Outline

- The Problem
- Ignoring Your Hosts
- Good Intentions (past/existing approaches)
- Emerging Biofilm Control Strategies
- Summary
BIOFILMS AND DEVICE CENTERED INFECTIONS

August, 2010

Biofilms and Device Centered Infections

Hospital Acquired

Nosocomial Infections

4th Leading Cause of Death in U.S.

1.7 million infections per year; 99,000 deaths/year

Adds ~ $1 BILLION Costs to Health Cost System per year

60-65% related to biomedical implants

Centers for Disease Control & Prevention, 2002

Artificial Ear
Cochlear Implant
Nasal Implants
Dental Materials
Mandibular Mesh
Artificial Heart
Vascular Grafts
Pacemaker

Glucose Biosensor
Hip Implant
Finger Joint

Artificial Liver
Artificial Skin
Dialysis Shunts, Catheters

Ocular Lens, Contact Lens
Artificial chin
Shoulder prosthesis
Birth Control Implant
Breast Prosthesis
Blood substitutes
Spinal fixation
Artificial Kidney
Testicular prosthesis
Temporary Tendons

Adsorbable Pins
Cartilage Replacement
Artificial Leg
Ankle Implant

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Biofilms and Device Centered Infections

Biofilm Formation

Examples: Devices INFECTIONS

Bacteria on
Cardiac Pacemaker  Bacterial Colonizing Intrauterine
Contraceptive Device (IUD)  Infections of Contact Lens ...

...leads to Keratitis of eye

Bacteria on a Artificial Hip Replacement
Examples: Devices INFECTIONS

A. *P. aeruginosa* corneal ulcer associated with extended-wear soft contact lens.
B. 3 months after treatment. Final visual = 20/50.
## Rates of Bloodstream Infections

<table>
<thead>
<tr>
<th>Device</th>
<th>Number of Device-Related Bloodstream Infections Per 100 Catheters</th>
<th>Number of Device-Related Bloodstream Infections Per 1000 Catheter-days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral venous catheters</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Arterial catheters</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Central venous catheters</td>
<td>3.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Pulmonary artery catheters</td>
<td>1.9</td>
<td>5.5</td>
</tr>
<tr>
<td>Hemodialysis catheters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-cuffed</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>cuffed</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Peripherally central catheter</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Subcut. Central venous port</td>
<td>5.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**BSI’s Mortality**
12-25%

### The Usual Suspects

**Central Venous Catheters:** *Staphylococcus epidermidis, Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterococcus faecalis*

**Mechanical Heart Valves:** *S. epidermidis, S. aureus, Streptococcus spp., gram-negative bacilli, diphtheroids, enterococci, and Candida spp.*

**Urinary Catheters:** *S. epidermidis, E. faecalis, E. coli, Proteus mirabilis, P. aeruginosa, K. pneumoniae, and other gram-negative organisms, Candida spp.*

**Orthopedic & Spinal Implants:** *S. epidermidis*

**Ventricular assist devices:** *S. epidermidis, S. aureus, Candida albicans, P. aeruginosa, K. pneumoniae, and E. faecalis*
**Processes Governing Biofilm Formation**

- **Biofilm Measure**
  - Adsorption
  - Macromolecules
  - Reversible Adhesion
  - Desorption
- **Nutrient Metabolism**
  - Cell Recruitment
  - Platelets
  - Nutrients, O2
  - Detachment
  - EPS matrix
- **Cell:cell Signaling**
  - EPS secretion
  - Platelets
  - Nutrient Metabolism
  - S U B S T R A T U M


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Antibody Mediated Phagocytosis

Opsonization

IgG Antibody

Fc receptor

Low pH, O\(^{•}\), H\(_2\)O\(_2\), dismutases

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Biofilms and Device Centered Infections  

Evading the Immune System

- Adhesion to substratum may prohibit COMPLETE OPSONIZATION of bacterial membrane; prevents phagocytosis.
- Polysaccharide matrix prevents opsonization or complement binding.
- Bacterial lipopolysaccharides (LPS) are endotoxins; inhibit phagocyte chemotaxis (Bignold et al., 1991).
- Secrete proteins that prevent ICAM selectin binding
- Secrete Chemotaxis inhibitory protein to prevent detection of formylated proteins & complement factor C5a
- Secrete enzymes to degrade IgG & Complement
- Secrete Protein A or G; binds IgG by Fc portion negating opsonization
- Secrete quorum signals which are apototic to macrophage
- Generate super-antigens preventing antibody generation
- D-alanine modifications to cell wall resist endosomal degradation
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Negating Biofilm Formation

- Substratum Pre-conditioning: Change surface chemistry; surface topography; non-fouling surfaces
- Cell Deposition: Maintain sterility ???
- Reversible Adhesion: Change surface chemistry or surface topography. Tethered or released toxic agent(s). Base material degradable. External toxic challenge.
- Irreversible Adhesion
- Desorption
- Cell Signaling: Signal analogs; receptor blocking.
- Cell Metabolism: Antibiotic challenge; starvation, chelator challenge; viral infection, device surgical removal
- Biofilm Removal

Medical community relies on antibiotics
Minimum Inhibitory Concentration of an Anti-biotic

A. 10 µg/mL
B. 1.0 µg/mL
C. 0.1 µg/mL

Time = 04 hrs.

Antibiotic Systemic Approaches

T=0.
T=2 hrs.
T=6 hrs.
T=12 hrs.
Antibiotic Futility: Why?

- Reduced Cell Metabolism
- Mass Transfer Reaction Limitations
- Phenotypic Changes/Altered Gene Expression

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Existing Approaches to Negate Biofilms

Surface modifications

Non-fouling coatings

Surface Chemistry Modifications

RF Plasma Gas Deposition


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Bovine serum albumin adsorption measured by surface plasmon resonance (SPR)

Surface Chemistry Modifications on *Ps. aeruginosa* Adhesion

“Glymes” = CH\(_3\)\(-(0-CH\(_2\)-CH\(_2\))\_N-O-CH\(_3\)
**Other “Non-fouling” Prospects**

Long-chain zwitterionic polymer-coated surfaces grafted via atom transfer radical polymerization (ATRP)

![Chemical structures of poly(Sulfobetaine methacrylate) pSBMA and poly(Carboxybetaine methacrylate) p(CBMA)]


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**“Non-fouling” Zwitterion polymer coatings**

**Existing Approaches to Negate Biofilms**

- Non-fouling coatings
- Antibiotics; Ag\(^{+2}\), Nitric Oxide
  - Controlled drug release
- Antibiotics
- Quaternary ammonium compounds

**Sustained Release Biomaterials**

<table>
<thead>
<tr>
<th>Concentration (Concn.)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bryers, JD E-mail: jbryers@u.washington.edu
Sustained Release Biomaterials: Assessment methods

Sustained Release Biomaterials: Preferred Method

Flow Cell Adhesion Studies

Residence time ~ secs

Flow cell with sample

Cell Conc., (cells/mL)

Live-Suspd.

Dead-Suspd.

Time, (days)
Parallel Plate Flow cell

Flow Cell for Adhesion & Biofilm Studies
**Sustained Release Biomaterials**

**Example:** Plasma deposited Barrier Membrane controlled Ciprofloxacin release

PEU = base material

pBMA = barrier membrane

**Different pBMA coating Effects on Ciprofloxacin Release Rates.** Different coatings as a function of plasma conditions. Plasma pressure = 150 mT. Power setting/application durations are: **a.** 40W/20min; **b.** 40W/15 min; **c.** 20W/15 min; **d.** 40W/5 min; **e.** 20W/5 min; **f.** Uncoated.

Biofilms and Device Centered Infections

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pBMA-PEU Ciprofloxacin™ Film at Time = 24.0 hr.
Initial Shear Stress= 2.0 N/m²; Suspended Cell Conc.,
5x10⁸ cells/mL.


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Sub-lethal doses of Anti-Biotics

A. 
EIOH ppt of EPS from PA Biofilm. LEFT exposed to imipenem (1.0 μg/mL) for 24 hours. RIGHT = No antibiotic.

B. 
PA biofilm exposed to 0.5 μg/mL imipenem (a) never, (b) 18hr, and (c) 37h.

C. 
P. aeruginosa biofilm
0.  0.1  0.2  0.3  0.4 µg/mL tobramycin

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## Emerging Alternatives

- **Bacteriophage**
- **Bacteriophage lytic enzymes**
- **Photo-activated surface catalysts**
- **Antimicrobial peptides (defensins, histatins)**
  - Inhibit Quorum Sensing
  - Iron Disruption of Biofilm Formation
  - Adhesion Blockers
  - Engineered Innate Response (Enhanced Phagocytosis)
  - Engineered Infection Immunity
  - Healing

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Kaneko, Y. et al., The transition metal gallium disrupts Pseudomonas aeruginosa iron metabolism and has antimicrobial and antibiofilm activity, J Clin Invest. 2007 Apr;117(4):877-88.

Gallium or Zn replacement of Iron
(Ga does not undergo redox reactions like iron; thus stops many enzymatic reactions)
Ga-ion or Ga-siderophore (bacterial metal chelator)

Emerging Alternatives

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Bacteriophage lytic enzymes
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Inhibit Quorum Sensing
Iron Disruption of Biofilm Formation
Adhesion Blockers
Engineered Innate Response (Enhanced Phagocytosis)
Engineered Infection Immunity
Healing
Enhanced phagocytosis

Adhesin receptor

Bacteria

Monocyte/macrophage

Enzyme-Linked Immunosorbent Assay

Enhancing Innate & Adaptive Immunity

Activated dendritic cell

Prime CD8+ T and CD4+ T/B cells

Immature dendritic cell

Vaccine against bacterial adhesin

Enhanced phagocytosis

- Artificial "Opsonins"

Bi-functional chemical linker

Bi-specific fusion molecules

MØ

Mabs, F(ab')2, Fab, or aptamers


Enhanced Phagocytosis - Artificial “Opsonins”

Vancomycin
M.W. 1485.7

Vancomycin recognizes peptides terminated in D-Ala-D-Ala on most Gram-positive bacteria

Broad & Specific Recognition by Vancomycin

Vancomycin-BODIPY binding to various SA & SE strains; PA negative control

At 50 nM vancomycin-BODIPY, competitive inhibition of binding by cell wall peptide analog, acetyl-Lys-D-Ala-D-Ala
MØ + BiFM treated SE; stained with pHrodo

Enhancing Innate & Adaptive Immunity

Prime CD8+ T and CD4+ T/B cells

Vaccine against bacterial adhesin

Immature dendritic cell

Activated dendritic cell

Monocyte/macrophage

Adhesin receptor

Fc receptor

Enhanced phagocytosis

Bi-specific fusion molecules

Vaccine against bacterial adhesin
## Biofilm "Factors" as Potential Antigen Targets

### S. epidermidis
- Auto-adhesions, AltE & Aae
- FGN-binding protein, SdrG
- Collagen binding, GehD
- Polysaccharide intercellular adhesin, PIA
- Accumulation association protein, Aap

### Ps. aeruginosa
- IIII mannose binding lecition
- FN binding protein
- binding to G4 glycolipid on cornea

### Enterococcus faecalis
- Collagen Type 4 binding Ace
- LN Type 1 binding Ace

### S. aureus
- FN receptors (FN_a & FN_b)
- FGN-binding protein
- Collagen binding CnA receptor
- Bap

### Candida albicans
- Als adhesin

### Group B Streptococcus
- FN receptor
- CSa peptidase
- Keratin binding Srr-1
- LN-binding Lmb
- Human C4 binding protein BibA

### P. gingivalis
- Arg-specific (RgPA) proteases
- Lys-specific (KgP) proteases

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## Directed Antigen Presentation

<table>
<thead>
<tr>
<th>CD+8 T Cells</th>
<th>CD+4 T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC-I Complex</td>
<td>MHC-II Complex</td>
</tr>
<tr>
<td>DNA or mRNA Vaccine generated Protein</td>
<td>Exogenous Antigen</td>
</tr>
<tr>
<td>Endogenous Antigen</td>
<td>MHC-I Pathway</td>
</tr>
<tr>
<td>Proteasome peptides</td>
<td>MHC-II Pathway</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>Endoplasmic Reticulum</td>
</tr>
</tbody>
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Emerging Alternatives

- Bacteriophage
- Bacteriophage lytic enzymes
- Photo-activated surface catalysts
- Antimicrobial peptides (defensins, histatins)
- Stop Quorum Sensing: Analogs vs Degraders
- Iron Disruption of Biofilm Formation
- Adhesion Blockers
- Engineered Innate Response (Enhanced Phagocytosis)
- Engineered Infection Immunity
- Healing

Prevention: Things to Consider

- **Device type:** Short-term vs. Long-term, Indwelling
- **Device cost:** Catheters vs. Tissue-engineered scaffold
- **Bacterial Source:** Airborne vs. Fluid phase, systemic
- **Biofilm Culture:** Pure vs. Mixed culture
Summary

- Biofilm bacteria are phenotypically different vs. floating bacteria
- Current health care approaches to clean & sterilize have done little to prevent nosocomial infections.
- Biomaterials technologies using disinfective rinses, tethered or released antibiotics have also done little to reduce the nosocomial epidemic.
- Current “delivery” methods of anti-microbial therapies are passive diffusion or “controlled-barrier” + diffusion. No technology exists to sense and respond to colonizing bacteria.

National Institute of Allergy and Infectious Diseases (NIAID)
- 1R01AI074661 - Biomaterials that Promote Infection Immunity
- 1R03AI079461 - Effects of Shear on Specific Adhesion of Staphylococcus epidermidis

National Institute of Biomedical Imaging and Bioengineering (NIBIB)
1R01EB007575 - Biomaterials that Prevent Biofilm Colonization and Infections using Anti-virulent Approaches (BRP)

National Institute of Dental and Craniofacial Research (NIDCR)
1R01DE018701 - Periodontal Biomaterials with BITE (Biofilm Immunity via T-cell Enhancement)
1R01DE021373 - Metal-titanates as Novel Inhibitors of Cariogenic Biofilms

National Institute of General Medical Sciences (NIGMS)
1R01AI084856 - Resolving Biomaterial Inflammatory Response by Controlling Macrophage Phenotype