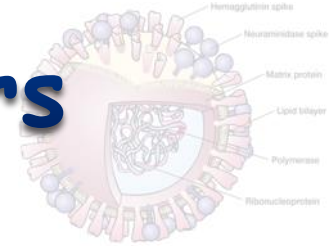
Attachment and Formation Variables

Properties of the substratum	Properties of the bulk fluid	Properties of the cell
Texture or roughness Hydrophobicity Conditioning film	Flow velocity pH Temperature Cations Presence	Cell surface hydrophobicity Fimbriae Flagella Extracellular polymeric substances of antimicrobial agents

Source: Ghazay,F., Alotaaibi,G., and Bukhari,M.; ***Factors Influencing Bacterial Biofilm Formation and Development***, Research Gate, 2020

Establishment Factors





Species Colonization Efficiency

Anti-infective/Hostile Forces

Genotypic Factors

Physio-Chemical Environment - (Eh)

Cyclic Stage

Biofilm Community Structure and Function

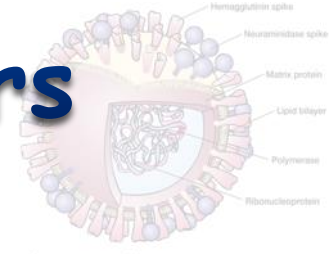
Mechanical Factors/Shear Force

Substratum/Abiotic Surface

Nutrient Energy/Resource

Source: Zeuko'O Menkem,E.; **The Mechanisms of Bacterial Biofilm Inhibition and Eradication: The Search for Alternative Antibiofilm Agents;** 5/24/22 In "**Focus on Bacterial Biofilms**"

Establishment Factors

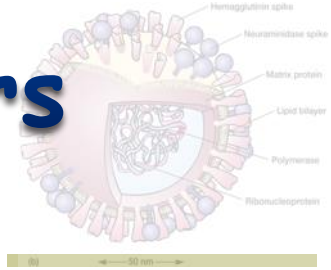


Some common microorganisms associated with HUMAN biofilms

stage in biofilm formation	bacterial pathogen		
	V. cholerae	***P. aeruginosa***	***S. aureus/ epidermidis***
attachment	flagella motility, type IV pili, adhesins and chitin-binding factors (e.g. GbpA, ChiRP, FrhA, CraA)	flagella/ twiching motility, type IV pili, Cup fimbrial adhesins and lectins	hydrophobic surface, teichoic acids, adhesins (e.g. Atl, Bap, MSCRAMMs, SERAMs)
maturation	exopolysaccharide (VPS), eDNA, proteinaceous factors (RbmA, RbmC, Bap1), lipids	exopolysaccharide (alginate, Psl, Pel), eDNA, proteinaceous factors (e.g. CdrA, LecA/B), rhamnolipids	exopolysaccharide (PIA), eDNA, proteinaceous factors [e.g. SasG, Aap, and other adhesins (see above)], teichoic acids
detachment	nucleases (Dns and Xds), proteases, predicted sugar lyase (RbmD)	Alginate lyase, rhamnolipids, cell lysis	exoproteases (e.g. SspA/ Esp, SspN/ SepA, SplA-F, ScpA)

Source: Adina Schulze, Fabian Mitterer, Joao P. Pombo, et.al ; Biofilms by bacterial human pathogens: Clinical relevance – development, composition and regulation – therapeutical strategies; Microbial Cell, Vol. 8, No. 2, pp. 28 - 56; doi: 10.15698/mic2021.02.741

Establishment Factors

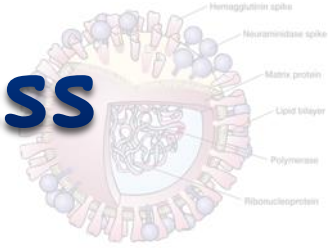


- **Some other Examples**

Components	Percentage of matrix	Functions in biofilm
Microbial cells	2–5%	Cohesion of the structure
DNA and RNA	<1–2%	Nutrient source Exchange of genetic information
Polysaccharides	1–2%	Cohesion of the structure Nutrient source Water retention Protective barrier Absorption of organic compounds and inorganic ions
Structural Proteins	<1–2%	Cohesion of the structure Nutrient source Protective barrier Absorption of organic compounds and inorganic ions Electron donor and acceptor
Enzymes	<1–2%	Enzymatic activity Nutrient Source
Lipids and biosurfactants	<1–2%	Nutrient source
Water	Up to 97%	Lubricates the environment, simple circulatory system distributing nutrients to microcolonies

Source: Zeuko'O Menkem,E.; **The Mechanisms of Bacterial Biofilm Inhibition and Eradication: The Search for Alternative Antibiofilm Agents;** 5/24/22 In "Focus on Bacterial Biofilms"





Medical Biofilm Process

- **Step 1.**
- Surface conditioning[SEP]
  - The first substances associated with the surface are not bacteria but trace organics. Almost immediately after any device comes into contact with any part of the human body, an organic layer deposits on the water/solid interface (Mittelman 1985)
  - These organics are said to form a "conditioning layer" which neutralizes excessive surface charge and surface free energy which may prevent a bacteria cell from approaching near enough to initiate attachment. In addition, the adsorbed organic molecules often serve as a nutrient source for bacteria

frontiers in Microbiology

Source: Bhagwat,G., O'Connor,W., Grainge,I, et.al.; ***Understanding the Fundamental Basis for Biofilm Formation on Plastic Surfaces: Role of Conditioning Films***; Front. Microbiol., 24 June 2021, Sec. Microbial Physiology and Metabolism;Volume 12 - 2021

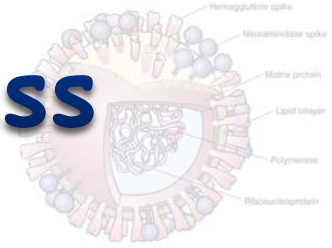
Medical Biofilm Process

- **Surface conditioning**
- Adsorption of organic molecules on a clean surface forms a conditioning film
  - (Characklis 1990)

Step. #	Components	Percentage (%)
1	Microbial cells	2–5
2	Water	Up to 97%
3	Polysaccharides	1–2
4	Proteins	< 1–2 (including enzymes)
5	DNA and RNA	< 1–2

Source: Rather,M., Gupta,K., and Mandal, M.; ***Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies***; Braz J Microbiol. 2021 Dec; 52(4): 1701–1718

Medical Biofilm Process

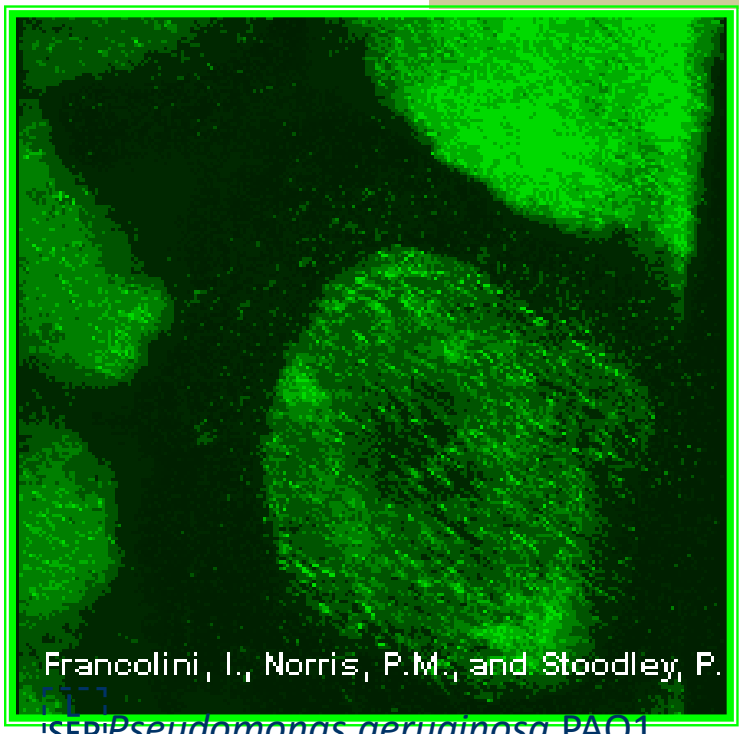


- **Step 2.**
- Adhesion of "pioneer" bacteria SEP
  - After the planktonic (free-floating) bacteria "reversably" (polar) attach and become entrained within the "boundary layer"
    - A quiescent zone forms where *flow velocity falls to zero*
  - Some of these cells will strike and adsorb to the surface for some finite time, and then desorb
    - = reversible adsorption
    - This attachment is based on:
      - electrostatic attraction
      - physical forces
      - not chemical attachments
  - What follows is "irreversible" attachment, which leads to "microcolonies"
    - Converts naïve planktonic cells (bacteria that exhibit low concentration of c-di-GMP and have not encountered surfaces initially) to surface sentient planktonic cells (bacteria that exhibit a high concentration of c-di-GMP and have encountered surfaces initially)

Source: Rather,M., Gupta,K., and Mandal, M.; ***Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies***; Braz J Microbiol. 2021 Dec; 52(4): 1701–1718

Medical Biofilm Process

- **Step 3.**
- Glycocalyx Formation
- Biofilm bacteria excrete extracellular polymeric substances
  - = sticky polymers
  - hold the biofilm together
  - cement it to the device wall
  - trap scarce nutrients
  - protect bacteria from biocides
  - EPS(Extracellular Polymer Substrate)

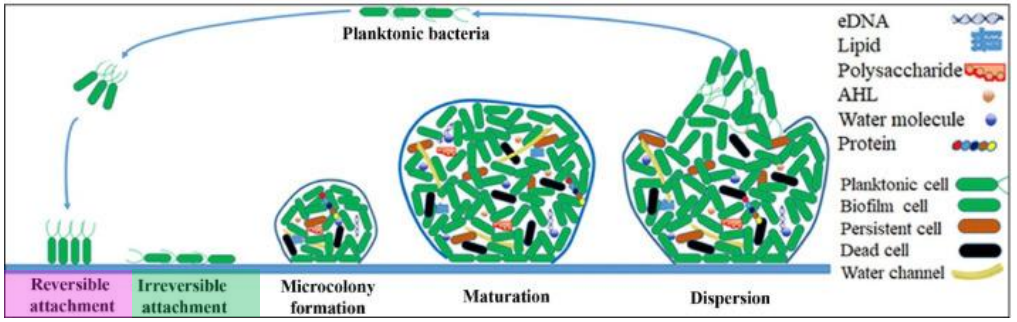


Pseudomonas aeruginosa PAO1 (pMF230) biofilm grown on untreated polyurethane

Source: Rather,M., Gupta,K., and Mandal, M.; ***Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies***; Braz J Microbiol. 2021 Dec; 52(4): 1701–1718

Medical Biofilm Process

- Glycocalyx
  - All bacteria secrete some sort of "Glycocalyx", this is used to aid in the formation of the protective "bubble" of a biofilm
- Complex exopolysaccharides
- Adhesion
  - By Planktonic bacteria
  - Pil-Chp surface-sensing system present on microbial surfaces
- Protection from
  - Biocides
  - Antibiotics
  - Bacteriophage
  - free-living amoebae
  - WBC

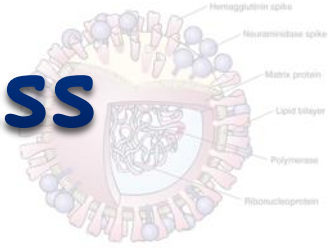


- Reversable adhesion initially
- Then "flat-lying" (non-Polar)
  - Bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is an intracellular signaling molecule that plays a vital role in early events of biofilm formation by restricting flagella-mediated swimming motility

Source: Rather,M., Gupta,K., and Mandal, M.; ***Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies***; Braz J Microbiol. 2021 Dec; 52(4): 1701–1718

Medical Biofilm Process



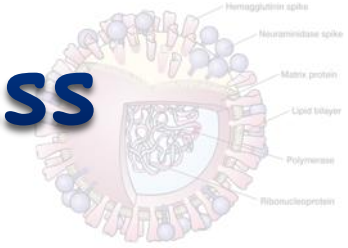


- EPS (Self-produced)
  - Greatly assists in the microcolony formation, aided by;
    - High concentration of c-di-GMP
    - Flagella and type IV pili-mediated motilities
      - Important for interactions between;
        - Microorganisms
        - Surfaces
        - cell–cell aggregations
- EPS is crucial in;
  - Biofilm maturation
  - Stabilizing the 3-D structure
  - Grouping cells together
  - Protecting from various stresses (host immune system response)
  - Antimicrobial protection
  - Oxidative damage

Source: Rather,M., Gupta,K., and Mandal, M.; ***Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies***; Braz J Microbiol. 2021 Dec; 52(4): 1701–1718

Medical Biofilm Process





- **Step 4.**
- **Climax** Biofilm
  - Reaches **optimal size**
  - External organisms become **planktonic**, leave to colonize elsewhere
  - Cells nearest the surface become **quiescent** or die due to limited O_2 and nutrients, increased waste

Microbial cells	2–5 %
Water	**Up to 97 %**
Polysaccharides	1–2 %
Proteins	< 1–2 % (including enzymes)
DNA and RNA	< 1–2 %

- **Dispersion**
  - Climax biofilm Actively ruptures;
    - motility and EPS degradation-dependent dispersion)
  - OR, Passively
    - physical factors
      - liquid flow-dependent dispersion to disperse the microorganisms
      - Starts a new cycle of biofilm formation
    - Some other factors;
      - outgrown population
      - intense competition
      - lack of nutrients etc.

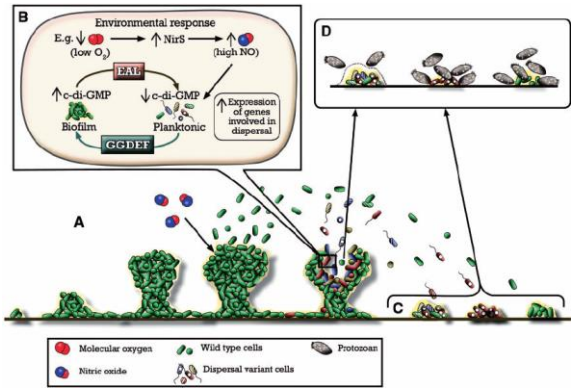
Source: Rather,M., Gupta,K., and Mandal, M.; ***Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies***; Braz J Microbiol. 2021 Dec; 52(4): 1701–1718

Biofilm Communication

- Quorum sensing
  - Cells secrete molecules
  - Nearby cells sense population density
  - Regulates biofilm architecture
  - Regulates gene expression
  - Horizontal gene transfer increases 10-600x
  - Conjugation
  - Transfection
  - Transduction

Quorum-sensing systems

- Acyl-homoserine lactone quorum sensing system (AHL) in Gram-negative bacteria,
- Autoinducing peptide (AIP) quorum sensing system in Gram-positive bacteria
- Autoinducer-2 (AI-2) system in both gram-neg. & positive

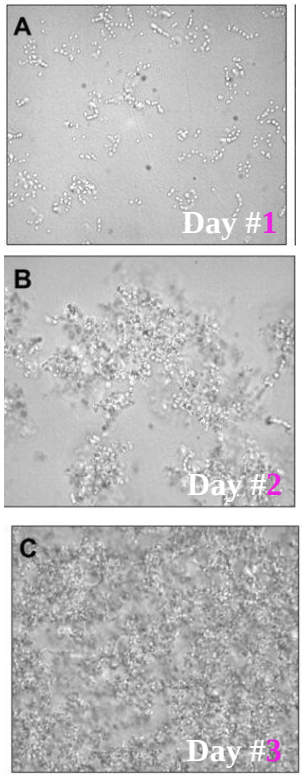


Source: Zeuko'O Menkem,E.; **The Mechanisms of Bacterial Biofilm Inhibition and Eradication: The Search for Alternative Antibiofilm Agents;** 5/24/22 In "Focus on Bacterial Biofilms"

Biofilm Communication

- Stp. pneumoniae reside in biofilms on the mucous membranes of the oral cavity and nasopharynx
- Communicate with each other by secreting strain specific peptide pheromones
  - monitor the density of the strain population
  - when a critical number of cells are present they decide to exchange DNA
  - A subfraction of the cells lyse and release DNA (the donors), whereas the majority of the cells (the recipients) become competent for natural transformation and take up the DNA released by the donors

Streptococcus pneumoniae biofilm formation under continuous flow Phase-contrast micrographs at a magnification of x1,000

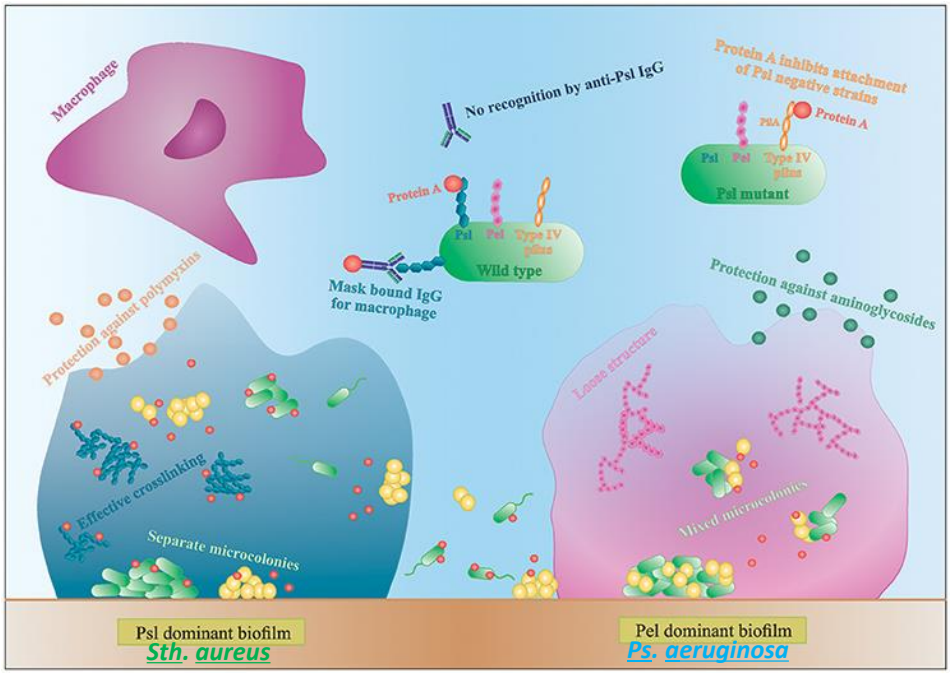


AMERICAN SOCIETY FOR MICROBIOLOGY

•**Source**: Allegrucci,M., Hu,F.Z., and Sauer,K.; **Phenotypic Characterization of Streptococcus pneumoniae Biofilm Development**; April 2006
•Journal of Bacteriology188(7):2325-35

Biofilm Genetics

- Change in gene expression of attached cells
- Increased production of glycocalyx
- Change in energy metabolism
  - Pseud. aeruginosa changes expression of 40 genes
  - Sth. aureus increases expression of genes for glycolysis, fermentation (low O_2)
- Increased antimicrobial resistance



Differences in biofilm formation by; *Sth. aureus* with a Psl- dominant, vs. *Ps. aeruginosa* Pel-dominant strain
IgG, immunoglobulin G

frontiers in Microbiology

Source: Hotterbeekx, A., Kumar-Singh, S., Goossens, H., et.al.:, *In vivo* and *In vitro* Interactions between *Pseudomonas aeruginosa* and *Staphylococcus* spp., Front. Cell. Infect. Microbiol., 03 April 2017.

Impact on Healthcare

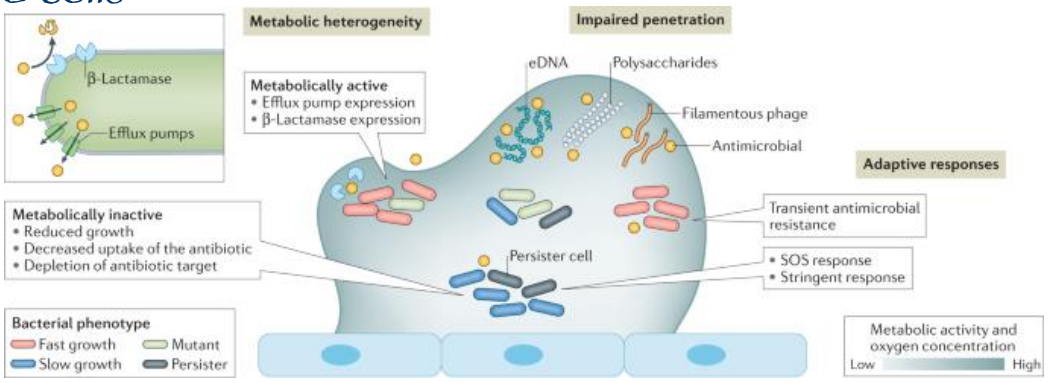
- In American hospitals alone, healthcare-associated infections (i.e., nosocomial infections) account for an estimated 1.7 million infections and 99,000 associated deaths / Yr.
- 32 % of all healthcare-associated infection = UTI
- 22 % = Surgical site infections
- 15 % = Pneumonia
- 14 % = CVS infections
- The European Centre for Disease Prevention and Control (ECDC) (2007) said that every year some 3 million people in European Union countries catch an infectious disease associated with healthcare and that around 50,000 die as a result

- Nosocomial (hospital acquired) infections are the *fourth leading cause of death in the U.S.* with 2 million cases annually (or ~10% of American hospital patients) leading to more than $5 billion in added medical cost per annum
- About 60–70% of nosocomial infections are associated with some type of implanted medical device
- It is estimated that over 5 million medical devices or implants are used / yr. in the U.S. alone

Source: Monina Klevens R, editor. Centers for Disease Control and Prevention Public Health Reports. ***Healthcare-associated infections and deaths in U.S. Hospitals.*** Vol. 1 2007. European Centre for Disease Prevention and Control. ***Annual epidemiological report on communicable diseases in Europe.*** Stockholm; Sweden: 2007.

Biofilm Biology

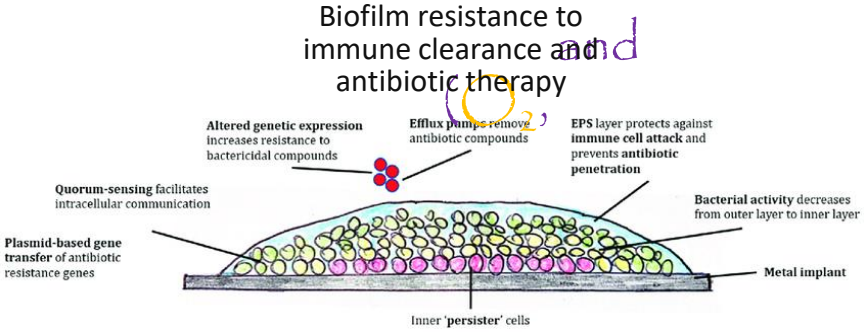
- 10^3-10^4x ↑ doses of antimicrobials needed to kill biofilm (sessile organisms) compared with planktonic organisms
- Biofilm is a molecular filter
- Low metabolism of attached cells
- Reduces drug activity



Source: Castaneda,P., McLaren,A., Tavaziva,G, et.al.; *Biofilm Antimicrobial Susceptibility Increases With Antimicrobial Exposure Time*; Clin Orthop Relat Res. 2016 Jul; 474(7): 1659–1664

Resistance to Immune System Clearance

- Phagocytosis (macrophages, PMNs)
  - Surface binding
  - Engulfed
  - Killed with digestive enzymes reactive oxygen molecules (H_2O_2, NO)
- Vaccinated rabbit to bacteria
  - Increased antibody levels
  - No increase in phagocytosis
- Biofilm E. coli are less likely to be killed by human PMN in vitro
  - Resistant to active oxygen species produced by PMNs



Source: Steadman,W., Chapman,P., Schuetz,M., et.al.; Local Antibiotic Delivery Options in Prosthetic Joint Infection, Research Gate, Apr., 2023

Some Medically Important Biofilms

- CDC estimate:
  - 65% of human bacterial infections involve biofilms
    - Dental plaque
      - Biofilm made visible on teeth after chewing
      - "Dental Plaque Disclosing Agent" tablet
    - Infectious kidney stones
    - Endocarditis
    - Catheters
    - Cystic fibrosis

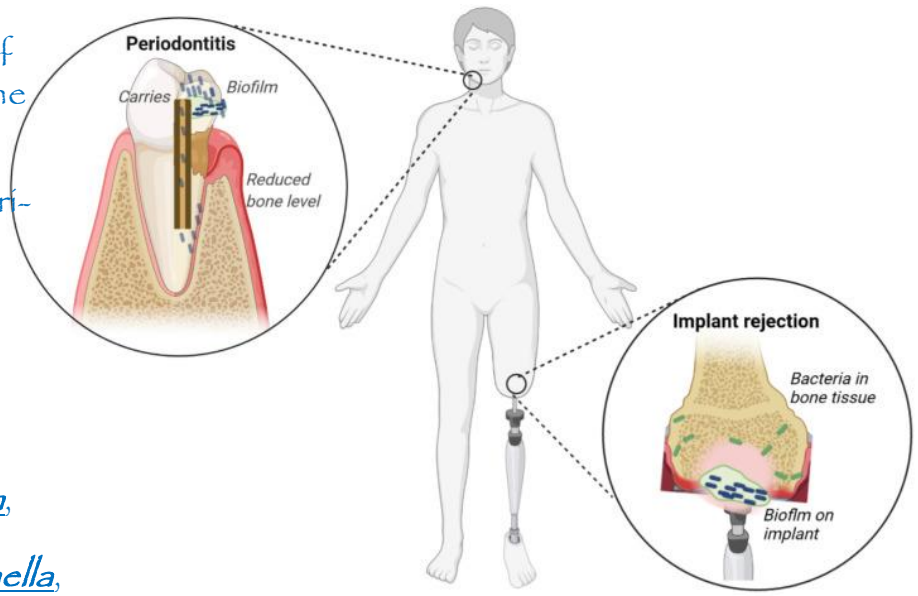
Before Tablet

Biofilm Reveal

Source: **Guided Biofilm Therapy (GBT)**, 2021

Some Medically Important Biofilms

- In pulpitis (dental)
  - = infection is localized in the root canal of the tooth and does not pass into the bone
- In apical periodontitis
  - = inflammation and the destruction of peri-radicular tissues (e.g., root cementum, periodontal ligament, and alveolar bone) eventually leading to total pulp necrosis
- Likely causative organisms;
  - *Streptococcus mutans*, Actinomyces, *Lactobacillus*, *Dialister*, *Eubacterium*, *Olsenella*, *Bifidobacterium*, *Atopobium*, *Propionibacterium*, *Scardovia*, *Abiotrophia*, *Selenomonas*, and *Veillonella*, including carbohydrate-fermenting oral streptococci

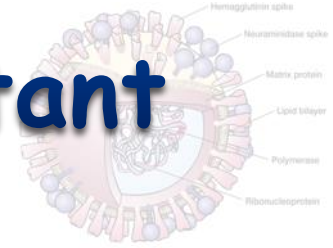


~ 500,000 types of medical implants are available on the global market

Source: Krukiewicz, K., Kazek-Kesik,A., Brzychczy, M.; *Recent Advances in the Control of Clinically Important Biofilms*; Int J Mol Sci. 2022 Sep; 23(17): 9526

Some Medically Important Biofilms





- Orthopedic implant-related infections have been linked to;
  - Joint arthroplasty (joint replacement) failure
  - Osteosynthesis (bone repair) failure
- Main pathogens causing these infections include;
  - *Staphylococcus aureus*
  - Coagulase-negative staphylococci (e.g., *Staphylococcus epidermidis*),
  - *Cutibacterium acnes*
- Offending organisms use **distinct mechanisms** to attach to the implants, form biofilms, persist, and avoid a host's defenses
- The resulting biofilms are not only localized on the prosthetic, but can also spread to the synovial fluid, fibrous tissue, bone cement, and the bone itself
  - Note: *Sth. aureus* and *Staphylococcus lugdunensis* can invade osteoblasts
  - Secretion of staphylococcal superantigen-like proteins 3 and 4 allows *Sth. aureus* to circumvent recognition by the host's toll-like receptor 2

Source: Krukiewicz, K., Kazek-Kesik,A., Brzychczy, M.; ***Recent Advances in the Control of Clinically Important Biofilms***; Int J Mol Sci. 2022 Sep; 23(17): 9526

Some Medically Important Biofilms

- Scanning Electron Micrograph of tarter on a tooth
- The mixture of bacteria, saliva and carbohydrates is known as plaque It is a cause of tooth decay (caries) and can also lead to the formation of tartar (pink) (calculus)

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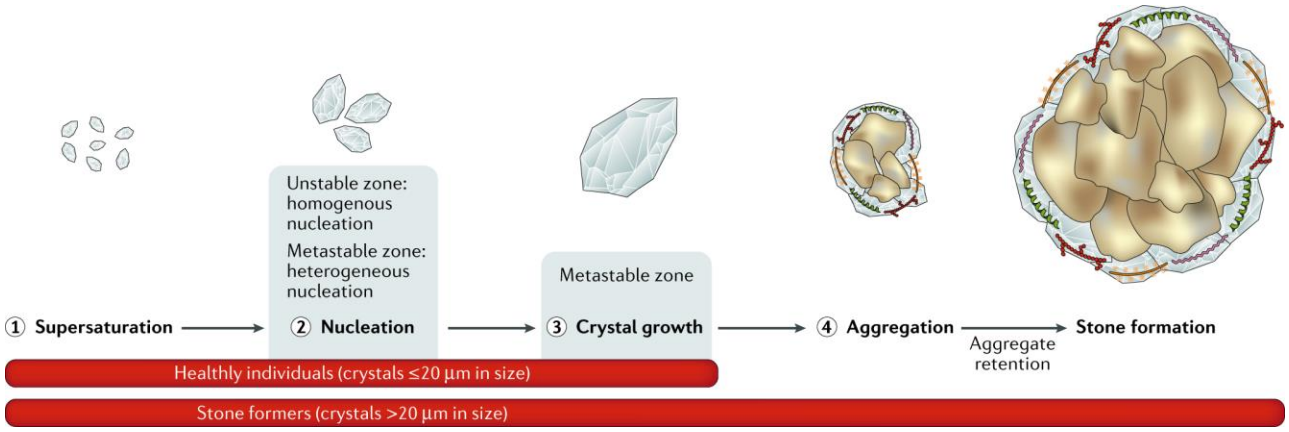
Source: STEVE GSCHMEISSNER / SCIENCE PHOTO LIBRARY; 38.6 x 34.3 cm · 15.2 x 13.5 in (300dpi), 2024

Some Medically Important Biofilms

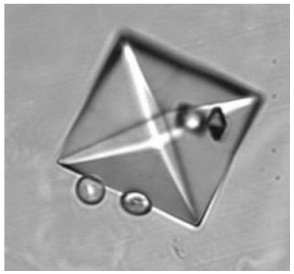
- Infectious Kidney Stones
  - 15-20% involve UTIs
  - Bacterium --> biofilm --> Mineralization
  - Causative organisms have Urease
    - Urea → $NH_4 + H_2CO_3$
  - Biofilm **concentrates** Urease → Crystal formation

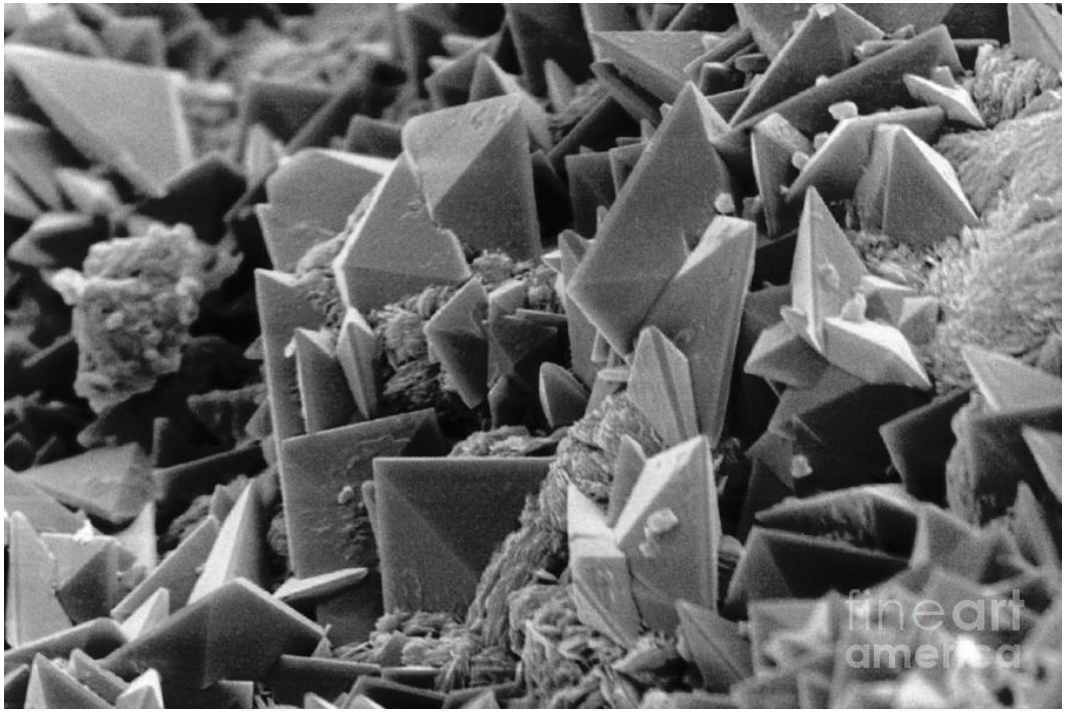


Source: Espinosa-Ortiz, E., Eisner,B., Lange,D., et.al.; ***Current Insights Into The Mechanisms and Management of Infectious Stones***, Nature Reviews Urology, 11/18

Some Medically Important Biofilms

- Calcium Oxalate + bacterial biofilm
- Infectious kidney stone
- You may be used to seeing them like this;





Source: THOMAS DEERINCK, NCMIR / SCIENCE PHOTO LIBRARY ; Kidney Stone SEM, 40.9 x 30.7 cm · 16.1 x 12.1 in (300dpi), 2024

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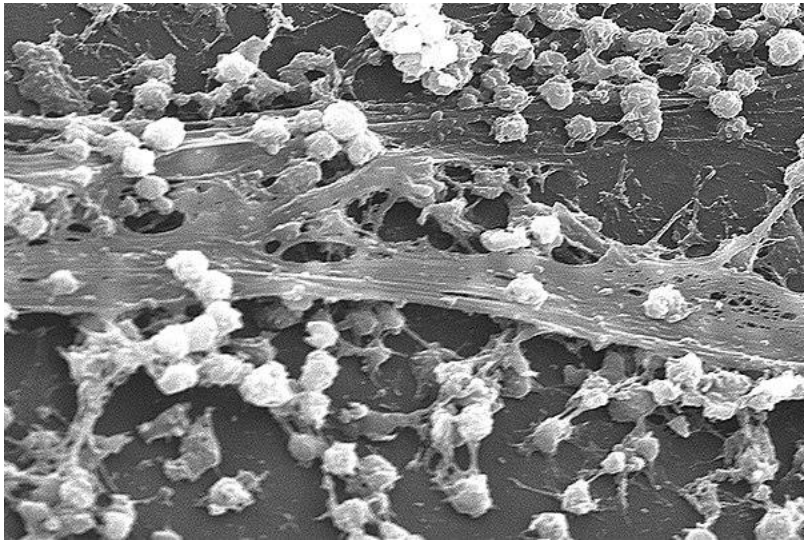
Some Medically Important Biofilms

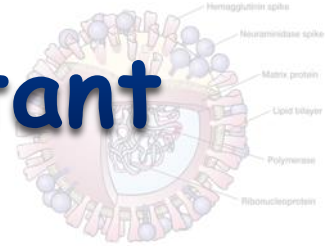
- Endocarditis
  - Biofilm of bacteria + host components on valve = vegetation
  - Requires prior valve injury
  - 200x increase in antibiotic resistance
- Rabbit model:
  - block biofilm formation --> acute virulent infection



Source: Research Gate, 2021, Indwelling Cardiac Catheter

Some Medically Important Biofilms





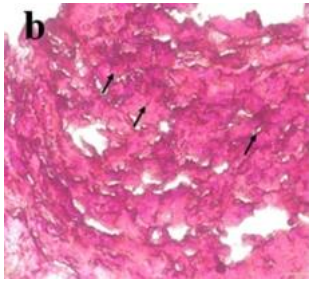
- Cystic Fibrosis
  - Mutation in chloride channel in epithelial cells
  - 1st stage:
    - Intermittent infections
  - 2nd stage:
    - Permanent infection with *Pseudomonas aeruginosa*
    - Mucoid type - overproduce alginate
    - Antibiotic resistance

Source: Research Gate, 2021, Indwelling Cardiac Catheter

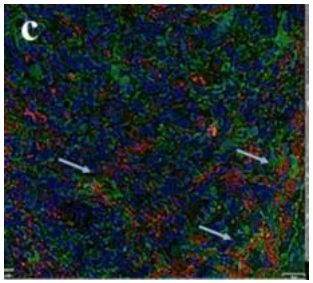
A Medically Curious Biofilm

- Cutaneous Leishmaniasis
  - More than half of all CL wounds are colonized with biofilms;
    - *Ps. areuginosa*
    - Other Enterobacteriaceae

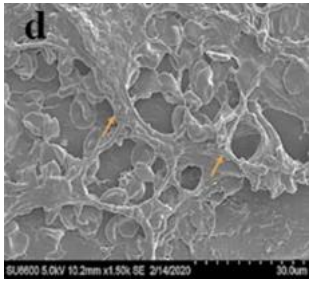
Wet Ulcer



Gram stain, the extra-polymeric substances (EPS) is stained in pinkish orange with the Safranin dye



Fluorescence in situ hybridization—bacteria in red due to Cyanine 3-tagged Eu-bacterial rRNA probe, EPS in green due to Concanavalin A-conjugated Alexa Fluor 488 and tissue nuclei in blue due to DAPI staining



SEM Biofilm

Source: Kaluarachchi, T.D., Campbell,P., Wickremasinghe, R, et.al.;', ***Possible clinical implications and future directions of managing bacterial biofilms in cutaneous leishmaniasis wounds***; *Tropical Medicine and Health* **volume 50**, Article number: 58 (2022)

Some Medically Important Biofilms

- Microorganisms commonly associated with biofilms on indwelling medical devices;
- CVC
- Artificial hip/voice
- Urinary Catheter

Microorganism	Has been isolated from biofilms on
Candida albicans	Artificial voice prosthesis Central venous catheter Intrauterine device
Coagulase-negative staphylococci	Artificial hip prosthesis Artificial voice prosthesis Central venous catheter Intrauterine device Prosthetic heart valve Urinary catheter
Enterococcus spp.	Artificial hip prosthesis Central venous catheter Intrauterine device Prosthetic heart valve Urinary catheter
Klebsiella pneumoniae	Central venous catheter Urinary catheter
Pseudomonas aeruginosa	Artificial hip prosthesis Central venous catheter Urinary catheter
Staphylococcus aureus	Artificial hip prosthesis Central venous catheter Intrauterine device Prosthetic heart valve

Source: Research Gate, 2021, Indwelling Cardiac Catheter

Some Medically Important Biofilms

CHARACTERISTIC	CYSTIC FIBROSIS	PERIODONTITIS	CENTRAL VENOUS CATHETER INFECTION	CHRONIC WOUNDS
Form preferentially on foreign bodies, dead or damaged tissue	The genetic defect in the chloride ion channel predisposes the lung to infection	The tooth surface is not as well-defended as are vascularized tissues	Indwelling plastic and metal surfaces are very vulnerable to microbial colonization	Necrotic tissue could provide nidus for biofilm formation
Slow to develop	Persistent infection takes years to establish	Typically manifests gradually, later in life	Symptoms may take weeks to manifest	Symptoms such as pain, exudate and size wax and wane over weeks to months
Respond poorly or only temporarily to antimicrobials	Lung is never cleared of bacteria despite aggressive chemotherapy	Tetracycline, antiseptic mouthwashes have little efficacy	Preferred therapy is removal of the infected catheter	Marginal response to antibiotics; may deteriorate when antibiotics are stopped
Collateral damage to neighboring healthy tissue	Massive neutrophil invasion contributes to gradual loss of lung function	Host responses and bacterial virulence factors lead to progressive bone loss; teeth fall out	Infection may disseminate to blood and other locations in body	Normal healing process of cell differentiation and migration is arrested

Source: DiDomenico,E., Oliva,A., and Guembe,M.; **The Current Knowledge on the Pathogenesis of Tissue and Medical Device-Related Biofilm Infections;** *Microorganisms* **2022**, *10*(7), 1259

Medical Biofilm Process

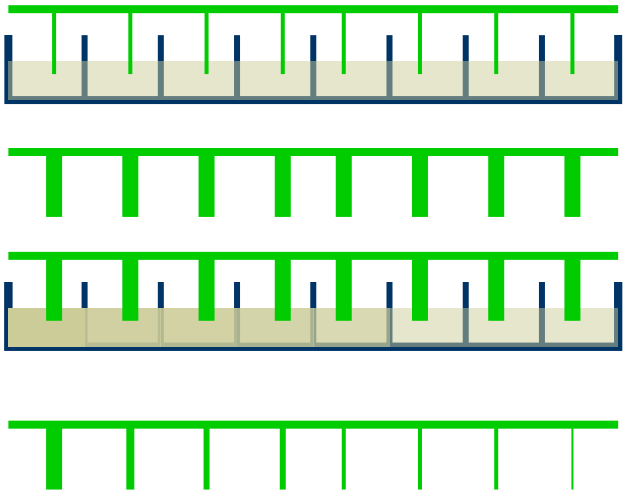
- + Blood Cultures =
  - Failed organism
    - Was trying to live somewhere and couldn't
  - Planktonic organism seeking new focal point
  - Ratio of P^P:P^{BF} may determine success or failure in treating some patients

Note: P^P = Planktonic,
P^{BF} = Biofilm planktonic

Source: DiDomenico,E., Oliva,A., and Guembe,M.; **The Current Knowledge on the Pathogenesis of Tissue and Medical Device-Related Biofilm Infections;** *Microorganisms* **2022**, *10*(7), 1259

Medical Biofilm Process

- Better way to test for antimicrobial susceptibility
- MBEC
  - Minimal Biofilm Elimination Concentration



Lid with pins placed (shake) in troughs containing media and bacteria (test biofilm)

Pins with biofilm

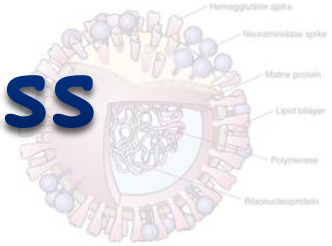
Lid with pins and biofilm placed various concentrations of antimicrobials

Biofilm eliminated from some pins

frontiers in Microbiology

Source: Okae,Y., Nishitani,K., Sakamoto,A., et.al.; **Estimation of Minimum Biofilm Eradication Concentration (MBEC) on *In Vivo* Biofilm on Orthopedic Implants in a Rodent Femoral Infection Model**; Front. Cell. Infect. Microbiol., 01 July 2022, Sec. Bacteria and Host, Volume 12 - 2022





Medical Biofilm Process

- Most chronic or long-term diseases are polymicrobic
  - CVS
    - #1 = Sth. epidermidis
      - Best biofilm producer in the world
    - #2 = Can. albicans
  - OT
    - Underside of a denture
  - Catheter
    - Biofilm @ 8 sec.

frontiers in Microbiology

Source: Okae,Y., Nishitani,K., Sakamoto,A., et.al.; **Estimation of Minimum Biofilm Eradication Concentration (MBEC) on *In Vivo* Biofilm on Orthopedic Implants in a Rodent Femoral Infection Model**; Front. Cell. Infect. Microbiol., 01 July 2022, Sec. Bacteria and Host, Volume 12 - 2022

Medical Biofilm Process

Clinical Lab Consequences

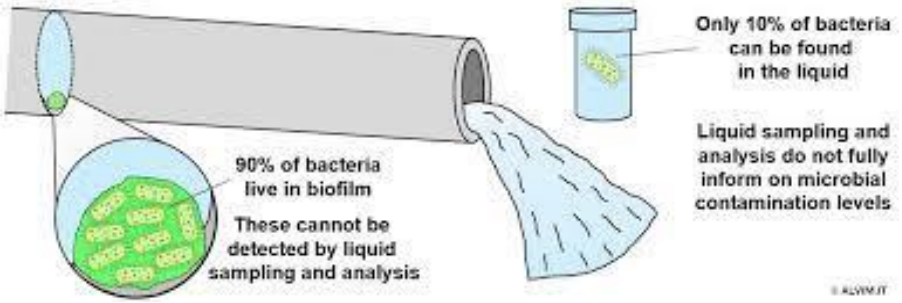
General Problems		Specific Examples	
False Negative:	Swab and plate	MAKI cultures	Simultaneous blood
Viable, Non-Cultivable	Biofilm (P^{BF}) Phenotype	CRBSI	Luminal Brush
Underestimated	Colony Count	Reporting Blood Cultures VAP	
Numbers = 0.1% of total population	Planktonic cells		
Loss of Susceptibility	MBEC 1,000x MIC	MBEC>MIC (always for Biofilms)	

frontiers in Microbiology

Source: Okae,Y., Nishitani,K., Sakamoto,A., et.al.; **Estimation of Minimum Biofilm Eradication Concentration (MBEC) on *In Vivo* Biofilm on Orthopedic Implants in a Rodent Femoral Infection Model**; Front. Cell. Infect. Microbiol., 01 July 2022, Sec. Bacteria and Host, Volume 12 - 2022

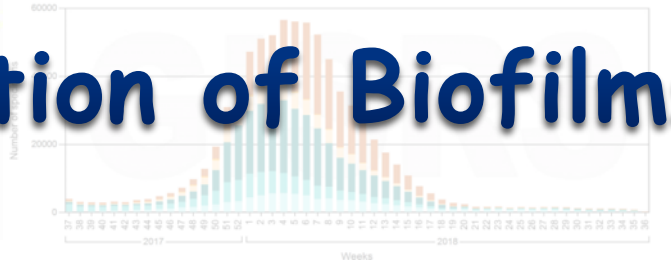
Detection of Biofilms

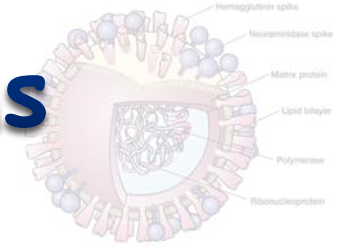
- Many of the initial ideas for the detection of biofilms came from the problem of "Biofouling" in the mechanical world
- General categories include;
- Physical: when the total biomass of the biofilm can be obtained from dry or wet weight measurements.
- Chemical: Use dyes or fluorochromes that can bind to or adsorb onto biofilm components.
- Microscopical: An imaging modality is used to detect the formation of biofilm (i.e., whenever a microscope is used) (CLSM, SEM, AFM, TEM, ESCM, STXM)
- Biological: Estimation of cell viability in measuring and detecting biofilm formation (QPCR)
- Most of these cannot work in medicine



Source: Achinas,S., Yska,S., Charalampogiannis, et.al.; **A Technological Understanding of Biofilm Detection Techniques: A Review;** *Materials* **2020**, *13*(14), 3147, 7/15/2020

Detection of Biofilms





	Advantages	Disadvantages
Models (*Some examples*)		
Static (microtitre plates)	Cheap, easy, quick Batch culture Different substrates can be added and removed for imaging	Not true mature biofilms Limited nutrient availability
Dynamic	Flow cells – Constant nutrient flow, equipment is autoclavable, cheap and easy to set up. Bioreactors – Constant nutrient flow, additional biofilm analysis, ability to expose biofilms to different nutrients/antimicrobials etc. Microfluidics – Mimic in vivo biofilms in vitro, real-time imaging and growth dynamics, small inoculating and growth medium volumes, exposing biofilms to different nutrients/ antimicrobials etc.	Flow cells – Contamination can be introduced easily. Bioreactors – Unacceptably large variation between biofilm of the same inoculum composition or sample type. Microfluidics – Risk of contamination , can be significantly more expensive than basic models
Single species, **mixed** species and **microcosm** models	Single species biofilms optimise biofilm models, multispecies models mimic in vivo/infections, microcosm patient samples model infection directly from infection site.	Single species are not always the way bacteria grow naturally, chosen multispecies are representative and not complete microbiome.
In Vivo modelling	More realistic and translational	Moral and ethical issues with animal testing
Ex Vivo modelling	Explanted material more easy to work with, preservation of tissue structures, ability to detect host-responses.	Donor availability, deterioration of tissue samples, difficult to image biofilms deep in tissue samples (Grivel and Margolis, 2009) .

Source: Cleaver,L., and Garnett,J.; **How to study biofilms: technological advancements in clinical biofilm research ;** Front. Cell. Infect. Microbiol. 13:1335389

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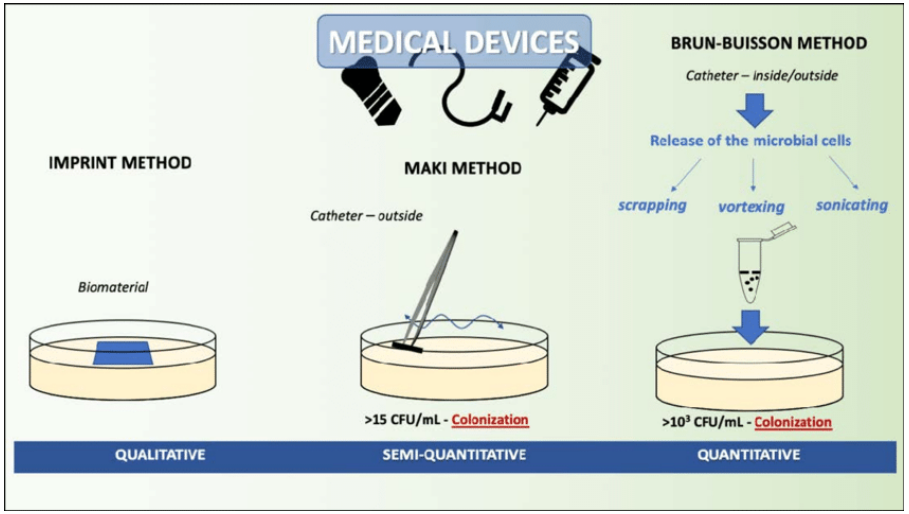
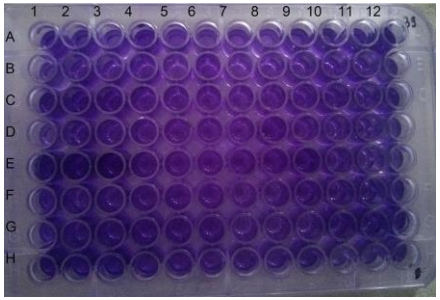
Detection of Biofilms

- Generally fall into three (3) categories;
  - Qualitative
    - Levine technique / syringe
  - Semi-Quantitative
    - Maki Method
  - Quantitative
    - Brun-Buisson Method



Microtiter Plate Method

Trypticase Soy Broth (TSB) @37 C. , diluted 1:50 with TSB-1% glucose medium and 150 µL
washed with 200 µl of phosphate-buffered saline (PBS; 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, and 130 mM NaCl, pH 7.4)
Adherent bacteria fixed with methanol (p.a.) and stained with 0.1% crystal violet (150 µL/well) for 15 min.

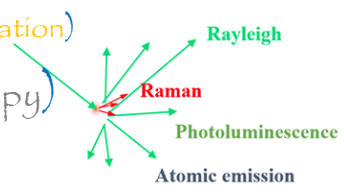


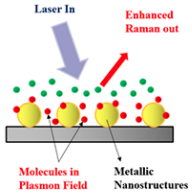
Tube Adherent Method
Better

Source: Kunwal,A., Shrestha,P., Shrestha,S.,et.al.; Detection of biofilm formation among Pseudomonas aeruginosa isolated from burn patients; Burns Open; Volume 5, Issue 3, July 2021, 125-9

Detection of Biofilms

- Some other isolation methods; (Molecular)
  - Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)
    - Enhanced LDI (MALDI-2, IR MALDI-2, Fs-LDPI, MetA-LDI, NIMS)
  - Congo Red Agar (CRA) assay (Slime productivity)
    - BHI agar supplemented with 0.8 g/L Congo Red and 50 g/L of saccharose and incubated @ 37 C, 24 hr., phenotypic ID
  - Detection of biofilm-associated genes (icaACD, bap, etc.)
  - NanoDESI (nanospray desorption electrospray ionization)
  - SERS (Surface Enhanced Raman Spectroscopy)
  - SR-FTIR
  - Fluorescense
  - SIMS (Secondary Ion Mass Spectrometry) Solid or Liquid



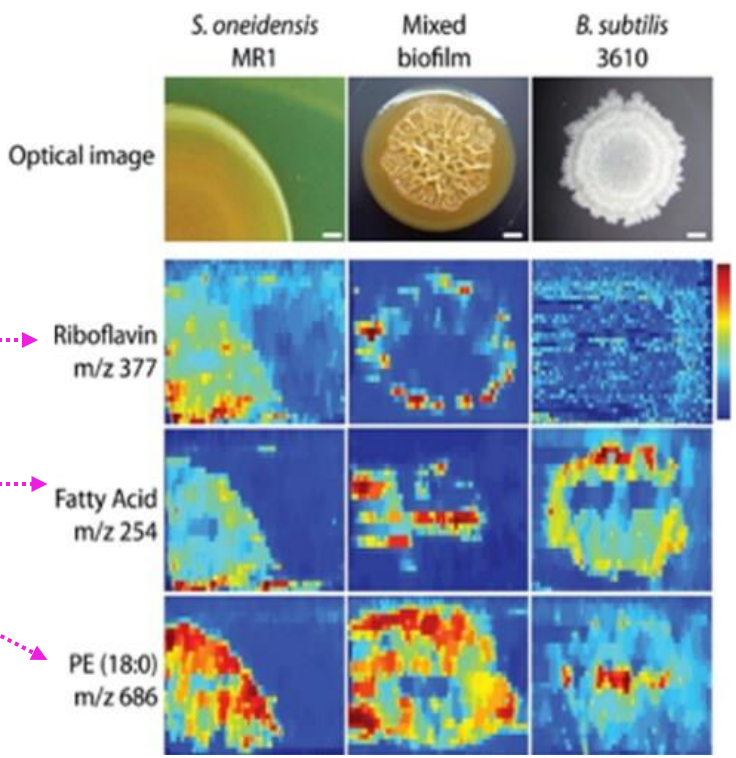


Source: Achek,R., Hotzel,H., Nabi,I, et.al.; **Phenotypic and Molecular Detection of Biofilm Formation in *Staphylococcus aureus* Isolated from Different Sources in Algeria**, Pathogens. 2020 Feb; 9(2): 153, 2020

Detection of Biofilms

- nanoDESI
- Nanospray Desorption Electrospray Ionization mass spectrometry

- nanoDESI IMS biofilm images showing;
  - Riboflavin (vitamin B12, which plays an essential role in extracellular electron transfer by *Shewanella oneidensis* MR-1)
  - Fatty acids
  - Phosphatidylethanolamines (PE)



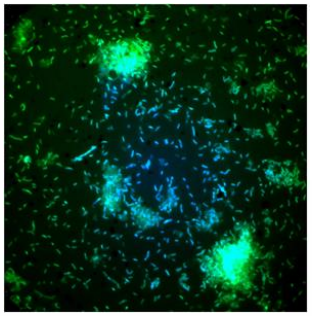
Source: Zhang J, Brown J, Scurr DJ, Bullen A, MacLellan-Gibson K, Williams P, Alexander MR, Hardie KR, Gilmore IS, Rakowska PD, et al. 2020. **Cryo-OrbiSIMS for 3D molecular imaging of a bacterial biofilm in its native state**. Anal Chem. 92(13):9008–9015

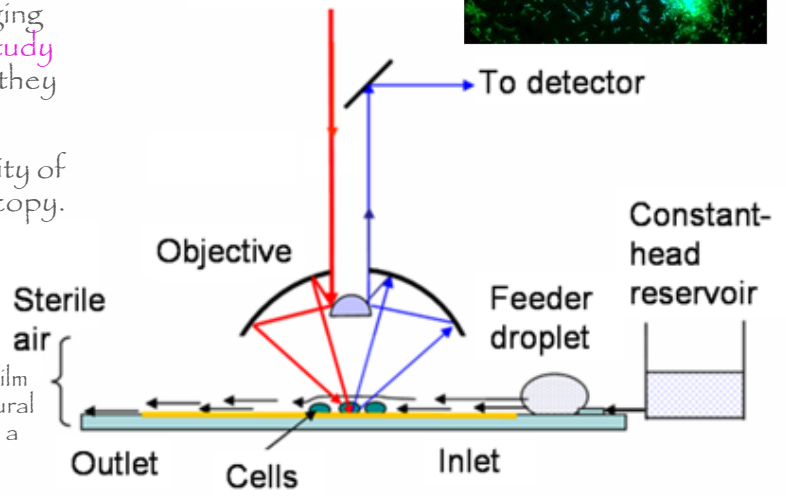
Open-channel microfluidic platform, SR-FTIR spectromicroscopy

- A robust, label-free method to probe the chemical underpinnings of developing bacterial biofilms—dynamic communities of cells that stick to other bacteria or surfaces in water.
- Coupling synchrotron radiation–based Fourier transform infrared (SR-FTIR) spectromicroscopy from ALS Beamline 1.4.3 with the first open-channel microfluidic platform could impact several scientific disciplines.
- SR-FTIR spectromicroscopy continuously monitors the changing contents in living samples without labeling, a technique used to study dynamic processes in bacteria living in aqueous environments as they respond to stimuli and form evolving biofilms.
- The open-channel microfluidic platform maintains the functionality of living cells while enabling high-quality SR-FTIR spectromicroscopy. It minimizes IR signal interference by controlling the water film dimensions.
- To evaluate this technique's potential, researchers studied:
  - Antibiotic resistance in biofilms. By maintaining living bacteria in biofilm over a long period of time, researchers captured molecular and structural changes in *E. coli* biofilms during adaptation to mitomycin antibiotics, a potent DNA crosslinker.

Lawrence Livermore National Laboratory

Berkeley UNIVERSITY OF CALIFORNIA



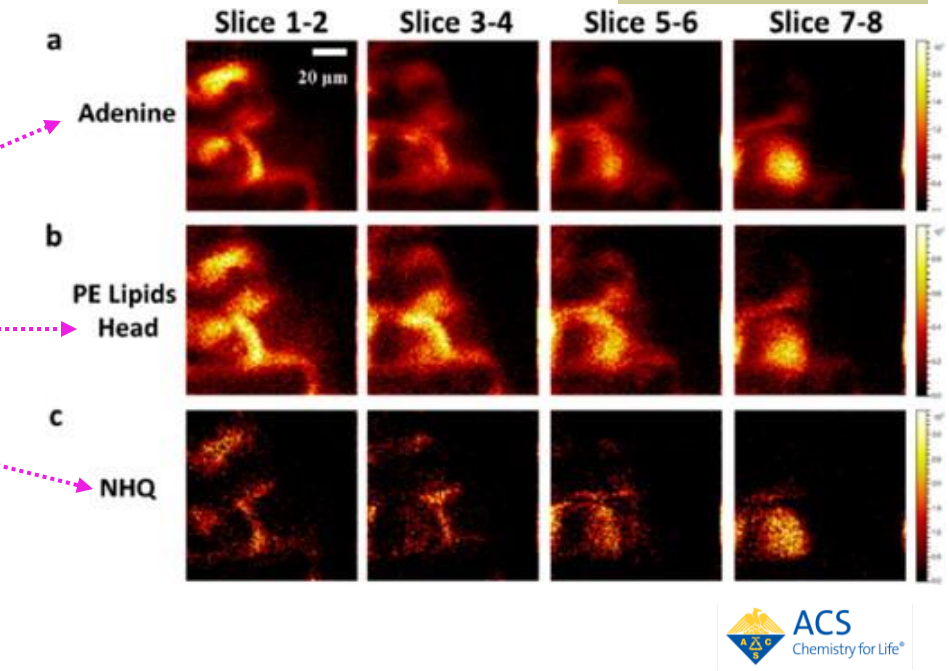


Berkeley Lab, Lawrence Livermore National Lab, and UC Berkeley

Source: H-Y. Holman, et al., *Anal. Chem.* **81,** (20) 8564 (2010) , http://www-als.lbl.gov/index.php/science-highlights/science-highlights/466

Detection of Biofilms

- Orbitrap MS (Mass Spectrometry)
- Frozen-hydrated *Pseudomonas aeruginosa* biofilm.
  - (a) Adenine, a nucleic acid marker that can originate from both the bacterial cytoplasm and the extracellular DNA present in the extracellular matrix.
  - (b) PE lipid head groups, markers for the bacterial membrane and only associated with bacterial cells and macrovesicles.
  - (c) NHQ (5-Nitro-8-Hydroxy Quinoline) is an extracellular signaling molecule, but because of its physical properties, a high proportion is associated with the cell envelope and any macrovesicles that had been shed into the biofilm matrix



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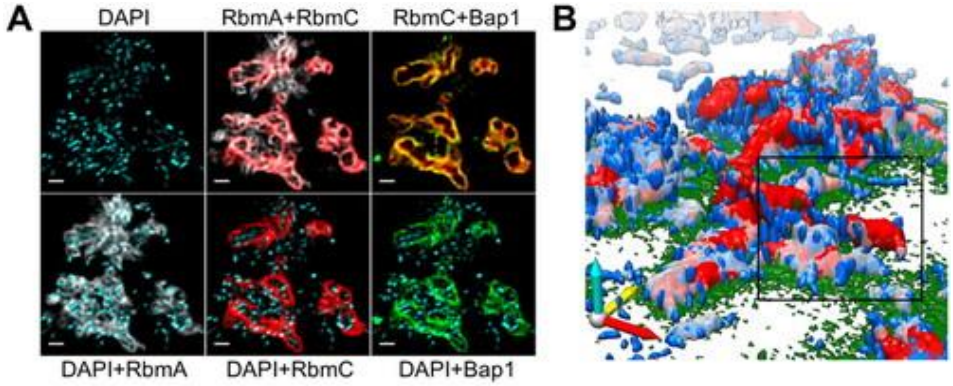
Source: Zhang J, Brown J, Scurr DJ, Bullen A, MacLellan-Gibson K, Williams P, Alexander MR, Hardie KR, Gilmore IS, Rakowska PD, et al. 2020. **Cryo-OrbiSIMS for 3D molecular imaging of a bacterial biofilm in its native state**. Anal Chem. 92(13):9008–9015

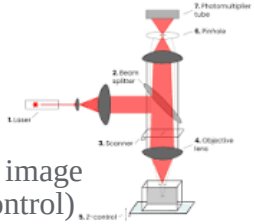
Detection of Biofilms

- Some other isolation methods;
  - Microscopy
  - NMR
  - µCT (Micro Computed-Tomography)
- Imaging
  - MSI
  - nanoDESI
  - Spectroscopy (SERS, SR-FTIR)
  - Fluorescence Imaging (CLSM)
  - µCT
  - NMR
  - LDI (Laser Desorption Ionization)



(**A**) **CLSM** images of *Vibrio cholerea* *biofilm* visualizing pseudo-colored blue (cells), grey (RbmA), red (RbmC), and green (Bap1)
(**B**) **3D biofilm** architecture with colors as in (A)

Confocal Laser Scanning Microscopy

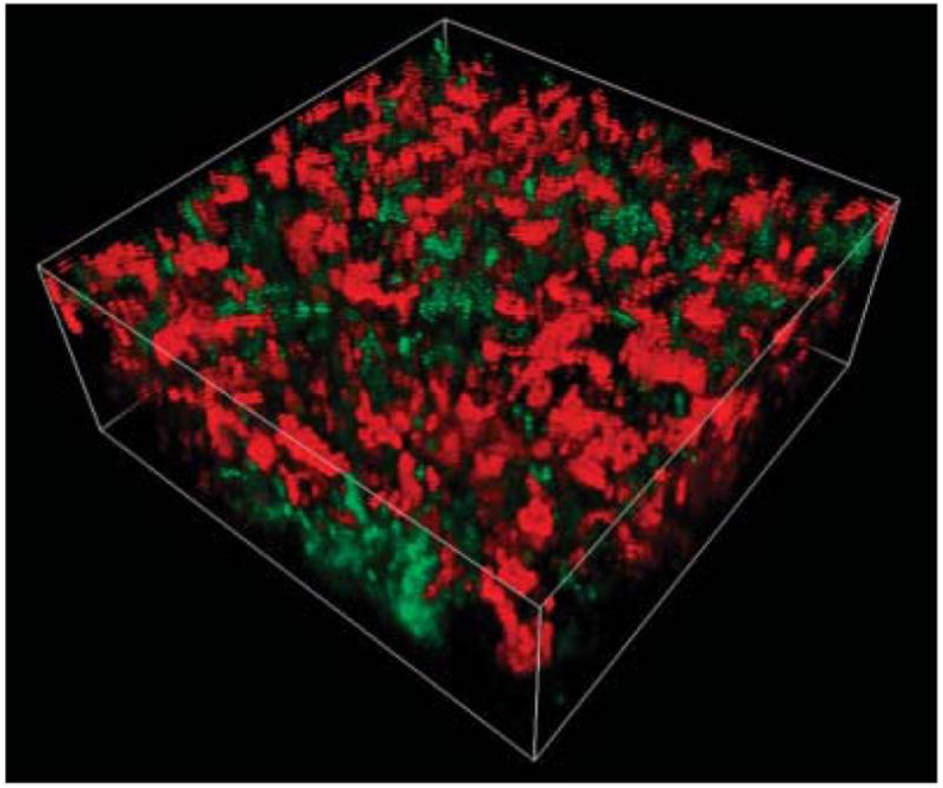


2D, results in a 3D image (pin-hole and Z-control)

Source: Achek,R., Hotzel,H., Nabi,I, et.al.; **Phenotypic and Molecular Detection of Biofilm Formation in *Staphylococcus aureus* Isolated from Different Sources in Algeria**, Pathogens. 2020 Feb; 9(2): 153, 2020

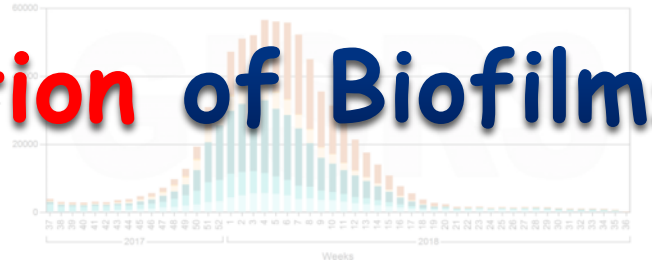
SSMACQD

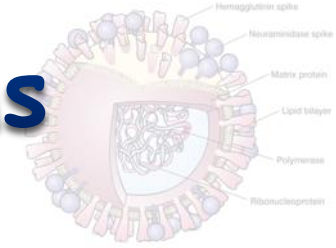
- Three-dimensional reconstruction of a binary culture biofilm collected by confocal laser scanning microscopy. Biofilm z-direction "height" is 25 µm. The biofilm comprises the bacterial species, *Klebsiella pneumoniae* (green) and *Pseudomonas aeruginosa* (red), which have been visualized using;
- Species-Specific Monoclonal Antibody-Conjugated Quantum Dots (QDs)
- SSMACQD
- Unlike traditional fluorochrome stains, quantum dot luminescence is photostable and size tunable, = multi-color emitted light from QDs of varying size (with a single excitation wavelength)



Source: James D. Bryers; Medical Biofilms; Biotechnol Bioeng. 2008 May 1; 100(1): 1–18.

Inhibition of Biofilms

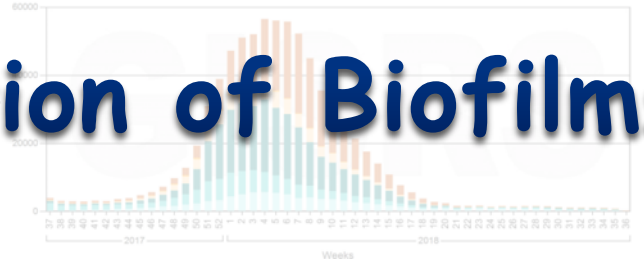


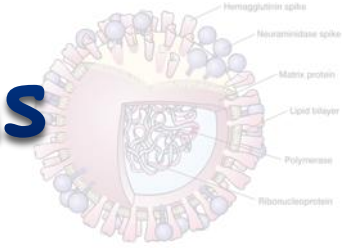


Resistance Mechanism	Characteristics
Glycocalyx	The capsule is an important part of the biofilm in both Gram positive and negative bacteria. Its contribution to the maturation step relies on the electrostatic and hydrogen bonds established on the matrix and the abiotic surface. The composition in glycoprotein and polysaccharides varies with biofilm progression, permitting pathogens to live in difficult environments. The antimicrobial resistance is supported by the glycocalyx with the external layer acquiring antimicrobial compounds, serving as adherent for exoenzymes and protecting against antibacterial activity.
Enzyme mediated resistance	The presence of heavy metals, such as cadmium, nickel, silver, zinc, copper, cobalt induces diversity of resistant phenotypes. This causes the enzymatic reduction of ionic particles mediating the transformation of toxic molecules to nontoxic or inactive.
Metabolism and growth rate heterogeneity	The bacterial metabolic activity and growth rate are influenced by the nutrients and oxygen concentrations within biofilms. = can limit the metabolic activity inside the biofilm resulting in the reduction of the growing rate of strains. These microbial communities increase the level of antimicrobial resistance inducing the expression of certain genes in different conditions.
Cellular persistence	Cells of the Biofilm's persistent strains are responsible for eliciting multidrug forbearance. The glycocalyx improves protection of the immune system inducing the growth of bacterial biofilm competing for antibiotic targets with multi-medicament resistance (MDR) protein synthesis.

Source: Zeuko'O Menkem,E.; **The Mechanisms of Bacterial Biofilm Inhibition and Eradication: The Search for Alternative Antibiofilm Agents;** 5/24/22 In "**Focus on Bacterial Biofilms**

Inhibition of Biofilms





Resistance Mechanism	Characteristics
Metabolic state	The inaccessibility of nutrients (Biofilm exposed to bactericidal agent), = modifies bacterial envelope The genetic profile. The **mar operons** are involved in the control of various genes' expression in *E. coli* assisting the MDR phenotype. The stress response cells display increase resistance to impaired factors within hours of exposure. The exposure of bacterial strains to molecular oxidants causes the diversified regulatory genes (oxyR and soxR) to exhibit persistence of the intracellular redox potential and the activation of stress response.
Quorum sensing (QS)	QS regulates the heterogeneous organization with nutrient supply during the cell migration procedure. QS deficiency is linked with thinner microbial biofilm growth consequently lowering the EPS production.
Stress response	The stress response acts as a preventive factor for cell damage more than repair. Biofilm stress = starvation, ↓ or ↑ temperature, high osmolality and ↓ pH. All = altered gene expression due to the stress response in immobilized strains result in increased resistance to antibiotics.
External membrane structure	The lipopolysaccharide layer prevents hydrophilic antimicrobials from entering through the outer membrane while the external membrane proteins reject hydrophobic molecules
Efflux systems	The efflux pumps = bacterial endurance, inherent and gained resistance to diverse antimicrobials. = overproduction of efflux pumps regulating the multi-medicament non-compliances. Efflux pumps are major player in the MDR of Gram-negative bacteria.

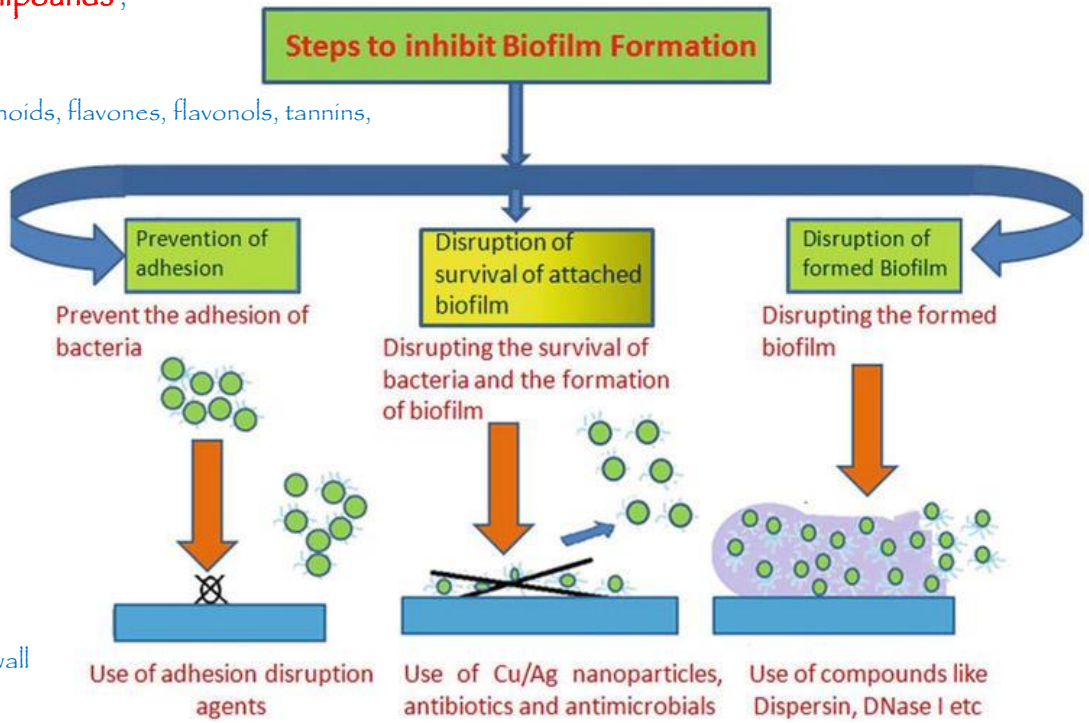
Source: Zeuko'O Menkem,E.; **The Mechanisms of Bacterial Biofilm Inhibition and Eradication: The Search for Alternative Antibiofilm Agents;** 5/24/22 In "Focus on Bacterial Biofilms

Inhibition of Biofilms

- There are six (6) broad classes of natural compounds ;
  - 1) Phenolics
    - Including: phenolic acids, quinones, flavonoids, flavones, flavonols, tannins, and coumarins
  - 2) Essential oils
  - 3) Terpenoids
  - 4) Lectins
  - 5) Alkaloids
  - 6) Polypeptides & polyacetylenes

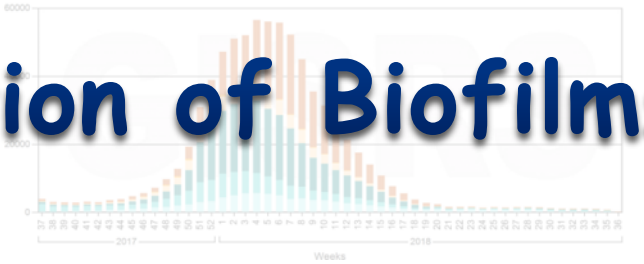
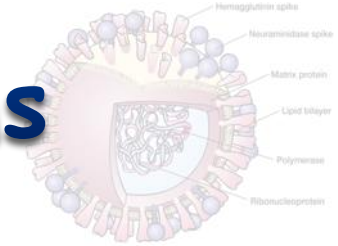
These act on biofilms by six main mechanisms

1) Substrate deprivation
2) Membrane disruption
3) Binding to the adhesin complex and cell wall
4) Binding to proteins
5) Interacting with eukaryotic DNA
6) Blocking viral fusion



Source: Rabin N, Zheng Y, Opoku-Temeng C, et.al.; ***Biofilm formation mechanisms and targets for developing antibiofilm agents***; Future Medicinal Chemistry. 2015;7(4):493-512

Inhibition of Biofilms

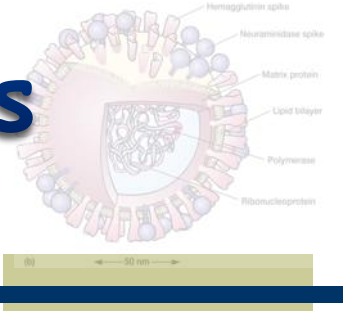




Compound	Source	Pathogenic species	Experimental details	Molecular mechanism
Allicin	*Allium sativum* L. (Garlic)	*Pseudomonas aeruginosa*	*In vitro* (1 pqsABCD knockout strain)	It decreases the bacterial attachment in the initial stages of biofilm formation as it reduces EPS formation It controls the expression of virulence factors hence interfere with the QS system
Ajoene		*Ps. aeruginosa* *Ps. aeruginosa* *Staphylococcus aureus*	*In vitro* (PMNs killing assays) and *in vivo* (Pulmonary infection mice model)	It downregulates rhamnolipid production It inhibits small regulatory RNA molecules (rsmY, rsmZ, and rnaIII) that operate in the later phase of QS signaling
Carvacrol (monoterpenoid)	*Origanum vulgare* L. (Oregano)	*Ps. aeruginosa*	*In vitro* (qPCR for relative expression of lasI/lasR genes) and bocking modeling of proteins LasI and LasR	It mainly acts on QS Machinery. The posttranslational inhibition against LasI, which effects AHL production.

Source: Rabin N, Zheng Y, Opoku-Temeng C, et.al.; ***Biofilm formation mechanisms and targets for developing antibiofilm agents***; Future Medicinal Chemistry. 2015;7(4):493-512

Inhibition of Biofilms

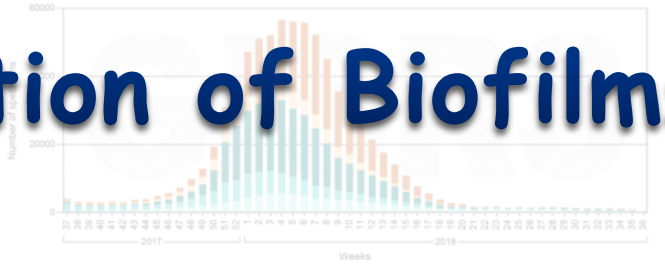
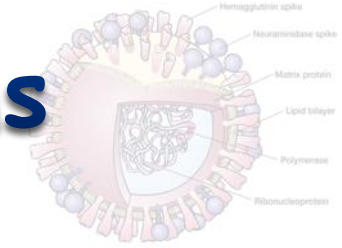




Compound	Source	Pathogenic species	Experimental details	Molecular mechanism
Emodin (anthraquinone)	*Polygonum cuspidatum* Siebold & Zucc. *Rheum palmatum* L.	*Sth. aureus*	*In vitro* crystal violet biofilm assay and SEM analysis	It decreases the release of eDNA and downregulates the expression of biofilm-forming related genes like cidA, icaA, dltB, agrA, sortaseA, and sarA
Emodin (anthroquinone)	*Rh. palmatum* L. (Chinese Rhubarb) (a Knotweed)	*Candida albicans* *Candida krusei* *Candida parapsilosis* *Candida tropicalis*	*In vitro* (microdilution assay, kinase assay) and molecular docking for emodin in CK2 (Autodock Vina)	The biofilm formation is inhibited by targeting cellular kinase signaling It acts on planktonic cells by reducing hyphal formation. It acts as a competitive inhibitor of CK2
Aloe-emodin	*Rheum officinale* all. (IndianRhubarb) (a Knotweed)	*Sth. aureus*	*In vitro* (CLSM assays and Congo red assay)	It reduces the production of extracellular proteins and polysaccharide intercellular adhesin It inhibits biofilm formation on polyvinyl chloride surfaces

Source: Rabin N, Zheng Y, Opoku-Temeng C, et.al.; ***Biofilm formation mechanisms and targets for developing antibiofilm agents***; Future Medicinal Chemistry. 2015;7(4):493-512

Inhibition of Biofilms

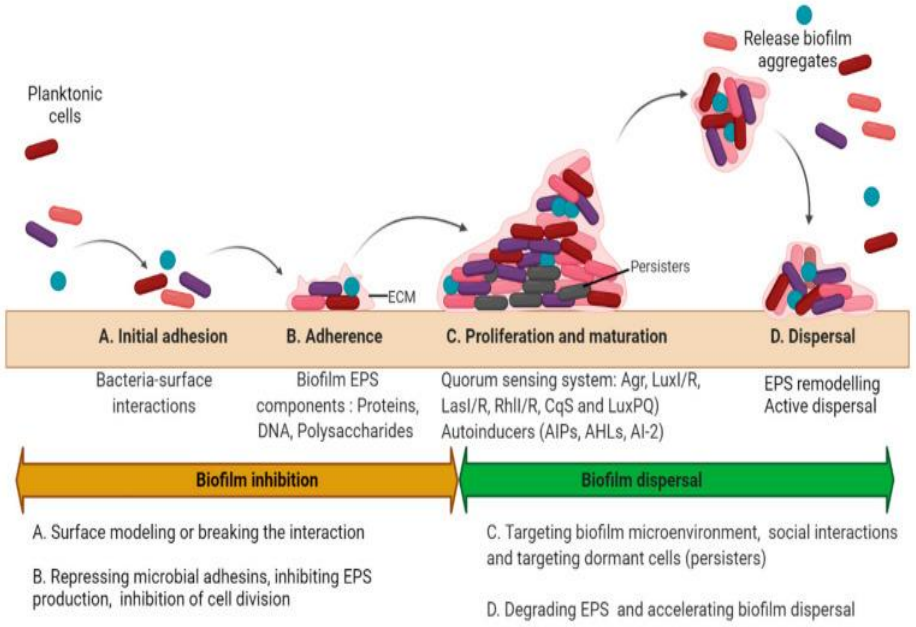




Compound	Source	Pathogenic species	Experimental details	Molecular mechanism
Hordenine	*Hordeum vulgare L.*(sprouting) (Barley)	*Ps. aeruginosa*	*In vitro* (SEM and CLSM assays, qPCR for QS-related genes)	It decreases AHL production It reduces the exhibition of virulence factors (proteases, elastase, pyocyanin,rhamnolipid, alginate, and pyroviridine) It impedes the swimming and swarming activity It negatively regulates the expression of lasI, lasR, rhlI and rhlR genes
Pulverulentone A	*Callistemon citrinus*(Curtis) skeels leaves	*Sth. aureus*	*In vitro* broth microdilution assay, CLSM, TEM analysis Methicillin-resistant	It reduces styphyloxanthin production, thus inhibiting biofilm formation It disrupts the cell membrane

Source: Rabin N, Zheng Y, Opoku-Temeng C, et.al.; ***Biofilm formation mechanisms and targets for developing antibiofilm agents***; Future Medicinal Chemistry. 2015;7(4):493-512

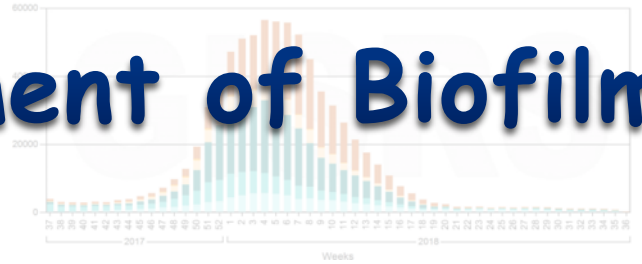
Treatment of Biofilms

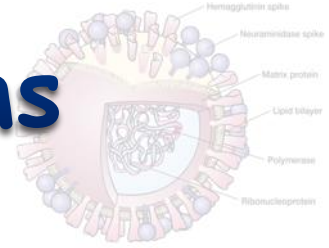
- Antibiotic tolerance in biofilms is markedly different than that of planktonic cells
- Currently available antimicrobials are woefully inadequate to treat biofilm-associated infections
- Strategies relate to four major stages in biofilm development;
  - Surface modeling, breaking the planktonic attachment
  - Repressing microbial adhesins
  - Targeting the biofilm microenvironment
  - Degrading EPS, accelerate dispersal



Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms





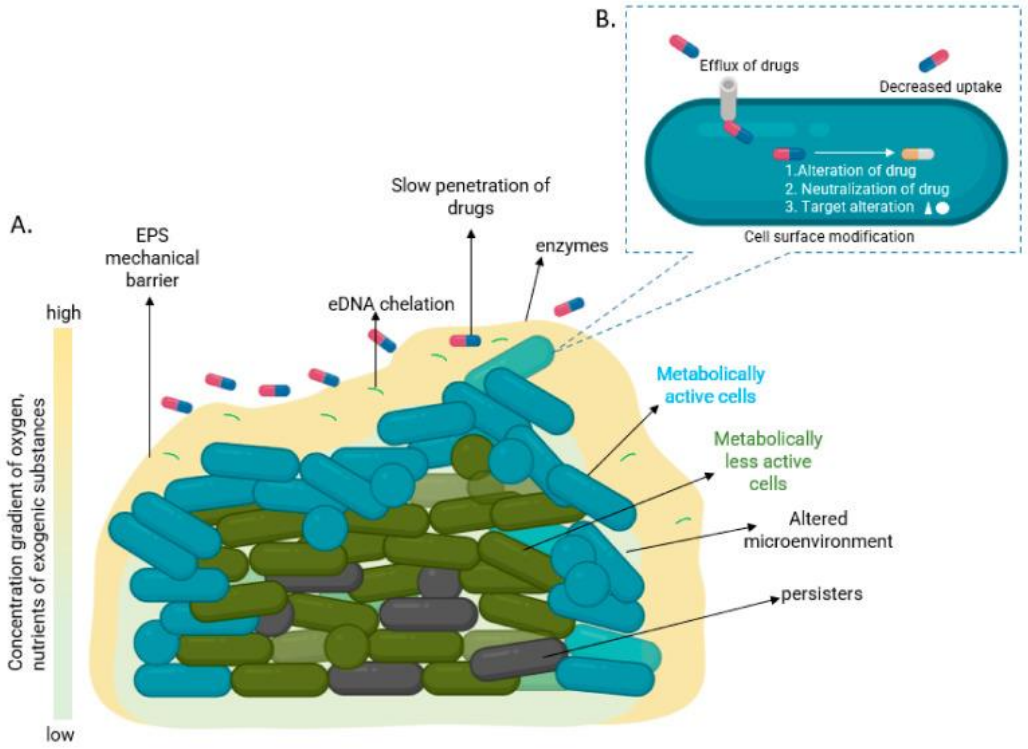
- Some Factors;
  - *Staphylococcus epidermidis*, *Sth. aureus*, and *Pseudomonas aeruginosa* are the **leading organisms** to contend with
  - Any **rupture in the mucous layer** exposes bacteria to the host epithelium and infection of mucosal surfaces
  - **Invading organisms** have had to to **overcome** the epithelial wall, host-microbiome, a variety of leukocytes, and complement
  - Biofilms decrease the efficiency of both macrophages and PMNs
- Rx: = 1. Aggressive physical removal of biofilms = encourage dispersal
- 2. Localized delivery of high and sustained antimicrobial chemotherapy
- = Inhibit formation
  - Intravenous catheters are usually treated using a "lock therapy" which involves the treatment of a high dose of antibiotics into the lumen of the catheter for several hours

MDPI

Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms

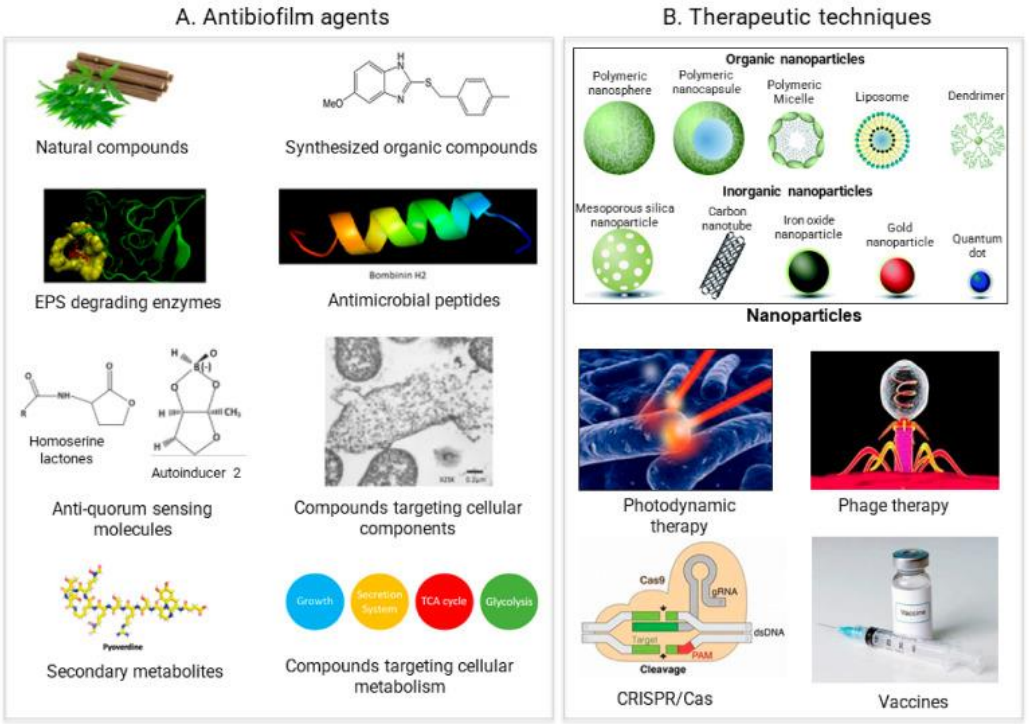
- Biofilm Antibiotic Tolerance;
  - = a physiological state of biofilm cell populations that is temporary and non-heritable
  - Community level
  - Cellular level



Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms

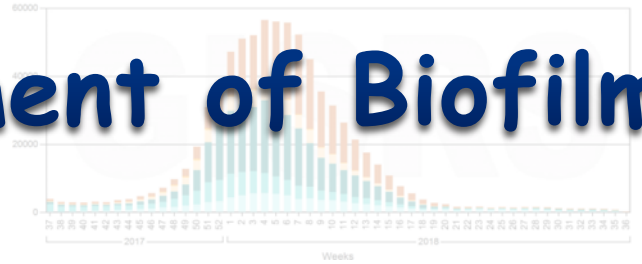
- Biofilm Treatment;
  - Antibiofilm Agents
    - Natural Compounds
    - Synthetic Organics
    - EPS Degraders
    - Antimicrobial Peptides
    - Anti-QS
    - Anti-cellular
    - Secondary Metabolites
  - Dispersants
    - Organic Nanoparticles
    - Photodynamic Rx
    - Phage Rx
    - CRISPR/Cas
    - Vaccines

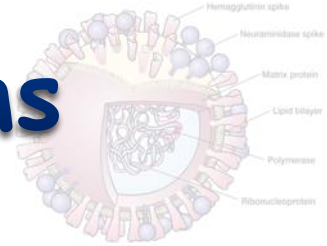


MDPI

Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms





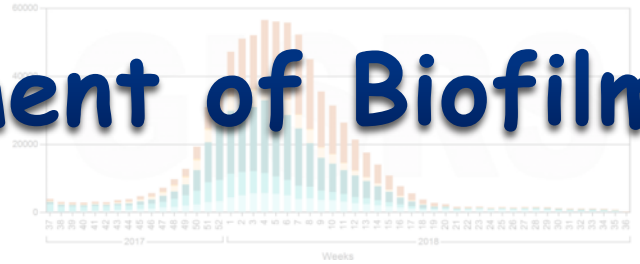
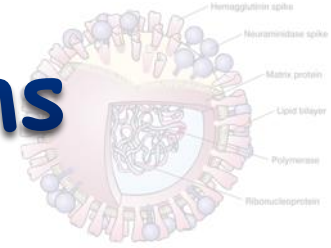
- Anti-Biofilm Agents;
  - Mostly extracted from natural sources

Agent Class	Functions
Class I	Penetrate the biofilm EPS and decrease the growth of cells
Class II	Interfere with the adherence of bacteria and the formation of biofilm phenotype
Class III	Controls both the growth of bacteria with biofilm phenotype as well as the EPS synthesis
Class IV	Disperse the mature biofilms

MDPI

Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms

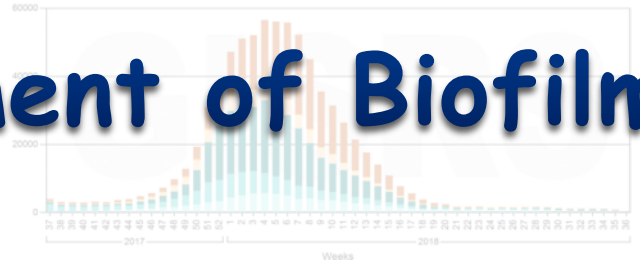
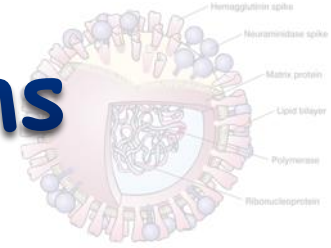
- Anti-Microbial Agents

Name of the Compound	Type	Mode of Action	Effective Against
Triton X-100	surfactant	autolysis, targeting EPS	*Sth. aureus*
Tween 80	surfactant	N/A	*Ps. aeruginosa*, *Sth. aureus*
Quarternary ammonium compounds	surfactant	Cell lysis and death	several bacteria
Poloxamer containing non-ionic surfactant	surfactant	EPS metalloproteinase modulation	*Ps. aeruginosa*
Rhamnolipids	bio-surfactant	N/A	*Sth. aureus*, *Salmonella enteritidis*, and *Listeria monocytogenes*
EDTA	chelators	damage to cell wall	*Ps. aeruginosa*
Chitosan	biomaterial	membrane damage	*Ps. aeruginosa*
Secondary metabolite from Citrus limonoids	secondary metabolite	quorum sensing	*Vibrio harveyi* (Marine fish)
Cyclo(l-Tyr-l-Leu)	secondary metabolite	inhibit EPS	*Sth. epidermidis*
Cahuitamycins	secondary metabolite	N/A	*A. baumanii*
Phlorotannin	secondary metabolite	damaging membrane permeability/ cell lysis	MRSA

MDPI

Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms

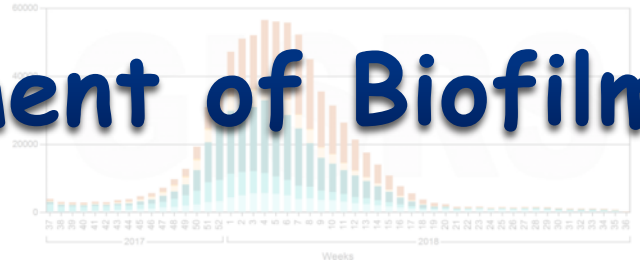




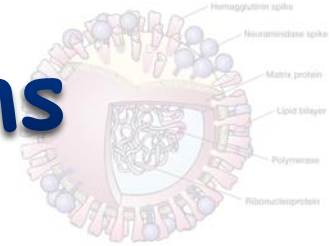
- Anti-Microbial Agents

Name of the Compound	Type	Mode of Action	Effective Against
α-amylase	enzyme	degrade EPS	MRSA
Polyamine norspermidine	polyamine	interacts with EPS	*B. subtilis*, *E. coli* and *Sth. aureus*
D-amino acids	amino acid	target YqxM	*E. coli*, *Sth. aureus*
N-acetylcysteine/NAC	amino acid	degrade EPS polysaccharide	Rapidly growing *Mycobacterium*
Esp (Serine protease)	enzymes	degrade EPS protein content	*Sth. aureus*
DNase I	enzymes	degrade eDNA	*E. coli*, *Sth. aureus*
tea-tree oil	secondary metabolite	metabolism	*Sth. aureus*
Protease from *Ps. aeruginosa*	enzymes	degrade EPS protein content	*Sth. aureus*

Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms





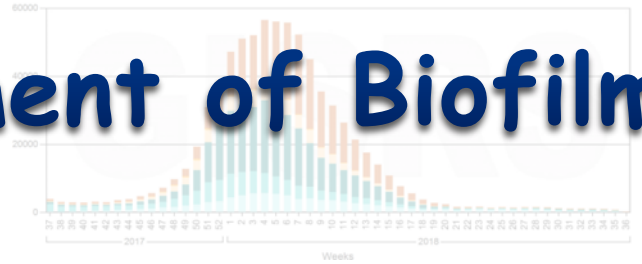
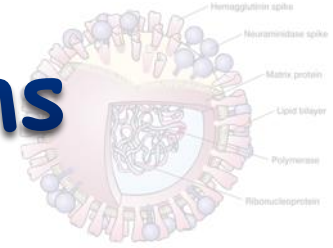
- Anti-Microbial Agents: Natural Compounds

Compound/Molecule	Mode of Action	Effective Against
Garlic extracts	inhibits QS	*Ps. aeruginosa*
Garlic extracts	inhibit LasR and LuxR	*Ps. aeruginosa*
Isolimonic acid	cell-cell signaling	*E. coli*
Isolimonic acid	reduce LuxR DNA binding	*Vibrio spp.*
Cinnamaldehyde	swimming motility	*E. coli*
Hordenine	decrease in signaling molecule, inhibition of QS-related genes	*Ps. aeruginosa*
Autoinducing peptide type I (AIP-I)	inhibit QS	*Sth. aureus*
RNAIII-inhibiting peptide (RIP)	inhibit QS	*Sth. aureus*
Querentin	decrease LasI/R, RhlI/R expressions	*Ps. aeruginosa*

MDPI

Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

Treatment of Biofilms

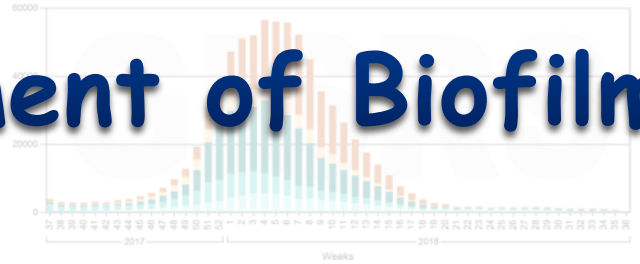
- One Example;
- Triclosan; (Acting as an adjuvant)
  - Broad-spectrum
  - Prevents type-II fatty acid synthesis
    - USFDA approved antibacterial and antifungal agent used in toothpaste
- Combination of triclosan and tobramycin led to a 100-fold ↓ in viable *Ps. aeruginosa*-persistent cells during 8 h of incubation, and resulted in complete eradication after 24 h
  - Triclosan alone had no appreciable effect
- Triclosan ↑ tobramycin's efficacy in terms of killing multiple *Burkholderia cenocepacia* and *Sth. aureus* clinical isolates grown as biofilms
- Triclosan exhibited synergy with gentamicin and streptomycin

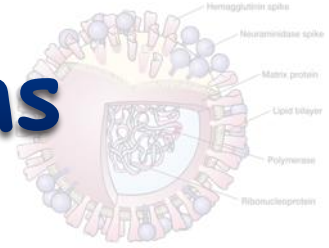
•Note: *Burkholderia cepacia* complex (Most common human organisms of the 20 in the Complex) *Bur. cenocepacia*, *Bur. multivorans*, *Bur. vietnamiensis*, *Bur. dolosa*, *Bur. cepacia*
(Cystic Fibrosis)

MDPI

Source: Krukiewicz,K., Kazek-Kesik,A., Brzchczy-Wioch,M..; et.al.; Recent Advances in the Control of Clinically Important Biofilms; Int J Mol Sci. 2022 Sep; 23(17): 9526

Treatment of Biofilms



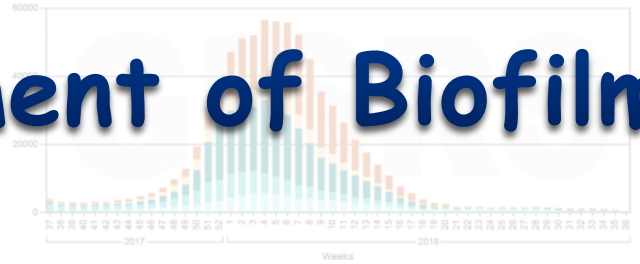


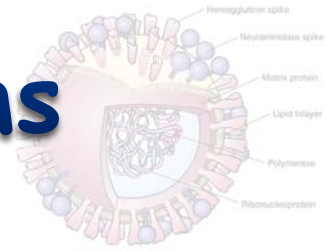
- Other Examples;
- Antimicrobial Peptides (AMPs);
  - Short (12-100 AAs)
  - Cationic
  - Amphipathic
  - Part of the innate immunity of bacteria, animals, and plants
  - MOA = inhibition of attachment, killing of planktonic cells, and/or eradication of mature biofilms
- Nisin; (Another Peptide)
  - FDA-approved, GRAS (generally recognized as safe)
  - Active against *Streptococcus pneumoniae*, *Clostridioides difficile*, *Sth. aureus*(MRSA)
  - Nisin Z = GN oral pathogens, such as *Por. gingivalis*, *Por. intermedia*, *Aggregatibacter actinomycetemcomitans*, and *Treponema denticola*

MDPI

Source: Krukiewicz,K., Kazek-Kesik,A., Brzchczy-Wioch,M..; et.al.; **Recent Advances in the Control of Clinically Important Biofilms**; Int J Mol Sci. 2022 Sep; 23(17): 9526

Treatment of Biofilms





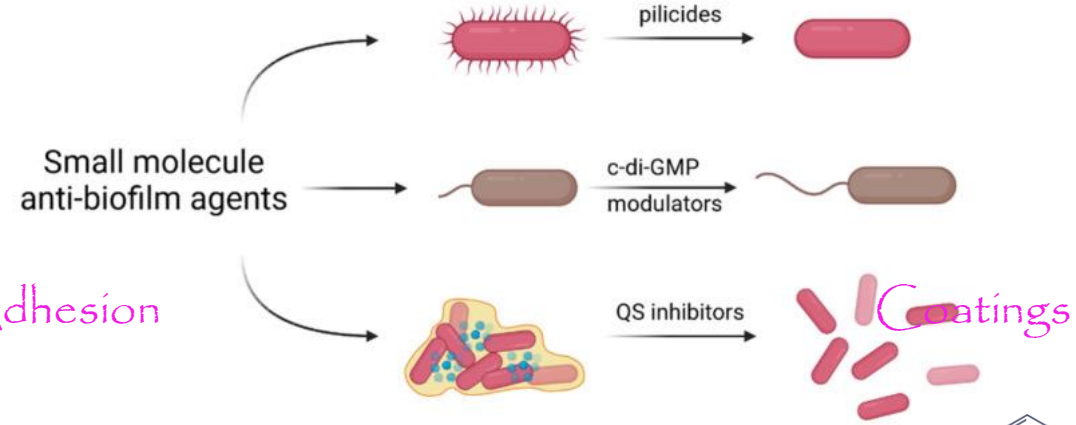
- Other Examples;
- Proteolytic Enzymes;
  - Attachment surface = polysaccharides, proteins, or nucleic acid
  - ∴ = Protease/enzyme may interfere
  - Bromelain, actinidin, papain, proteinase K, and trypsin = Dental Biofilms
  - DNAses = antibiofilm for, *Sth. aureus*, *Ps. aeruginosa*, *E. coli*, *Acinetobacter baumannii*, *Haemophilus influenzae*, and *Klebs. pneumoniae*
  - Ficin, (sulfhydryl protease) from the latex of fig trees, was shown to disrupt *Sth. aureus* and *Sth. epidermidis* biofilms and act as an adjuvant for anti-biofilm effects of antibiotics

MDPI

Source: Krukiewicz,K., Kazek-Kesik,A., Brzchczy-Wioch,M..; et.al.; **Recent Advances in the Control of Clinically Important Biofilms**; Int J Mol Sci. 2022 Sep; 23(17): 9526

Treatment of Biofilms

- Other Categories of Actions:
  - Vaccines
  - Biomaterials and Nanoparticles
  - Photodynamic Therapy
  - Synthetic Microbiology (Fabricated Devises)
  - Bacteriophage Rx
  - Small Molecule
  - Pilicides
  - Curlicides
  - Biomimetic Anti-Adhesion

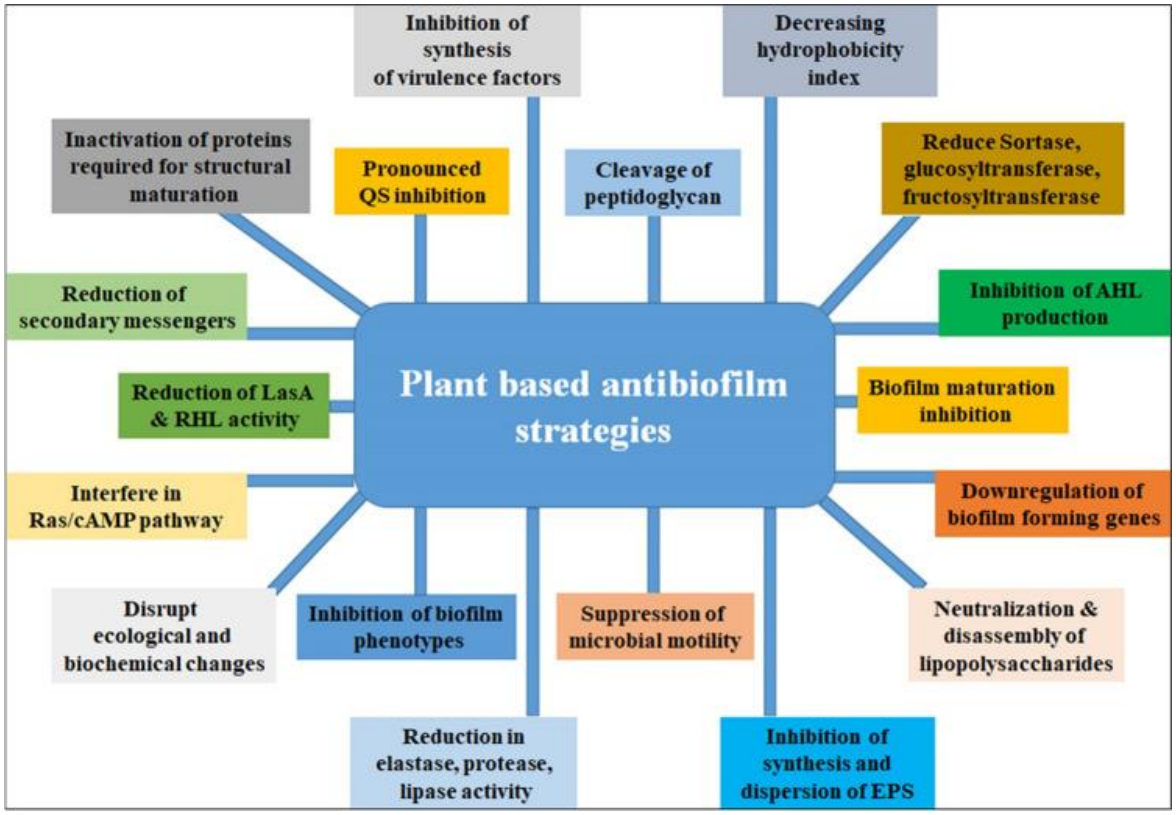


MDPI

Source: Krukiewicz,K., Kazek-Kesik,A., Brzchczy-Wioch,M..; et.al.; Recent Advances in the Control of Clinically Important Biofilms; Int J Mol Sci. 2022 Sep; 23(17): 9526

Treatment of Biofilms

- Plant-based Approaches:
  - Non-human and medical applications

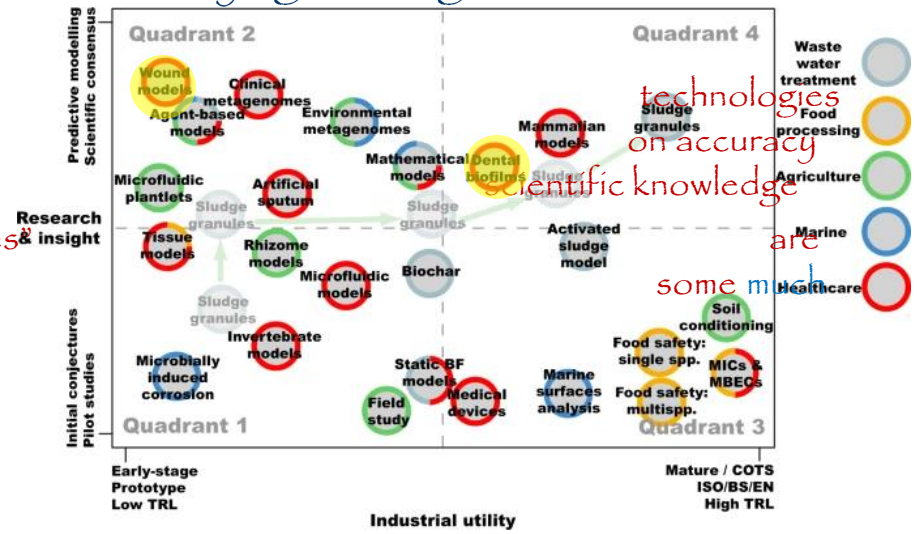


MDPI

Source: Shrestha,L., Fan, H.M., Tao, H.R.; et.al.; Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches; Pathogens. 2022 Mar; 11(3): 292

The Future

- Biofilms may have been with life on Earth as long as there has been life on Earth
- The estimated global economic impact of biofilms is over $5 trillion / Yr.
- There is a 2-dimensional framework, termed the Biofilm Research-Industrial Engagement Framework (BRIEF) for classifying existing biofilm technologies
  - This system organizes biofilm technologies across sectors based on accuracy and conformation to scientific knowledge
  - Note: Some of the "medical technologies" are more robust (Dental biofilms), and some much less (Wound models)



Source: Flemming, H.-C. & Wingender, J.; *The biofilm matrix*; Nat. Rev. Microbiol. 8, 623–633 (2010)

The Future

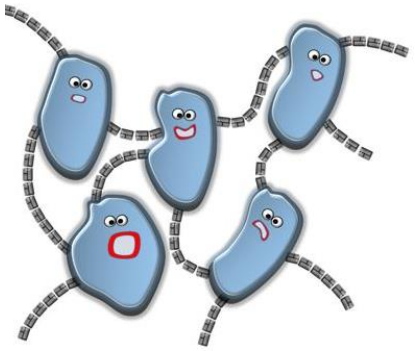
- Biofilm technologies of the near-term;
  - Imaging
    - Super high resolution (beyond the diffraction limit of light) live cell imaging is now becoming increasingly available with resolutions from 100 nm to 20 nm (in the XY plane)
    - Label free imaging techniques such as Coherent Anti-Stokes Raman Spectroscopy (CARS)
    - MALDI-MS
  - Sensors
    - Microelectrodes and planar optodes ??, old, fiddly
    - Nanobots – on their way
  - Artificial intelligence (AI)/machine learning (ML)
    - Omics, complex data sets
    - ? 4-D imaging
    - Cryo-EM
    - µCT

SPACE BIOFILMS
BIOSERVE • NASA • DLR • MIT • U SAARLAND • UVG

Source: Coenye,T..Kjellerup,B., Stoodley,P, et.al.; ***The future of biofilm research – Report on the '2019 Biofilm Bash'***; Biofilm. 2020 Dec; 2: 100012

The Future

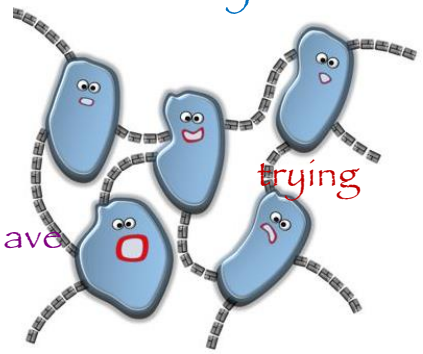
- Questions at the edge of the envelope;
  - Antimicrobial/biofilm interaction
    - We still do not know how this works
    - ? Add 'adjuvant'
    - ? Add 'potentiators'
    - ? 'immunomodulation'
    - ↓ bacterial adhesion and biofilm build up
    - ? 'probiotics'
- Biofilms are the predominant



Source: Coenye,T..Kjellerup,B., Stoodley,P, et.al.; ***The future of biofilm research – Report on the '2019 Biofilm Bash'***; Biofilm. 2020 Dec; 2: 100012

The Future

- Biofilms are the predominant lifestyle of the predominant life-forms on earth (microorganisms) !!
- Through this modality, microorganisms thrive in every spot on our planet that can sustain any definition of life
- When we encounter microorganisms, we are hoping to defeat 3Billion years of evolution with our few million years of evolution
  - It is just **unlikely**
  - We have just covered MANY ways that we are trying
  - But it is taking almost EVERY technology we have
  - Good Luck . . . . .



Source: Coenye,T..Kjellerup,B., Stoodley,P, et.al.; ***The future of biofilm research – Report on the '2019 Biofilm Bash'***; Biofilm. 2020 Dec; 2: 100012

Questions