Inflammation and the human microbiome –
Stunning revelations about how microbes actually create disease

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What is a biofilm?

A 3-Dimensional "community" of microbes attached to a surface
- fluid interaction
- channels / pores
- complex structure

Emerging Trends in Oral Care - Philips Oral Healthcare Symposium 2003

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What is a biofilm?
Where do biofilms form?
- solid sub-strata in contact with moisture
- soft tissue surfaces in living organisms
- liquid-air interfaces - the surface of the ocean

Biofilm facts
- rapid regeneration via signaling
- tenacious - difficult to remove mechanically
- resistant - immune system / antimicrobials
- high adhesion to saliva-coated enamel

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Strength
- multi-species
- inter-species cooperation
- intracellular matrix
- FEP - functionally equivalent pathogenicity

Mixed biofilm community - proportions
- mixed biofilm community - Candida albicans (opportunistic fungal pathogen)

Mixed biofilm community - Candida albicans (opportunistic fungal pathogen)
Moving biofilm “ripples” in mixed species biofilm

Viscoelastic Properties of S. aureus Biofilm

Growth and spontaneous detachment of cell cluster

Shear induced creep and failure

Understanding the trouble makers - microbial defenses that make it hard to treat disease
Layers

- surface microbes - most active
- most susceptible - antimicrobials/host defenses
- deeper layers - sheltered
  - less metabolically active
  - more resistant - antimicrobial therapies
  - can reconstitute biofilm (persisters)

pH and oxygen levels vary in biofilms

Resistance

- adapts to stresses
- resistant to
  - ultraviolet light
  - biocides
  - antibiotics
  - host defenses
- management - multiple, concurrent strategies

Antibiotic resistance - 1,000x greater

- as compared planktonic
- to antibiotics and biocides
- biofilm-related infections can’t be treated with conventional antibiotic therapy

Chronic wounds

- anaerobes - chronic wounds
- molecular techniques
- diverse populations
  - barrier to healing
  - susceptible to contamination
  - available substrate
  - surface for attachment

Host impairment

- repetitive trauma
- poor perfusion
- hyperglycemia
- presence of a foreign body
- white blood cell dysfunction

Chronic wound infections

- immune system can’t clear
- antimicrobial resistant
- systemic / topical
- frequent debridement critical
Chronic wound infections
- polymicrobial biofilms
- clinical aspects resemble other biofilm infections

Oral biofilm survival – making inflammation a lifestyle

Ecological plaque hypothesis – inflammatory periodontal disease

Inflammation
- originates - bacterial assault
- inflammatory response - most damage
- we used to think........... Only a handful of oral bacteria were implicated in periodontal disease

Red complex microbes
- T. denticola - long thin spirochete creates bridges
- P. gingivalis - grape-like cocobacillus surface vesicles
- T. (Bactericides) forsythus larger fusiform rod

Periodontitis
- requires susceptible host
- dysbiotic microbial communities
- inflation-philic
- inflammation - provides nutrients

Inflammation
- fosters dysbiotic growth
- selects for certain pathogens
- dysbiosis and inflammation support each other control of inflammation - critical
Polymicrobial synergy - dysbiosis (PSD) - model of periodontal disease etiology

Keystone pathogens

- P. gingivalis (Pg)
- T. denticola (Td)

Fusobacterium nucleatum (Fn)

- anaerobic oral commensal
- perio and endo pathogen
- increases with disease severity, inflammation, pocket depth
- higher in smokers, diabetics

Synergy

- motile
- creates pores - biofilm matrix
- moves through highly viscous environment
- pores enhance nutrient flow

Polymicrobial synergy

- P. gingivalis and T. denticola
  - superficial layers - subgingival plaque
  - chronic periodontitis lesions
  - increase biomass and thickness

Inflammation

- P. gingivalis
  - 4 hours after scaling
  - early, middle and late stage biofilm

Keystone pathogen

- P. gingivalis
  - low abundance microbe
  - modulates host response
  - impairs host immune system

Polymicrobial synergy - dysbiosis (PSD) - model of periodontal disease etiology

- tip the balance to dysbiosis
- pro-inflammatory microbial community
- elicit non-resolving, tissue-destructive host response

Keystone pathogens

- P. gingivalis

Polymicrobial synergy - dysbiosis (PSD) - model of periodontal disease etiology

- changes in microbiota composition
- increased biomass and thickness
- enhanced tissue-destructive host response
First parasitic relationship discovered

- Bdellovibrio - RNA fragment
- 700 genes - can’t make amino acids
- lives on Actinomyces odontolyticus (2,200 genes)
- nutrients from host, kills host, holes in A. odontolyticus


Parasitic outcomes?

- A. odontolyticus evades macrophages
- resistant to streptomycin
- high concentration - periodontitis and cystic fibrosis

Improving the patient’s outcome

- individual clinical expertise
- best external evidence
- patient values & expectations

http://med.hsu.edu/index.cfm?Page=medicalinformatics.alternative

Health history clues

- medications - poly pharmacy
- antibiotics - prolonged use compromise intestinal flora
- acid reducers - alter intestinal tract pH

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Saving lives
Links to systemic disease......what can a clinician do?

Evaluate individually
  › their risks
  › willing to do
  › motivated to change

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Individualize your recommendations
  › establish a dialogue with other healthcare providers

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Characteristics of biofilm infections

<table>
<thead>
<tr>
<th>General Characteristics</th>
<th>Unique Characteristics</th>
<th>Matrix Constituents</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of bacteria and extracellular matrix</td>
<td>Biofilm formation on surfaces</td>
<td>Extracellular polymeric substances (EPS)</td>
<td>Tissue, teeth, devices, implants</td>
</tr>
</tbody>
</table>

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Aging/inflammation....Gut dysbiosis?

Gut dysbiosis......Chronic inflammation?

Oral microbes ......impact

Oral microbes - systemic infections / inflammation

- cardiovascular disease
- adverse pregnancy outcomes
- rheumatoid arthritis
- inflammatory bowel disease
- colorectal cancer
- respiratory tract infections
- meningitis - brain accesses
- lung, liver, spleen accesses
- appendicitis

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Perio disease - bacteremia
- greater microbial diversity
- extra-oral link - association
- virulence varies
Cardiovascular disease – atherosclerosis

- Systemic spread of oral microbes - 1954
- Periodontal pathogens - Aa, Pg, Tt, Td, and Fn
- Acute and chronic inflammation
- Periodontal instrumentation (40%)
- Extractions (35%)
- Brushing (24%)
- Mastication (17%)

Cardiovascular disease - medical condition with a dental solution

- High risk PD pathogens
- Pathogen burden
- Causal - clinical grounds
- Contribute to ASVD
- Therapy - reduce risk

Inflammation - Pre-term birth

- Independent risk factor
- Severe or generalized - promotes PTB
- Young
- HIV-infected
- Preeclampsia
- Genetic predisposition

Pregnancy - Low birth weight - Association

- Bacteremia
- Inflammatory mediators
- Vagina to placental microbiome

Erectile dysfunction - positive association

- Common risk factors - diabetes, smoking, alcohol, heart disease
- Endothelial dysfunction - inflammation
- Periodontal - possible risk factor for ED

Macular Degeneration - Association

- Macular Degeneration
- Healthy eye
- Eye with degenerated macula
- Pg - can invade human retinal pigment epithelial cells
- Pg - elevate AMD-related genes

Pancreatic cancer risk

- Oral dysbiosis precedes cancer
- P. gingivalis - 59% higher
- A. actinomycetemcomitans - 50% higher

- Findings are not causal
- Susceptibility to inflammation?
- Microbes causing inflammation?
Esophageal squamous cell carcinoma risk

- P. gingivalis - 61% cancerous tissues
- 12% surrounding tissues
- associated with progression on ESCC
- findings are not causal
- PG a disease biomarker?


Alzheimer's disease

- Parkinson's, Alcoholic dementia
- Lewy body disease
- vascular dementia
- exposure to oral pathogens is associated with systemic inflammation, cognitive decline, and AD.
- inflammation - microbes pass through compromised blood vessels?


Exposure to oral pathogens is associated with systemic inflammation, cognitive decline, and AD. In the cascade of events causing AD, oral microorganisms may play a role, particularly anaerobic bacteria such as Treponema, P. gingivalis, Porphyromonas, Fusobacterium and Actinomyces, but also facultative anaerobic Candida species.

It is important to recognize that infection can occur decades before the manifestation of dementia.

Health implications - aspirated biofilm

- cuff on endo tube - biofilm
- oral gel reduced contamination
- small scratches - hard to clean
- retained microbes - proliferate - appliance reinserted

Health implications Aspirated biofilm

Links to systemic disease......what can a clinician do?

Evaluate individually
- their risks
- willing to do
- motivated to change

Individualize your recommendations
- establish a dialogue with other healthcare providers

10 FOODS THAT FIGHT INFLAMMATION
- Nuts
- Avocados
- Spinach
- Tart cherries
- processed meats
- fried food
- soft drinks, high fructose corn syrup
- meat and dairy
- gluten
- casein
- oils - safflower, sunflower, corn
- fast foods
- olive oil
- Orange vegetables and fruits
- Pineapple
- Turmeric, ginger, onions, garlic

Health implications

Aspirated biofilm


New ideas - about caries biofilm

- Ecological plaque hypothesis - caries
  - Health
  - Disease

  - Medium/high levels of cariogenic microbes
  - Heavy / visible plaque accumulations
  - Snacking - 3+ times / day

New news about caries biofilm
- Strept mutans not the only organism - Different microbes colonize
  - White spots
  - Dentin lesions
  - Root caries
  - Primary and secondary dentition
  - Specific tooth surfaces

Decay in children and adolescents
- 28% age 2-5
- 50% by age 11
- 68% at age 19

Predisposing risk factors
- Medium/high levels of cariogenic microbes
- Heavy / visible plaque accumulations
- Snacking - 3+ times / day

Predisposing risk factors
- New research - caries biofilm more complex
- Bacteria on different surfaces
- Mutans streptococci dominate fissures

Ecological plaque hypothesis - homeostasis
- Disease sites species differ from health
- Caries - shift - acidogenic / aciduric
- Imbalance in resident microflora

Ecological plaque hypothesis
- Increase mutants streptococci (gateway)
- Dentin lesions = lactobacillus + proteolytic microbes
- Sustained low pH - select for Ms and Lb
More news - caries biofilm

- §. Mutans - early colonizer
- 30% rampant caries - do not test positive for §.Mutans
- children with ECC
- high levels of §. mutans and C. albicans

Early Childhood Caries

- low income children - 2 X decay
- 4 X more common than asthma (42% versus 9.5%)
- 52,000,000 lost school hours/year - dental disease


Oral appliances

- dentures / partials
- bite guards
- orthodontic aligners
- sports mouth guards
- whitening trays
- surface roughness
- Strep and Candida adhered
- reinsert - microbes proliferate

Cigarettes

- increase C. albicans adhesion/growth/biofilm formation
- metal bands, brackets, acrylic resin, elastic rings
- cigarette smoke condensate
- increased adhesion - §. mutans - bands, acrylic and brackets
- C. albicans - bands, acrylic, brackets, elastics
- increased biomass - §. mutans - only brackets
- C. albicans - on brackets and acrylic
- nicotine §. mutans - increase growth and ESP


Micro Ultrasonics...using today's technology

- disrupt plaque biofilm and calculus
- reduce bacterial level below immune system threshold
- pocket disinfection
- improved access – complex anatomy, deep, narrow pockets

Clinical benefits - contemporary ultrasonic scalers

Structural differences sensitive and non-sensitive dentin

- Non-sensitive
  - No of open tubules: x
  - Diameter of tubules: 0.43
  - Fluid Flow (Poiseuille's law): y

- Sensitive
  - No of open tubules: 8 x
  - Diameter of tubules: 0.83
  - Fluid Flow (Poiseuille's law): 36 y


Other reasons to use a polishing handpiece

- applying desensitizing medicaments
- pre-procedural or site specific application
- pre-scaling biofilm reduction
- reduce ultrasonic aerosol bioburden
How cavitation works
- cavitation = ultrasonic energy + water
- creation of micro-bubbles
- acoustic micro-streaming
- implosion of micro-bubbles
- destruction of biofilm and calculus

![Image of cavitation process]

When are we done?
- all root surfaces - contact with activated tip
- no visible debris
- no clinically detectable deposits

Blending scaling techniques
- power and hand or hand and power
- factors include: type of power scaler, condition of tips, clinical challenge, type of deposit, patient comfort
- skill level / familiarity with power scaling techniques

Subgingival biofilm disruption - glycine
- glycine powder
- soft, small particle size
- reduced surface damage
- lowers biofilm adhesion

Subgingival biofilm disruption - glycine
- comfortable
- 5-9 mm pockets
- lower P. gingivalis counts
- more effective in removing subgingival biofilm than SCP alone

Silver diamine fluoride 38%
- natural antibacterial
- hypersensitivity relief
- carious dentin lesion turns black
- no anesthesia

Healthy biofilm - getting rid of pathogens
Silver diamine fluoride 38%

- arrests caries
- less than $1 / 1-2 teeth
- 8ml bottle
- anyone who can apply fluoride

Noncavitated

Cavitated†

In keeping with the concept of informed consent, all nonrestorative and restorative treatment options and their potential side effects (such as blackened tooth surfaces treated with SDF) should be offered and explained to all patients.

Silver diamine fluoride 38%

- no known bacterial resistance
- very low biocide resistance
- high biocide resistance
- 2-4 days - fully mature
- 5-7 days - partially mature
- >7 days - necrotic

Hydrogen Peroxide

- antiseptic
- debridement
- reduces inflammation

- naturally occurs - breast milk, saliva, liver
- no allergic reactions
- no known bacterial resistance

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**Hydrogen peroxide - comparison**

- 3% liquid
- pH 3
- rapid spike - bubbling
- 1.7% gel
- pH 5.5 - 5.8
- 15 min active time

**Prescription trays**

Problem: “Therapeutic delivery of H_2O_2 to prevent periodontal disease required mechanical access to subgingival pockets.”

- trays create hydraulic seal
- eliminates dilution
- up to 9mm
- 10 min therapeutic
- 15 min full O_2 release

**1.7% peroxide gel – S. mutans biofilm**

- S. mutans aerobic - will not die in oxygen
- thick biofilm mass
- live/dead dyes: intact cell walls - green
degraded, lysed wall - red

**Prescription tray therapy**

- July 2016
  - tray delivery - 4/2016
  - only PerioGel
  - twice daily - 15 min

- Prescription tray therapy
  - June 8, 2016
  - July 7, 2016
  - 3 times a day
  - 15 min per session
  - PerioGel only

**Making it hard on the bad bugs…… encouraging healthier microflora**

- Encouraging healthier microflora

**Ecological plaque hypothesis - caries**

**Prebiotics – prebiotics stimulate growth or activity of certain healthy bacteria**

**Probiotics – a microorganism introduced into the body for its beneficial qualities**
Interesting herbal approach

- licorice root extract
- 2x per day
- 10 days
- 2-4 times annually
- stimulates saliva
- reduces Lactobacillus and S. mutans

What is arginine?

- natural amino acid
- naturally found in saliva
- bipolar molecule - + and - groups
- net positive charge

Arginine - mode of action

Urea - few bacteria
- saliva & crevicular fluid
- broken down by urea
- byproduct-ammonia

Arginine - many bacteria
- low in saliva/abundant in peptides
- ADS - 3 enzyme system
- byproduct - ammonia
- action produces ATP

Arginine - mode of action

- exogenous source - toothpaste
- enhances alkaline pH - saliva and plaque
- 4 weeks
- higher alkali production - plaque samples - caries active subjects
- CA subjects - bacterial shift - healthier

Arginine bicarbonate calcium carbonate tooth paste - lower DMFS

- 6,000 children - low to moderate risk - 6-12 years old
- double blind, randomized - 3 groups - 2 year study
- 1,450 ppm F paste
- 1,450 Fl + 1.5% arginine/calcium carbonate or dicalcium phosphate
- 16.5% lower DMFS - arginine/calcium/flouride groups than FL paste alone

Emerging research - Arginine calcium carbonate

- inhibits bacterial adhesion - tooth surfaces
- reduces biofilm thickness
- reduces EPS matrix density
- arginine + fluoride - suppresses S. mutans and P. gingivalis
- suppresses C. albicans growth
- facilitates microbial resistance - acidic environment

Arginine-based products

- Sugar free chocolate covered soft chews
- Discontinued!

Arginine bicarbonate calcium carbonate - keeping saliva neutral

Remineralization strategies
**Tubule occlusion**

- Stannous fluoride
- High fluoride
- Precipitating salts
- Restorative materials
- Laser

**Fluoride platforms**

- toothpaste, gel, rinse
- varnish, gels
- calcium phosphate
- arginine bicarbonate
- adhesives
- resins
- silicates
- hydroxyapatite

**Varnish recommendations**

- Benefits
  - less time
  - less patient discomfort
  - patient acceptance
  - preschool / adolescents / geriatrics

**Patient-centered choices**

- 24 hour uptake time
- application - moist or in plaque
- horizontal swipe
- surface migration

**Mechanical disruption**

- Feel a new level of clean

**Chemotherapeutic strategies**

- Mouth rinses – non-selective
  - Essential oils
  - Chlorhexidine
- Toothpaste – non-selective
  - Stannous fluoride
- Toothpaste – gels

**More solutions**

- biofilm turns green
- mint / berry bubble gum
- .24% sodium fluoride

**Application**

- 2+ times a year
- caries prevention
- high risk populations
"The results were almost impossible for me to believe the first time through," commented. "One of the difficulties with plaque biofilm is that you really can’t see it, it's clear. So we didn’t have visual evidence of complete removal. But now with these direct methods, the scanning electron microscopy, you apply the Waterpik to plaque on a surface of a tooth and you look with a scanning scope and it’s gone. It’s simply gone. And that's unequivocal and unarguable.”
Understanding biofilm – impacting lives

Multiple concurrent strategies
- debridement: frequent and aggressive
- selective biocides: silver, Iodosorb, Hydrofera Blue
- antibiofilm agents: lactoferrin, xylitol, farnasol
- plant products: fatty acid gel
- antibiotics (25-32% effective): adjunct, strong and long

Wound healing – anti-biofilm strategies
- lactoferrin – bactericidal / block surface attachment / works with PMNs / binds iron even at a low pH
- xylitol – interferes with biofilm formation
- enzymes: Dispersin B – causes biofilm detachment
- gallium, EDTA, hyperbaric oxygen, bacteriophages, glucose oxidase, pulsed electric fields
- ultrasound / ultrasonics

Honey
- broad spectrum antimicrobial
- anti-inflammatory
- first recorded use over 4,500 years ago
- effective against P. gingivalis
- domestic / Manuka (tea tree plant) honey
- both planktonic and biofilm organisms
- inhibited formation / reduced viable numbers - both groups

Ancient ideas and emerging technologies

Medical biofilm-based wound care

Wound healing anti-biofilm agents
- ionic silver: minute concentrations / broad spectrum / used in dressings
- iodine: prevents wound infection / aids healing / povidone iodine preferred
- medicinal honey: antibacterial

Wound healing antimicrobial agents
- ionic silver: minute concentrations / broad spectrum / used in dressings
- iodine: prevents wound infection / aids healing / povidone iodine preferred
- medicinal honey: antibacterial

Ancient ideas and emerging technologies
Red wine
- 5-species supragingival biofilm
- regular red wine / de-alcoholized / extract
- regular solutions / solutions spiked with grape seed extract

Tannins – 72 hour study
- Pseudomonas aeruginosa - opportunistic pathogen
- bacteriostatic
- bacterial membrane damage
- inhibited biofilm formation 6-24 hours
- impaired EPS matrix production


Silver – natural antimicrobial
- natural antibacterial
- bacteriocidal
- anti-fungal

Products
- bandages
- catheters
- dental implants
- endotrachel tubes
- toothbrushes
- orthodontic wires


What’s the take home message?
- biofilms are complex
- Mother Nature rules
- better health is possible
- take responsibility for your role
- new information emerges every day
- share with your colleagues

What do we owe our patients?
- current scientific information
- patients must make the final choice

How would you treat your Mom?

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