Biofilms in root canals – can we get rid of them?

ESSENTIAL CONCEPT
What are biofilms?

BACTERIAL BIOFILMS: FROM THE NATURAL ENVIRONMENT TO INFECTIOUS DISEASES
Laurence Biek, Scott A., J. William Gansner, and Paul S. Sarnow
NATURE REVIEWS | MICROBIOLOGY
2004

- A structured community of bacterial cells
- Enclosed in a self-produced polymeric matrix
- Adherent to a biological or non-biological surface

“Biofilm” versus “Planktonic”

Biofilms are attached to a surface

Planktonic microorganisms are in suspension

Cells and clusters of cells in biofilms can detach into the surrounding fluid

How biofilms form

Illustration: S. Freese. From Hermann et al. 2002

ESSENTIAL CONCEPT
Quorum sensing = how bacteria talk

- Means of regulating gene expression and diverse physiological activities within community

- Regulation is dynamic:
  - Stressors
  - Population density

- Spatial wisdom of crowds:
  - iPhone apps
  - GoogleMaps etc

http://blogs.halcrow.com/geospatial/?p=131
Biofilms in root canals – can we get rid of them?

**ESSENTIAL CONCEPT**

**EPS - Extracellular Polymeric Substance**

- matrix of polysaccharides, DNA, lipids, proteins
- multi-functional

**Biofilms are complex communities**

**Food-borne Enterococci Integrate Into Oral Biofilm: An In Vivo Study**

**Biofilms and peri-implantitis**

“A loss of mechanical integrity of the dental implant may occur as a consequence of the decrease in friction caused by biofilm formation.”

“First observed by Nair using microscopy in 1987!”

Described as clusters of “self-aggregating” colonies of one distinct type or “coaggregating” communities of several types

Not termed “biofilms” until more recently

**Identification of Microbial Biofilms in Osteonecrosis of the Jaws Secondary to Bisphosphonate Therapy**


**Biofilms in infected root canals**
Biofilms in root canals – can we get rid of them?

Participation of Bacterial Biofilms in Refractory and Chronic Periapical Periodontitis

“Entombed biofilms” in the root canal system

Biofilms in dentinal tubules

Multi-species biofilms in root canals - ESEM

• *F. nucleatum* and *E. faecalis* biofilms on dentin

Biofilms in root canals – can we get rid of them?
Biofilms and Apical Periodontitis: Study of Prevalence and Association with Clinical and Histopathologic Findings

- Proposed that "apical periodontitis" be included in the set of "biofilm-induced disease"
- Observed biofilms mineralized and with relatively few bacteria

Confocal laser scanning microscopy (CLSM)

- Produces in-focus images of thick specimens (e.g. biofilms)
- Specimens need to be first treated with a fluorescent dye

CLSM allows 3-D reconstruction of tissue sections

- Fluorescence in situ hybridization (FISH) for direct visualization of bacteria in periapical lesions of asymptomatic root-filled teeth

- Probe-labeled microorganisms labeled in FISH-processed periapical lesions

Investigating biofilms

CLSM of biofilms grown in flow cells

- Investigating biofilms
- CLSM of biofilms grown in flow cells

Biofilm formation by root canal isolates

- Biofilm formation depends on microbes adapting physiology to new environmental conditions set by treatment

- E. faecalis clinical isolates from infected roots canals were not strong biofilm-formers

Gene transfer occurs in biofilms

- Can gene transfer occur between different species in infected root canals?
Biofilms in root canals – can we get rid of them?

Outline

- Biofilms update
- Biofilms in root canals
- Controlling biofilms in root canal

Microflora in teeth associated with apical periodontitis: a methodological observational study comparing two protocols and three microscopy techniques

- Chronic apical periodontitis cases
- PMNs walled off biofilm from the remaining canal lumen in all cases

Role of Polymorphonuclear Neutrophils in the Clearance of Enterococcus faecalis Derived from Saliva and Infected Root Canals

- PMNs cells can play an important role in killing E. faecalis
- Endo and saliva E. faecalis injected into mouse muscle recruited PMNs leading to inflammation via up-regulation of PMN IL-1α, TNF-α, MMP-8, and COX-2

Immunogenic Potential of Enterococcus faecalis Biofilm under Simulated Growth Conditions

- Cytokine release from macrophages exposed to E. faecalis in biofilm is less than in planktonic conditions

Proposed biofilm-resistance mechanisms

- Planktonic bacteria
- Biofilm matrix (glycocalyx) with pH, pH gradient
- Biofilm active growing bacteria
- Antibiotic chelator enzymes
- Biofilm persister bacteria
- Osmotic sensors
- Antimicrobial agent

Biofilms in root canals – can we get rid of them?
Christine Sedgley, AAE San Antonio 2011

Biofilms in root canals – can we get rid of them?

Possible strategies to treat biofilms

1. Substances that destroy biofilm matrix (e.g. dispersin B)
2. Substances that destroy persister cells
3. Quorum-quenching enzymes
4. Substances that cause biofilm self-destruction
5. Strategies to boost antimicrobial agent action (e.g. electrical current)

Mechanical disruption

Ultrasonics and sonic agitation

Clinical studies
- Ultrasonic irrigation removed debris from isthmuses
- One minute of ultrasonic irrigation was 7 times more likely to yield a negative culture
- No difference between sonic (EndoActivator) and control (conventional syringe) to eliminate cultivable bacteria from canals

Simultaneous irrigation and evacuation
- Canals of exo’d teeth infected with E. faecalis
- Biofilm formation confirmed using SEM
- Irrigated with apical positive or negative pressure
- No microbes detected in root canals irrigated using apical negative pressure irrigation

Biofilms in root canals – can we get rid of them?

- Conventional irrigation
- Activation of irrigant
  - Ultrasonics
  - Sonic
  - Irrigation/Evacuation
  - Lasers
    - Photodynamic endodontic disinfection

The Synergistic Antimicrobial Effect by Mechanical Agitation and Two Chlorhexidine Preparations on Biofilm Bacteria

- Biofilms exposed to 2% CHX or CHX-Plus with or without sonic agitation
- Low-intensity agitation did not disrupt disperse biofilm, but did enhance action of antimicrobials against biofilms

Photodynamic Treatment of Endodontic Polymicrobial Infection In Vitro

- Grew multispecies biofilms (4 species) in root canals
- Photodynamic therapy: up to 80% reduction of CFU

Clinical studies

- Gutarts et al. 2005
- Carver et al. 2007
- Huffaker et al. 2010
- Shen et al. 2010
- Fimple et al. 2008

Hockett et al. 2008

Sedgley 2011
Biofilms in root canals – can we get rid of them?

Chemical disruption

- NaOCl
- Chlorhexidine
- Calcium hydroxide
- EDTA
- Others

Sedgley 2011

NaOCl rendered bacteria nonviable and physically removed polymicrobial biofilms from root segments (EDTA, MTAD, CHX did not)

Clegg et al. 2006

1% & 6% NaOCl removed E. faecalis biofilms

Durantini et al. 2006

Mixed-species biofilms of F. nucleatum and P. micros showed time-dependent synergy in growth and resistance to NaOCl

Crook et al. 2007

NaOCl removed dual species biofilms. Gram-neg obligate anaerobes more susceptible than gram-pos facultatives

Bryce et al. 2009

Early stage of biofilm formation - adherence

How do irrigants affect adherence?

Pre-treated dentin blocks with different irrigants, then exposed samples to E. faecalis for one hour

Adhesion (pull-off) force measured using atomic force microscopy

Bacterial adherence significantly influenced by the last irrigant used: least adherence with NaOCl

Kohen et al. 2008

Clinical studies using molecular methods

- Used real-time PCR
- Compared 2% CHX gel and 2.5% NaOCl
- NaOCl killed more microorganisms and removed more cells from the canal

Viana et al. 2006

- Used PCR/reverse-capture checkerboard assay
- Compared 0.12% CHX and 2.5% NaOCl as irrigants
- Both reduced taxa and levels of microorganisms
- No difference between CHX and NaOCl

Roças and Siqueira 2011

Effects of endodontic treatment on microbiota:

Chlorhexidine

Clinical studies – culture methods

- CHX as an endodontic irrigant was no more effective than NaOCl
  - Ringel et al. 1982, Siqueira et al. 2007
- 2% CHX liquid as an endodontic medicament was not effective
  - Paquette et al. 2007
- CHX as an additional rinse reduced number of positive cultures
  - Zamary et al. 2003

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Influence of Irrigation Regimens on the Adherence of Enterococcus faecalis to Root Canal Dentin

Sedgley 2011

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Kohen et al. 2008

Effects of endodontic treatment on biofilms

Sodium hypochlorite

Effect on biofilms

In vitro

- 6% NaOCl rendered bacteria nonviable and physically removed polymicrobial biofilms from root segments (EDTA, MTAD, CHX did not)
  - Clegg et al. 2006
- 1% & 6% NaOCl removed E. faecalis biofilms
  - Durantini et al. 2006
- Mixed-species biofilms of F. nucleatum and P. micros showed time-dependent synergy in growth and resistance to NaOCl
  - Crook et al. 2007
- NaOCl removed dual species biofilms. Gram-neg obligate anaerobes more susceptible than gram-pos facultatives
  - Bryce et al. 2009

Ozok et al. 2007

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Roças and Siqueira 2011

Biofilms in root canals – can we get rid of them?
Reported in non-dental literature
Chlorhexidine and biofilms - problems?

- CHX-treated dual-species biofilm bacteria had a higher survival rate compared with CHX-treated single-species biofilms
  Kara et al. 2007 FEMS Microbiol Lett

- Sublethal exposure to cationic antiseptics may contribute to the persistence of staphylococci through biofilm induction
  Houari and Di Martino 2007 Lett Appl Microbiol

Response to alkaline stress by root canal isolates

- Bacteria isolated from infected root canals resisted alkaline stress more in biofilms than in planktonic cultures
  Chavez de Paz et al. 2007

Impact of Growth Conditions on Susceptibility of Five Microbial Species to Alkaline Stress

- Bovine dentin discs infected with A. naeslundi, F. nucleatum, S. sobrinus, E. faecalis, C. albicans
  Brändle et al. 2008

- Determined viability in 5 day biofilms exposed to saturated Ca(OH)₂
- Adherence to dentin and interspecies interactions in a biofilm differentially affected the sensitivity of microbial species to Ca(OH)₂

Effect of Ethylenediaminetetraacetic Acid and Sodium Hypochlorite Irrigation on Enterococcus faecalis Biofilm Colonization in Young and Old Human Root Canal Dentin: In Vitro Study

- Root canals of old (A, B) and young (C, D) human subjects at different biofilm depths (24 hr E. faecalis infection)
- Mean bacterial counts higher in old compared to young group (p < .05)
  Ozdemir et al. 2010

Biofilms in root canals – can we get rid of them?

- EDTA
  - Introduced to endo as a chelating agent
  Nygaard-Ostby 1957

- EDTA can also act as an antibiofilm acid for limiting biofilm attachment by decreasing iron availability
  Dunne et al. 1992, Al-Azehmi et al. 2011

Effectiveness of Chemomechanical Preparation with Alternating Use of Sodium Hypochlorite and EDTA in Eliminating Intracanal Enterococcus faecalis Biofilm

- Alternating use of EDTA and NaOCl was better than when all EDTA is used at the same time
  Soares et al. 2010
Biofilms in root canals – can we get rid of them?

Contemporary Root Canal Irrigants Are Able to Disrupt and Eradicate Single- and Dual-Species Biofilms

- Biofilms on membranes exposed to NaOCl, iodine, CHX, EDTA
- CLSM
- NaOCl was most effective

Bryce et al. 2009

Control
CHX

The Effects of Antimicrobials on Endodontic Biofilm Bacteria

- % of cells removed
  - Live/dead
  - Single-species biofilms
  - Miniflow
  - CLSM & analysis
  - Green – intact membrane
  - Red – damaged membrane

Chavez de Paz et al. 2010

Control
CHX
NaOCl

Nanoparticulates for Antibiofilm Treatment and Effect of Aging on Its Antibacterial Activity

- E. faecalis biofilm
- After treatment with ZnO-np
- After treatment with chitosan-np

Shrestha et al. 2010

Bactericidal Activity of Stabilized Chlorine Dioxide as an Endodontic Irrigant in a Polymicrobial Biofilm Tooth Model System

- Infected bovine dentin model
- Antimicrobial activity:
  - NaOCl > CHX > ClO2

Lundstrom et al. 2010

Removing root canal biofilms: Other “chemical” approaches…

  - Pretreatment with 5 min 5% IPI prior to obturation
- Maleic acid: Ferrer-Luque et al. 2010
- Liquorice: Badar et al. 2011
- 0.2% cetrirnide: Baca et al. 2011
- Propolis: Kayaoglu et al. 2011

In vitro:
- Ozone: Huth et al. 2009
- Nanoparticulates: Kühn et al. 2008
- MTA, bioaggregate: Zhang et al. 2009

Molander et al. 1999

Antibiotic-treated biofilms on dentin slices

- One day cultures from infected root canals grown on dentin slices for 12 days
- Exposure to ampicillin, doxycline, clindamycin, azithromycin or metronidazole for up to 8 days did not eliminate biofilm

Norrington et al. 2008

12 days + 8 days exposure to ampicillin
Biofilms in root canals – can we get rid of them?

What’s ahead?

- Identification of root canal biofilm targets
- Engineered peptides targeting “bad bugs” in biofilms?
  - Targeting Killing of Streptococcus mutans by a Phenomenon-Guided
    - "Nest" Antimicrobial Peptide
  - Engineered peptides targeting "bad bugs" in biofilms?
- "Natural" peptides targeting microbes in biofilms?
- Targeting biofilm matrix

Sanger sequencing vs. pyrosequencing of 7 specimens from endodontic infections
- Pyrosequencing showed 179 bacterial genera
- Found ~ 47 versus 28,590 sequences obtained per sample, representing a 600-fold difference

Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics

- Sang and Blecha 2008

Alternatives to antibiotics

Natural antimicrobial peptides and bacteriocins

- <100 amino acid residues
- Broad spectrum
- Rapid mechanism of action
- Permeabilize and/or form pores within the cytoplasmic membranes
- Relative selectivity towards their targets (microbial membranes)
- Low frequency in selecting resistant strains
- Have potential to act on slow growing or even non-growing bacteria

Plasmid pAM51-Encoded, Bacteriocin-Related “Siblicide” in Enteroococcus Faecalis

- Sedgley et al. J Bact 2009

Thank you

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Remmery and Wingender
Nature Reviews Micro 2010