Life on the Edge: The Clinical Implications of Gastrointestinal Biofilm

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Microbiology Laboratory Artifacts

Microbes do not normally live in enriched media in petri dishes and flasks.

Microbes Prefer Life in Biofilms

- Attached collaborative communities
- Metabolically & genetically distinct from free-living planktonic kindred
- Ubiquitous in nature
- May be beneficial or pathogenic

Common Biofilms

Dental plaque biofilm

Used dental floss biofilm

What is Biofilm?

- Gathering of sessile microorganisms
- One or more species; multikingdom
- Heterogeneous communities
- Encased in self-produced matrix
- Hydrated exopolysaccharides
- Criss-crossed by microchannels
- Analogized to multicellular organisms
**Biofilm Morphology**

Confocal microscopy green fluorescent protein labeled organisms.

*Pseudomonas aeruginosa* biofilm


**Biofilm Micromorphology**

Schmutzdecke ("filth cover") - Slow sand filter

Adhesive Mucilage (Exopolysaccharides)

**Biofilm Cycle**

Quorum Sensing

- Cell-to-cell communication
- Mediated by hormone-like molecules — Autoinducers
- Gram-negatives
  - N-acyl-L-homoserine lactones, 4-quinolones, fatty acids, and fatty acid methyl esters
- Gram-positives
  - Oligopeptides
  - *Candida*
    - Phenylethyl alcohol, tryptophol, isoamyl alcohol, morphogenic autoregulating substance (small <10 kD, amine ring)
- Autoinducer-2 — Interconvertible furones
- Autoinducer-3 — Poorly characterized aromatic
  - Cross-talks with eukaryotic hormones
  - Senses metabolic status & stress

**Biofilm Matrix**

- Exopolysaccharides, exoproteins, Mg**, Ca**
- Gram-positive exopolysaccharides - cationic
- Gram-negative exopolysaccharides - anionic
- Exopolysaccharides vary by species
  - *E. coli* - colanic acid
  - *Pseudomonas aeruginosa* - alginate
  - *Streptococcus mutans* - mutan, fructan
  - *Candida albicans* - β-glucan
- Cellulose and poly-β-1,6-N-acetylglucosamine ubiquitous
- Surface exoproteins homologous with *Staph. aureus*
  - Biofilm-associated protein (Bap)
- Free DNA - native & foreign

**Why Live in Biofilm?**

- Protection
  - Dislodgment, Predation, Competitors, Toxins, Immune Responses, Antimicrobials
### Biofilm Protective Functions

- Antibody
- Phagocytic Enzymes
- Macrophage
- Antibiotic

**Costerton et al.** Science 1999;284:1318-22

### Other Advantages of Life in Biofilm

- Improved reproductive fitness
- Horizontal gene transfer
- Assimilation of external DNA
- Specialization of cells
- Optimal nutrient utilization
- Host status sensing
  - Metabolic sensing
  - Stress

**Schloss Neuschwanstein**

### Biofilm-Associated Human Infections

1. Chronic Sinusitis
2. CSF Shunt Infection
3. Contact Lens Keratitis
4. Chronic Otitis Media
5. Infected Cochlear Implant
6. Infected Burns
7. Vascular Catheter Infection
8. Endocarditis
9. Pacemaker Pocket Infection
10. Cardiac Lead Wire Infection
11. Biliary Stent Infection
12. Peritoneal Dialysis Catheter Infection
13. Prosthetic Joint Infection
14. Urinary Stent Infection
15. Intravascular Stent Infection
16. Cystic Fibrosis Pneumonia
17. Ventilator-Associated Pneumonia
18. Breast Implant Infection

**del Pozo & Patel** Clin Pharmacol Ther 2007;82:204-9

### The Intestinal Microbiota Organ

- Unique & vital organ
- Weighs 1.5 Kg
- Metabolic activity rivals liver
- Immune and GI maturation
- Colonization resistance
- Immune response modulation
- Nutritional support
- Prefers Life in Biofilm

**Colonic mucosa**

**Scanning Electron Microscopy**

### Gastrointestinal Biofilm

- Myriad of microenvironments
- Associated with mucosa, mucus layer & food particles
- Usually multispecies
- Barrier to pathogens
- Site of microbiota/host interaction
- Disrupted normal biofilm leads to disease


### Loss of Normal Biofilm Barrier

- Enteric aggregative E. coli infection
- Loss of microvilli
- Edematous villus
Altered GI Microbiota Biofilm Consequences

- Increased gut permeability
- Deranged immune responses
- Increased susceptibility to pathogens
- Impaired GI motility & function
- Increased proinflammatory cytokines
- Diminished toxin clearance
- Reduced phytonutrient availability
- Diminished omega-3 PUFA availability

Biofilm-Associated Gastrointestinal Disease

Barrett's Esophagus

Inflammatory Bowel Disease

Helicobacter pylori

- Spiral-shaped Gram-negative
- Produces large amounts urease
- CO$_2$ & NH$_3$ neutralize acid
- Harbored by 50% of people
- Associated with:
  - Gastritis
  - Gastric and duodenal ulcers
  - Gastric carcinoma
- 10-20% treatment failure rate

Helicobacter pylori Biofilm

- Urease (+) patients
  - 97.3% gastric mucosa covered by biofilm
- Urease (-) patients
  - 1.64% gastric mucosa covered by biofilm
- May explain persistence and treatment resistance

Clostridium species

- Sporulating Gram-positive
- Toxin producers
- C. difficile
  - 25-50% AAD
  - 95% Pseudomembranous colitis
- C. botulinum and C. tetani
  - Non-necrotizing neurotoxins
- High relapse rate for C. difficile

Clostridium Biofilm

- Possess biofilm genes
- Make biofilm in vitro
- Make biofilm in animals
- Biofilm may partly explain relapses

C. difficile
Disordered Gut Microbiota in Autism

- Clostridium & Ruminococcus spp.

- C. bolteae 46-fold, Clostridium cluster I 9-fold & Clostridium cluster XI 3.5 fold

- Clostridium histolyticum

Oral vancomycin improves neuropsychiatric Sx

Chronic Fatigue Syndrome (CFS)

- Debilitating fatigue
- Tender lymphadenopathy
- Memory and cognition difficulties
- Persistent muscle pain
- Joint pain
- Sore throat
- Sleep disturbances
- Symptoms following exertion
- Overlap with major depression, irritable bowel syndrome and fibromyalgia

Disrupted GI Microflora in CFS

- ↓ Bifidobacterium, ↑ Enterococcus
- Higher Enterococcus levels associated with greater confusion, forgetfulness, memory deficits, & anxiety
- ↑ D(-)-lactate producing Enterococcus & Streptococcus spp.
- ↓ E. coli populations
- ↑ Candida albicans in acute phase

Candida albicans

- Diploid fungus (yeast)
  Blastospore → Hyphae
- Morphogenesis & virulence
- Commensal in 80%
- Highly immunogenic
- Opportunistic infections
- Candidiasis sensitivity syndrome

Candida albicans Biofilm

- Biofilm formation universal
- Activates resistance genes
- Upregulates drug efflux pumps
- Biofilm may explain systemic Yeast Syndrome Sx, CFS
- Biofilm may explain high recurrence rate post Rx

Candida and CFS: Possible Mechanisms

- 178 antigens
- Hyphal wall protein 1 homologous to α- & γ-gliadin
- Glycoproteins stimulate histamine release
- Glycoproteins stimulate prostaglandin E2
- Protease degrades IgA1, IgA2 & sIgA
- Protease induces polyclonal B cell response
Strategies to Disrupt Pathogenic Biofilm

- Enzymes
  - Carbohydrases
  - Proteases
- Chelating agents
  - EDTA
  - Lactoferrin
- Probiotics
- Prebiotics

Carbohydrases

<table>
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<tr>
<th>Cellulase Concentration (units/mL)</th>
<th>Areal Biomass (mg/cm²)</th>
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Cellulase inhibits *Pseudomonas aeruginosa* biofilm

* pH=5  * pH=7


Proteases

Serratia peptidase inhibits *Pseudomonas aeruginosa* biofilm

No Serratia peptidase  Serratia peptidase

37°C, 120 mL/h flow, MIC ofloxacin


Combination Enzyme Formulation*

- Carbohydrases
  - Cellulase
  - Glucoamylase
  - beta-Glucanase
  - Chitosanase
  - Hemicellulase/Pectinase Complex
- Proteases
  - Serratia Peptidase
  - Protease/Peptidase Complex
- Lysozyme

*US Patent Pending

Combination Enzyme Biofilm Testing

Inhibition of *Klebsiella pneumoniae* biofilm

Statistics*

<table>
<thead>
<tr>
<th>Log Reduction</th>
<th>Plate-Enzyme Log Reduction (GC - Test)</th>
<th>Log Reduction vs. GC</th>
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*US Patent Pending
Combination Enzyme Formulation* & *H. pylori

*US Patent Pending

H. pylori Biofilm

Post Enzymes

SEM x1000

SEM x150

SEM x5000

SEM x2500

Combination Enzyme Formulation* & *H. pylori

Use with Amoxicillin

*US Patent Pending

Amoxicillin (µg/mL)

Log Reduction

1.000 0.120 0.015 0.001

SEM x100

SEM x500

SEM x1000

Combination Enzyme Formulation* & *H. pylori

Use with Clarithromycin

*US Patent Pending

Clarithromycin (µg/mL)

Log Reduction

1.000 0.120 0.015 0.001

SEM x100

SEM x500

SEM x1000

Combination Enzyme Formulation* & *H. pylori

Use with Tetracycline

*US Patent Pending

Tetracycline (µg/mL)

Log Reduction

1.000 0.120 0.015 0.001

SEM x100

SEM x500

SEM x1000

Synergism of Enzyme Formulation & Tetracycline in *H. pylori* Biofilm

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Enzymes

Enzymes EDTA

25-32x

2000x

7000x

TCN 0.01 µg/mL

TCN 0.01 µg/mL Enzymes 12.5 mg/mL

Combination Enzyme Formulation* & *C. difficile

*US Patent Pending

Log Reduction

6.25 mg/mL

Enzymes

Enzymes EDTA

EDTA

108

1.00
**Combination Enzyme Formulation** & *C. difficile*

Use with Vancomycin

*US Patent Pending

**Log Reduction**

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<th>µg/mL Vancomycin</th>
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**Combination Enzyme Formulation** Spectrum

*Documented in vitro* Antibiofilm Activity Against:
- Escherichia coli O157:H7
- Helicobacter pylori ATCC 43504
- Gardnerella vaginalis ATCC 14018
- Klebsiella pneumoniae ATCC 4352
- Pseudomonas aeruginosa ATCC 27853
- Pseudomonas aeruginosa PAK FKCC4-003
- Clostridium difficile ATCC 9689
- Staphylococcus aureus ATCC 29213
- Staphylococcus aureus MRSA 399
- Staphylococcus aureus MRSA U of C #13
- Streptococcus pneumoniae ATCC 10015
- Streptococcus pyogenes ATCC 10096
- Candida paratropicalis ATCC 99916

*US Patent Pending

**Combination Enzyme Formulation** Spectrum

No Adverse Effect on Healthy Biofilm *in vitro*:
- Bifidobacterium bifidum PT131
- Bifidobacterium breve PT132
- Bifidobacterium longum PT136
- Lactobacillus casei PT116
- Lactobacillus rhamnosus PT112
- Lactobacillus salivarius ATCC 29602

*US Patent Pending

**Ethylene Diamine Tetraacetic Acid**

EDTA inhibits urinary catheter biofilm formation.

- EDTA catheter flush
- Saline catheter flush

Sheep model chronic indwelling urinary catheter.

**Lactoferrin**

Lactoferrin inhibits *Pseudomonas aeruginosa* biofilm.

- No lactoferrin
- Lactoferrin 20 mcg/mL

Singh et al., Nature 2002;417:552-5
Probiotics

Biofilm Inhibition by Surfactants from Lactobacillus acidophilus.

<table>
<thead>
<tr>
<th>Biofilm strain</th>
<th>Control</th>
<th>LAA H-1</th>
<th>LAA 336</th>
<th>LAA Ch-2</th>
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<tr>
<td>SAU-ATCC 29213</td>
<td>14.6 ± 1.32</td>
<td>6.74 ± 1.64</td>
<td>9.7 ± 1.64</td>
<td>10.1 ± 3.07</td>
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<tr>
<td>SAU-1474/01</td>
<td>12.7 ± 4.65</td>
<td>5.37 ± 1.32</td>
<td>11.3 ± 7.37</td>
<td>8.22 ± 2.42</td>
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<tr>
<td>SAU-A3</td>
<td>27.8 ± 5.97</td>
<td>9.87 ± 2.25</td>
<td>23.2 ± 2.59</td>
<td>11.1 ± 2.02</td>
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<tr>
<td>SEP-6756/99</td>
<td>36.3 ± 3.57</td>
<td>14.8 ± 2.02</td>
<td>18.9 ± 2.22</td>
<td>20.4 ± 3.63</td>
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<td>SEP-4Ac</td>
<td>15.8 ± 5.90</td>
<td>7.53 ± 1.54</td>
<td>8.20 ± 1.79</td>
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<td>SEP-RP12</td>
<td>35.5 ± 7.45</td>
<td>5.47 ± 1.69</td>
<td>9.06 ± 5.13</td>
<td>5.68 ± 5.35</td>
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Prebiotics

Alteration of Colonic Biofilm Communities using Inulin

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<th>Bacteria</th>
<th>Control</th>
<th>Prebiotic</th>
<th>P</th>
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<tr>
<td>Total anaerobes</td>
<td>8.7 ± 0.1</td>
<td>8.6 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Facultative anaerobes</td>
<td>6.4 ± 0.3</td>
<td>5.9 ± 0.4</td>
<td>NS</td>
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<tr>
<td>Bifidobacteria</td>
<td>5.2 ± 0.3</td>
<td>6.4 ± 0.3</td>
<td>P&lt;0.05</td>
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<tr>
<td>Eubacteria</td>
<td>4.6 ± 0.3</td>
<td>6.1 ± 0.3</td>
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<td>Clostridia</td>
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<td>Lactobacilli</td>
<td>3.1 ± 0.1</td>
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<td>Bacteroides</td>
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<td>Enterobacteria</td>
<td>6.4 ± 0.3</td>
<td>5.9 ± 0.4</td>
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</table>


Proposed Protocol for Managing Pathogenic Gastrointestinal Biofilm

- Disrupt pathogenic biofilm
  - Carbohydrase/protease enzyme complex
  - EDTA
  - Lactoferrin

- Kill pathogens
  - Saccharomyces boulardii
  - Oil of oregano, berberine, undecylenic acid, GTE
  - Prescription antimicrobials

- Administer enzymes plus antimicrobials fasting
- Begin with low dose & titrate up
- Duration of Rx depends on pathogen

Gastrointestinal Biofilm Conclusions

- Biofilm is the microbial preferred lifestyle
- Biofilms are ubiquitous
- Gastrointestinal microbiota lives in biofilm
- Healthy biofilm provides barrier function and modulates immune system
- Pathogenic biofilm is associated with GI and systemic disease
- Enzymes, chelating agents, pre- and probiotics may offer viable treatment modalities

Want to Know More About Biofilm?

To learn more about biofilm, contact:

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