Osteomyelitis:
The Slime Layer
(Biofilm)

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Overview

- Review the complex structure and dynamics of biofilms.
- Discuss the organisms that produce biofilms and the infections associated with biofilm formation.
- Describe how biofilms render antimicrobials and host defenses less effective and novel approaches to circumvent these obstacles.

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Biofilm

- ‘A structured community of bacterial cells enclosed in a self produced polymeric matrix, adherent to an inert or living surface.’
  - not only bacteria, fungi also form biofilms
  - most adhere to a surface but can also be free-floating
- Biofilm extracellular matrix is composed of:
  - Microbial derived products:
    - polysaccharides
    - nucleic acids (DNA &RNA)
    - proteins
    - lipids
  - Host products:
    - fibrinogen
    - fibronectin
    - platelets
    - collagen
- Extracellular Polymeric Substances (EPS)=Matrix=Glycocalyx=Slime Layer

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S. Aureus Biofilm on an Indwelling Catheter

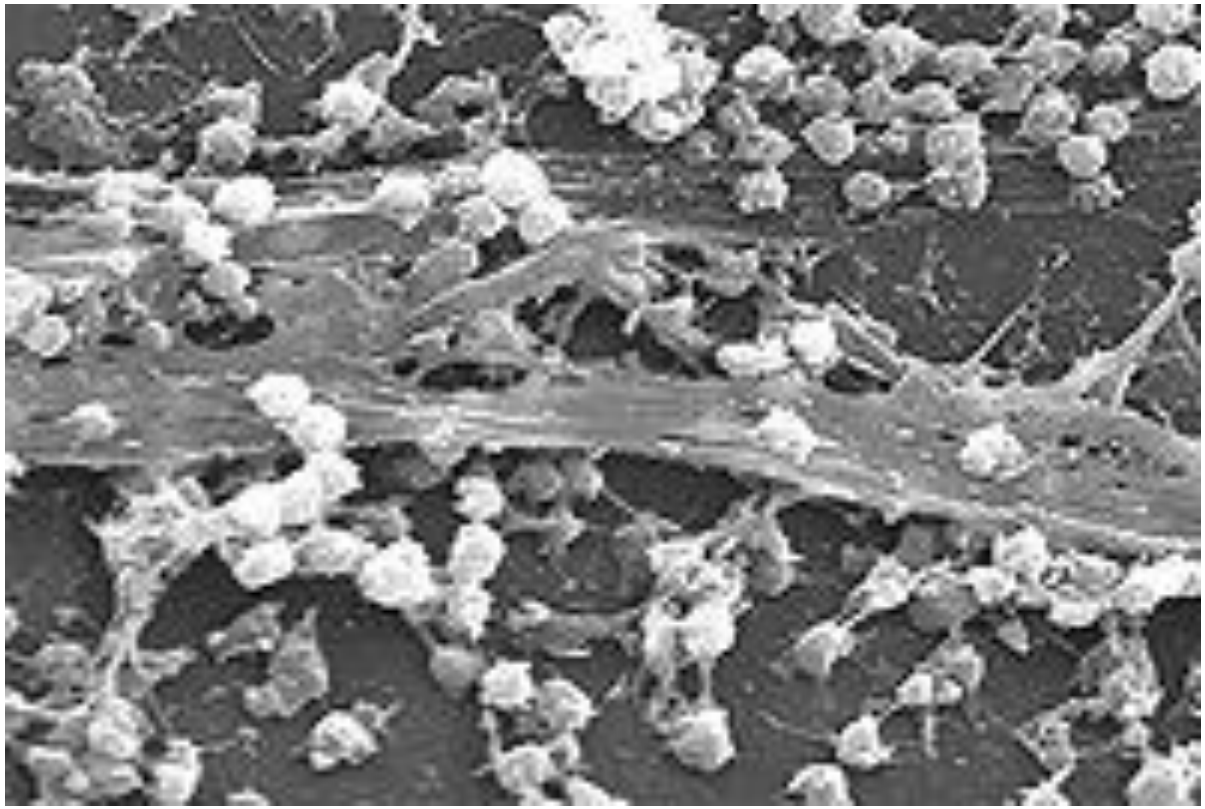

Image credit: CDC/ Rodney M. Donlan, Ph.D.; Janice Carr (PHIL #7488), 2005.

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Stages of Biofilm Formation

- Adhesion Stage
  - Planktonic (single, independent) microbes attach to surfaces (or to each other) via adhesins.
  - Surfaces can be:
    - Synthetic surfaces
      - medical devices
    - Damaged tissue
      - CF lungs
      - infected bone, sequestra
      - chronically infected sinuses
    - Healthy mammalian tissue

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Stages of Biofilm Formation

- Maturation Stage
  - Microbes multiply and secrete EPS and form micro-colonies which grow in size and coalesce to form macro-colonies.
  - The microbes and their extracellular matrix form a complex structure w/ channels enabling delivery of O2 & nutrients, removal of waste products & communication via signaling compounds.
  - This structure adapts to nutritional and environmental conditions.

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Quorum Sensing System

- A chemical cell to cell signaling mechanism used for the coordinated production of biofilms.
- Bacteria can sense each other by taking up small molecules secreted by other nearby bacteria.
- Quorum refers to the minimum number of bacteria aggregated within a specific volume that is required to switch on QS-controlled genes
- QS-controlled genes code for the production:
  - EPS components
  - Virulence factors
- Quorum sensing allows bacteria to act as a population instead of as individuals.

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Stages of Biofilm Formation

- Detachment/Dispersal Stage
  - Micro-colonies (or even a single motile cell) may detach under the direction of mechanical fluid shear or through a genetically programmed response that mediates the detachment process
  - These detached cell(s) then travel to other regions of the host, attach and promote biofilm formation in previously uninfected areas.
  - Maintains biofilm equilibrium (growth and dispersal)

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Biofilm Microbes

- Planktonic cells-single, independent cells that can float/swim in liquid medium
  - Inhabit biofilm surface
  - Exponential growth phase (actively replicating)
- Sessile cells – attached (persisters)
  - Found deeper in biofilms
  - Stationary (dormant) growth phase
    - likely induced by limited nutrients and oxygen w/in the biofilm
- Bacteria shift from sessile to planktonic modes of growth in response to different environmental cues
- Biofilms make in-vitro active antimicrobials less effective,
  - When the biofilm is disrupted, antimicrobial activity returns

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Biofilm Advantages

- The extracellular matrix seizes and concentrates environmental nutrients (carbon, nitrogen & phosphate)
- The biofilm acts as a barrier slowing down the diffusion & infiltration of some antimicrobial agents & host immune components
- The biofilm milieu renders antimicrobials and host defenses less effective
  - Binds free oxygen radicals secreted by PMNs
  - eDNA neutralizes aminoglycosides
  - Low ph inhibits activity of some antimicrobials
- Stationary growth phase limits effectiveness of antimicrobials that require actively replicating cells
  - Persisters can withstand concentrations of bactericidal abxs 1,000 fold above the MIC of their planktonic counterparts.
- The biofilm provides an enduring source of microbes that is resilient against antimicrobial agents and host defenses, while simultaneously enabling continuous shedding & bacterial spread.

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Organisms that Commonly Produce Biofilms

- Bacteria
  - Gram positive
    - S. aureus
    - S. epidermidis
    - E. faecalis
    - S. viridans
  - Gram negative
    - P. aeruginosa
    - E. coli
    - K. Pneumoniae
    - Proteus mirabilis
- Fungi
  - Candida spp.

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Biofilm-associated Infections

- Can be mono or poly-microbial
- Often chronic and recurrent
- Frequently associated with medical devices
  - Devices become coated w/ host proteins soon after placement and these proteins provide an excellent site of attachment for microbes.

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Biofilm-associated Infections of Indwelling Medical Devices

- Catheters
  - Central venous, urinary and PD
- Other tubes
  - Nephrostomies, cholecystostomies, shunts, Endotracheal, A-V grafts
- Prostheses
  - Joints, heart valves
- Other
  - Cardiac pacemakers and leads, orthopedic hardware, IUDs

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Biofilm-associated Infections of Native Host Tissue

- Chronic & recurrent infections:
  - osteomyelitis, wound infections, otitis media, rhino-sinusitis, prostatitis, UTIs, lung infections in CF pts.
- Native valve endocarditis
- Dental caries and peridontitis

Biofilm Microbe-Detection

- Biofilms make pathogen detection difficult.
- Isolation of bacteria using standard culture methods often fails.
- Biofilm bacteria are not unculturable, they have to be released from the surface, tissue or aggregate using ultrasound (sonication).
  - Prosthetic implants
  - Vascular catheters

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Treatment

- The most effective treatment for a biofilm infection is to mechanically remove the infected area &/or infected device.
  - In osteomyelitis, this means debridement of all infected bone.

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Antibiotic Treatment

- Most abxs kill planktonic bacteria released from the biofilm but not those embedded in the biofilm.
- Certain abxs are more effective against biofilm embedded bacteria.
  - Daptomycin
  - Tigecycline
  - Minocycline
- Addition of Rifampin improves efficacy
  - PVE
  - PJI
  - There is a lack of compelling clinical information to support using rifampin combination therapy for osteomyelitis.

Novel Treatment Approaches to Biofilm-associated Infections

- Interfere with Biofilm Production
  - Adhesion Stage: Anti-adhesins
  - Maturation Stage: Quorum sensing inhibitors
- Biofilm Disrupting Agents
  - render biofilm bacteria S to killing by abxs and host immune defenses by increasing penetration
  - interfere with quorum sensing
  - Degrade eDNA
  - Cause dispersal
    - sessile become planktonic microbes
- Alter ph-alkalinize biofilm environment to make abxs more effective
  - Aminoglycosides, Quinolones

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Prevention

- As with all things difficult to Rx, prevention is a good approach.
  - Surface modification of medical devices
    - Coat catheters & implantable medical devices w/ abxs, ions and/or anti-adhesins
      - Minocycline-Rifampin coated cvc's
      - Silver-coated urinary catheters
    - Make implants from material more R to biofilm formation
      - Titanium > stainless steel
  - Vaccine development against biofilm producing organisms
    - Most work done against S. aureus but unsuccessful to date in finding one that provides lasting immunity.

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Prevention

- The best example of prevention is our daily oral hygiene to combat dental biofilm formation. Caries are preventable if the teeth are cleaned mechanically to remove any biofilm using toothbrush, flush (waterpik) & floss.

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Summary

- Biofilm-associated infections pose a significant and difficult to address problem.
- Standard modes of detection and treatment are often unsuccessful.
- Physical removal of infected material is the current cornerstone of Rx.
- New approaches to Rx and prevention are being explored.

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