What is a Biofilm?

A structured community of bacterial, fungal, or other cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface.

Biofilms in the News

1. Evolution of Hyperswarming Bacteria Could Develop Anti-Biofilm Therapies
   Aug. 15, 2013
2. These Bacteria Are Wired to Hunt Like a Tiny Wolf Pack
   July 24, 2013
3. Bacteria Commonly Found in Drinking Water Creates Conditions Which Enable Other — Potentially Harmful — Bacteria to Thrive
   Aug. 14, 2013
4. Keeping beverage manufacture safe by preventing contamination
   Aug. 15, 2013
5. Coating being developed to reduce implant infections
   Aug. 1, 2013
6. Removing bacterial biofilms could help prevent and treat colon cancers, study suggests
   Aug. 1, 2013
7. Published on May 8, 2015
Outline

- Introduction
- Biofilm Development
- Implications in the Urinary Tract
- Prevention Strategies
- Treatment
- Future Directions

Discovery of Biofilms

- 1960s: Dental disease
- 1970s: Cardiac pacemaker
- 1976: Legionnaire's Disease
- 1980s: Lungs of patients with Cystic Fibrosis

Biofilms are Prevalent

- NIH estimates biofilm involvement in 65-80% of all infections
  - Chronic & acute infections
  - High incidence on catheters, stents, implants
- Most research done on planktonic (free-floating) bacteria
- Bacteria in biofilms
  - Altered metabolism
  - Altered protein expression
  - Ability to remain quiescent
  - Resistance to antibiotics
Benefits of Biofilm Formation

- A surface-bound community has several advantages over a planktonic lifestyle
- Pathogen survival strategy

Pros and Cons of Biofilm Formation

**ADVANTAGES:**
- Nutrients concentrate at surface
- Protection against predation & external environment
- Pooling of resources (enzymes) from various bacterial species

**DISADVANTAGES:**
- Limited access to oxygen & water
- Waste accumulation inside biofilm

Biofilm Formation

1. Attachment
2. Growth
3. Dispersal
Biofilm Formation

1) Attachment:
- Creation of conditioning film of molecules on a wet surface
- Serves as a nutrient base for bacteria
- Planktonic bacteria adhere loosely and reversibly to surface
- Electrostatic attraction, Van der Waals forces, cell hydrophobicity

2) Growth: formation of glycocalyx leads to irreversible bacterial adhesion
- Biofilm bacteria secrete polysaccharide extracellular polymeric substances (EPS)
- Increases adherence among bacteria
- Offers a protective barrier
- Traps nutrients within matrix
- Facilitates communication between bacteria

3) Maturation & Dispersal
- Multiple organisms often incorporated into biofilm
  - Bacteria
  - Fungi
- Promotes additional cell adhesion
- Deeper bacteria often remain quiescent
- Surface bacteria may return to planktonic form & help disseminate infection
Altered Bacterial Behavior

- Quorum sensing
  - Bacterial colony responds to a critical level of cell density
  - Auto-inducer molecules exchange information

- Altered biofilm activities
  - Surface attachment
  - EPS matrix production
  - Secretion of virulence factors

- Altered gene expression
  - Phenotypic changes
  - Enzyme production up-regulated/down-regulated

Altered Bacterial Behavior

- Persister cells
  - 1-10% of the population becomes quiescent
  - Persister cells increase as population reaches a steady state
  - Cells are resistant to anti-microbials
  - State of quiescence is reversible
    - Contributes to chronic infections

Biofilm Resistance to Treatment

Biofilm Resistance to Antimicrobials

- Slower rate of growth & metabolism
- Inter-bacterial transfer of resistance genes
- Poor penetration of antimicrobials into matrix
- Matrix inactivates antibiotics
- Biofilm bacteria up to 1000x more resistant than planktonic cells

Urinary Tract: Natural Host Defenses

- Urine voiding
- Epithelial cell sloughing
- GAG mucous layer
- Immune response
- Low iron availability
- Increase concentration of urea

Catheter-Induced UTIs

- Disruption of bladder mucosa/glycosaminoglycan layer
- Mechanical & chemical irritation
- Promotion of bacterial adherence
Animal Models of UTIs

- Simple UTIs
  - 50% infection rate with bacterial infusion
  - Based on clinical signs and bacteria in urine
  - UTIs cleared in 2-3 days

- Altered bladder (foreign object/implant)
  - Biofilm developed <24h
  - Created infectious nidus
  - Near 100% of rats developed chronic UTIs
  - Complications commonly develop

Catheter-associated UTIs in Dogs

Incidence of catheter-associated urinary tract infection among dogs in a small animal ICU
JAVMA 2004

- 4/39 (10.3%) of dogs developed UTIs

- Low risk of infection if catheterized less than 3 days

Catheter-associated UTIs in Dogs

Frequency of UTIs in catheterized dogs.
JAVMA 2007

- Most common bacteria isolated:
  - Catheterized dogs (n = 147): Enterobacter, Staphylococcus
  - Non-catheterized dogs (n = 99): E. coli, Proteus

- Odds of developing UTI increased:
  - 20% for each year of age;
  - 27% for each day increase of catheterization
  - 454% with antibiotic use
Urinary Catheter-Associated Biofilm

The Road to Colonization

Catheter attracts bacteria:
- Irregular surface & roughness of catheter
- Narrow central channels to trap bacteria
- Charge & chemical make-up of material

Urinary Catheters: Bacterial Colonization

Extraluminal route:
- Organisms ascend around catheter at time of & after insertion
- Endogenous bacteria
- Primarily from GI tract

Intraluminal route:
- Organisms gain access to internal lumen
- Organisms introduced from exogenous sources
- Health care workers (hands)
Urinary Catheters

- Ascending bacterial movement:
  - Rapidly dividing bacteria spread along catheter surface in glycocalyx
  - Planktonic bacteria move ahead of biofilm in urine

- Planktonic bacterial continually shed into urine (asymptomatic infection)

- Adherence of bacteria to bladder surface (symptomatic infection)

Catheter-associated UTIs

- Most frequently isolated bacteria:
  - Escherichia coli
  - Enterococcus faecalis
  - Pseudomonas aeruginosa

- Strongest biofilm producers:
  - Proteus mirabilis
  - Enterococcus faecalis
  - Staphylococcus aureus
  - Candida tropicalis

- Strongest biofilms often mixed-species

Biofilm Detection

- Difficult to diagnose

- Imaging
  - Bright-field microscopy
  - Scanning electron microscopy
  - Confocal laser microscopy

- Molecular diagnostics
  - PCR: biofilm specific proteins
Biofilms in the Urinary Tract

Biofilm infections should be suspected when:

- Low-bacterial cell count cystitis
- Antibiotic success with negative urine culture
- Catheter-associated colonization & infections
- Antibiotic failures in culture-directed treatment
- Relapses after theoretically successful treatment

Prevention

- Aggressive staff hygiene/hand washing
- Vigilance to avoid contamination when manipulating catheter connection sites
- Replacement of infected catheters
- Avoid in-dwelling catheters

Prevention: Catheter Selection

Hydrophilic-coated catheters:

- Intermittent catheterization:
  - Coated catheters: 64% developed UTIs
  - Uncoated: 82% developed UTIs

- Long-term catheterization (6 weeks)
  - No difference in symptomatic UTIs
  - No difference in microbiological urinalysis
Prevention: Catheter Selection


- Median CFU counts lower
- Less formation of biofilm
  - Evaluated by confocal laser scanning microscopy and electron microscopy
- No adverse events from catheter use

Prevention: Human Guidelines

2009 International Clinical Practice Guidelines from the Infectious Disease Society of America

- Reduction of inappropriate urinary catheterization
- Discontinuation of catheter as soon as possible
- Consider alternatives to indwelling urethral catheterization
- Systemic antimicrobial prophylaxis should not be utilized for catheterized patients

Current Biofilm Treatment

- Prolonged antibiotic use
- Increased antibiotic dosages
- Combination antibiotic therapy
- Removal of implants/foreign material
- Indwelling u-catheter care q 4 hr, exchange out q 48 hr
Biofilm Proposed Treatment Strategies

- Dissolution
  - Physical treatment
  - Photodynamic therapy
  - Low-energy surface acoustic waves (SAW)
  - Cytotoxic strategies

- Dispersion signals
  - Enzymatic activity
  - Presence of commensal flora

Photodynamic Therapy

- Uses non-toxic dye and low-intensity visible light
- In presence of oxygen produces cytotoxic reactive oxygen species
- Shows promise—especially in dental disease

Low-energy Surface Acoustic Waves

- Used in combination with antibiotics
- Period pulses of electrical energy transmitted to catheter
- Shown to prevent adhesion of planktonic bacteria
- Reduces size of biofilm
- Changes transcription pattern
SAW Affects on Bacteria

GM = Gentamicin, SAW = Surface Acoustic Waves

Kapel M., et. al., 2001

Signaling Networks

- Manipulation of enzymatic activities
- Expression of c-di-GMP-specific phosphodiesterases causes biofilm formation & suppresses motility
- Down-regulation may be key in biofilm treatment

Signaling Networks

- Inactivation of expression of key regulators in mature biofilms
  - bflS, bfmR, mifR
- Causes changes in phosphorylation patterns in Pseudomonas aeruginosa
- Results in architectural collapse & biomass loss

www.ScienceDaily.com, Jan 1, 2010
DispersinB

- Enzyme produced by periodontal “good” bacteria (Aggregatibacter actinomycetemcomitans)
- Causes hydrolysis of linkages in polysaccharide adhesions
- Alters biofilm integrity
- Results in biofilm degradation & bacterial dispersal

Other Considerations

- Beneficial Bacteria
  - Competitively inhibit bacterial growth
  - Prevent intrusion of pathogens
- Low-dose chemotherapy
  - Shown to affect biofilm formation
  - Azithromycin has been used for lung infections
  - Inhibits Pasteurella biofilm

Future Directions

- Evaluation of permanent implants:
  - Urethral stents
  - Ureteral stents
- MIC testing of biofilm-embedded bacteria
- Evaluation of new treatment modalities in animal patients
- Investigation of biofilms in spread of nosocomial infections
Conclusions

- Biofilms prevalent & contribute to chronic infections
- Catheters & other implants pose increased risk
- Treatment can be challenging
- New treatment modalities may offer new solutions for our patients

Special Thanks

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Questions?
References


