Endodontic microbiology

Christine Sedgley
MDS, MDSc(Endo), FRACDS, FRACDS(Endo), PhD
February 2018

Overview
Part 1 Basic microbiology update
Part 2 Methods used for evaluating endodontic microflora
Part 3 Microorganisms in primary endodontic infections
Part 4 Microorganisms and unsuccessful endodontic treatment
Part 5 Microorganisms in periapical lesions
Part 6 Microorganisms and regenerative endodontics
Part 7 Antimicrobial efficacy of endodontic treatment

Overview I
Part 1 (Very) basic microbiology update
Bugs Biofilms Virulence

Basic microbiology
Prokaryotes bacteria No nucleus
Eukaryotes fungi, animals Chromosomes in nucleus

Classification - cell wall
Gram positive and Gram negative

What are biofilms?
Aggregates of microbial cells enclosed in a self-produced matrix adherent to a surface

Biofilm Composition
| Bacterial cells | ± 20% by volume |
| EPS/glycocalyx, biofilm matrix | ± 80% by volume |
**EPS - Extracellular Polymeric Substance**

What’s happening in the EPS?

How is activity in the EPS regulated?

- **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:
    - Texts, tweets etc

**Biofilms and antimicrobial resistance**

- Microbes in biofilms can easily interact with each other and exchange genes encoding:
  - Virulence factors
  - Antibiotic resistance

- Biofilm microorganisms are more resistant to antimicrobial agents than planktonic bacteria

**Intracanal biofilms**

- 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

- Described as “biofilms” by Svensater & Bergenholtz (2005)

**Root canal biofilms are complex, polymicrobial and heterogenous**

Biofilm morphology can vary between and within root canal systems

“Entombed biofilms” in the root canal system

Proposed that “apical periodontitis” be included in the set of “biofilm-induced diseases”

**Biofilms and Apical Periodontitis: Study of Prevalence and Association with Clinical and Histopathologic Findings**

Ricucci and Sequeira 2010

Nair et al. 2005

LM, TEM Nair 1990

Nair et al. 2005

Ricucci and Sequeira 2010
What is virulence?

- The degree of pathogenicity or disease-producing ability of a microorganism
- The pathogenicity of an organism is determined by its virulence factors

Potential virulence factors associated with a bacterial cell

Microbial virulence

Specific virulence factors and endodontics

Endotoxin (LPS)

- Hemolysin (Sedgley et al. 2005)
- Lipoteichoic acid (Baik et al. 2008)
- Peptidoglycan (Hahn and Liewehr 2007)
- Gelatinase (Sedgley 2007, Sato et al. 2009)
- Short chain fatty acids (Ho and Chang 2007, Provenzano et al. 2015)
- Fimbriae (Figdor and Davies 1997, Rôças and Siqueira 2010)
- Proteases (Chow et al. 2006, Nandakumar et al. 2009)

Microbial virulence

Endotoxin and endodontics

Endotoxin is positively associated with:

- Pulpal pain and periapical inflammation

- Bone destruction

Microbial virulence

Gram negative cell wall - Endotoxin

Endotoxin is an established pathogenic factor in endodontic infections

Microbial virulence

What about host factors?

- Periapical pathology is multifactorial
- The host determines the response to microorganisms

Microbial virulence

Endotoxin and endodontics

Endotoxin is positively associated with:

- Pulpal pain and periapical inflammation

- Bone destruction

Microbial virulence

What about host factors?

- Periapical pathology is multifactorial
- The host determines the response to microorganisms
Periapical pathology is multifactorial

- Pulpal infection
- Immune response
- Cytokines
- Systemic factors
- Bone resorption

Adapted from Stashenko

Overview

Part 1: Basic microbiology update
Part 2: Methods used for evaluating endodontic microflora

Methods used for evaluating endodontic microflora

1. Histology
2. Culturing and biochemical tests
3. Microbial bioinformatics

Evaluating endodontic infections

1. Histology
   - Limited to observing morphology, and more recently, viability
   - Light microscopy (LM) (Ricucci and Bergenholtz 2003, Ricucci et al. 2015)
   - Transmission electron microscopy (TEM) (Nair 2005)
   - Scanning electron microscopy (SEM) (Leonardo et al. 2002)
   - Environmental SEM (Bergmans et al. 2005)
   - Confocal laser SEM (Parmar et al. 2011)
   - Combination LM, TEM, SEM (Richardson et al. 2009)

2. Culturing
   - Anaerobic culturing

3. Microbial bioinformatics
   - DNA Sequence Databases
   - Comparative genomics
   - Gene expression
   - Proteomics
   - Metabolomics

Evaluating endodontic infections – now and the future

3. Microbial bioinformatics
Sampling controls should be subjected to same investigations as root canals sample

“Critical evaluation and standardization of the disinfection methods and aseptic procedures in endodontics are needed”

“The high binding affinity between DNA and hydroxyapatite presents a special challenge for decontamination”

Evaluating endodontic infections

Genomics – DNA based identification

- Polymerase chain reaction (PCR)
- Nested PCR
- Multiplex-PCR
- Real-time PCR
- DNA-DNA hybridization
- Pyrosequencing

Genomics – DNA based identification

Pyrosequencing

Enables more comprehensive analysis than traditional (Sanger) sequencing

600-fold difference

Li et al. 2010

Advantages of genomic approaches

- More sensitive than culturing
- Many microbes that are not easily cultured, or are unculturable, have been identified
- Multiple studies have clarified and confirmed that flora is diverse and not limited to a single species

Disadvantages of using genomic methods to study endodontic infections

- Strong binding affinity between DNA and dentin may compromise root canal sampling (Brundin et al. 2014, Figdor et al. 2016)
- Methods are very technique sensitive
- Preparation methods destroy microorganisms so cannot do further phenotypic analyses
- Most methods used don’t address cell viability
- Many nucleic acid sequences are unidentifiable
Evaluating endodontic infections

3. Microbial bioinformatics

- Microbial proteins in 7 samples:
  - adhesins, autolysins, proteases, virulence factors, and antibiotic-resistance proteins

- Microbial and human proteins in 24 cases:
  - Microbial proteins: metabolism and housekeeping, adhesion, biofilm formation, antibiotic resistance, stress proteins, exotoxins, invasins, proteases
  - Human proteins: cellular processes and metabolism, immune defense

Proteomics

At what stage are we in the bioinformatics era?

Tip of the iceberg

Overview 3

Part 1: Basic microbiology update
Part 2: Methods used for evaluating endodontic microflora
Part 3: Microorganisms in primary endodontic infections

Are microorganisms the cause of apical periodontitis?

YES

Animal studies have proven that bacteria in the pulp are essential to the development of periapical disease

Used germ-free rats to prove that bacteria in the pulp are essential to the development of periapical disease

Kakehashi, Stanley and Fitzgerald 1965

Microbial etiology
Classic papers - Rats

- Relative number of obligate anaerobes increases with time
- Proportionally more anaerobes apically with time
- Mixed infections show greatest capacity for inducing apical periodontitis

Möller, Fabricius, Dahlén et al. 1981, 1982

Microbial etiology
Classic papers - Monkeys

Primary endodontic infections
Classic CULTURE studies - bottom line

- Mixed flora dominated by Gram negative anaerobes
- “Polymicrobial”

Möller 1966
Bergenholtz 1974
Sundqvist 1976
Cvek et al. 1976
Kantz and Henry 1974 and others

Specific microorganisms and symptoms

- Are specific symptoms associated with specific microorganisms?
  - Yes
  - No

Geographic location

- Microflora of infected root canals may vary according to geographic location

Siqueira et al. 2005, Rôças et al. 2006

Summary - Primary endodontic infections
Histology and culture-based studies

- Biofilms
- Polymicrobial
- Predominantly anaerobic Gram negative rods

<table>
<thead>
<tr>
<th>Facultative anaerobic</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Porphyromonas</td>
<td>- Bacteroides</td>
</tr>
<tr>
<td>- Prevotella</td>
<td>- Peptostreptococcus</td>
</tr>
<tr>
<td>- Eikenella</td>
<td>- Peptococcus</td>
</tr>
<tr>
<td>- Filifactor</td>
<td>- Eubacterium</td>
</tr>
<tr>
<td>- Dialister</td>
<td>- Propionibacterium</td>
</tr>
<tr>
<td>- Capnocytophaga</td>
<td>- Hypocreus</td>
</tr>
<tr>
<td>- Treponema</td>
<td>- Corynebacterium</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Candida</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>- HIV</td>
</tr>
<tr>
<td>- Epstein-Barr virus</td>
</tr>
<tr>
<td>- Human cytomegalovirus</td>
</tr>
<tr>
<td>- Herpes simplex virus-1</td>
</tr>
</tbody>
</table>

**Summary - Primary endodontic infections**

**Molecular-based studies**

- Multiple species (highly “diverse”)
- Significantly more species detected than in culture-based studies
- Many species as yet unidentified and unculturable

Pyrosequencing studies:

- Li et al. 2010
- Siqueira et al. 2011
- Saber et al. 2012
- Hong et al. 2013
- Tzanetakis et al. 2015

**Overview 4**

- Part 1: Basic microbiology of endodontics
- Part 2: Methods used for evaluating endodontic microflora
- Part 3: Microorganisms in primary endodontic infections
- Part 4: Microorganisms and unsuccessful endodontic treatment

---

**How well can root canal systems be cleaned in a single visit … or ever?**

Microorganisms remain after debridement in isthmuses

- Nair et al. 2005

---

**Microflora in primary and secondary endodontic infections differs?**

**Primary endodontic infections**

- Biofilms
- Polymicrobial
- Predominantly Gram negative anaerobic rods
- Many species as yet unidentified and unculturable

**Previously root filled canals**

- Biofilms
- Polymicrobial, reduced microbial load
- Frequently recovered in culture-based studies - Gram positive facultatively anaerobic cocci:
  - Enterococcus faecalis
- Many species as yet unidentified and unculturable

---

**Secondary endodontic infections**

**Enterococci**

- Enterococci *predominate* in culture-positive root-filled teeth with chronic apical periodontitis
- Enterococci are associated with persistence of infection during endodontic treatment
  - Siren et al. 1997

---

**Reasons *E. faecalis* could resist root canal treatment procedures and survive**

- Ability to *invade* dentinal tubules and *adhere* to collagen in the presence of human serum
  - Luse et al. 2001
- A *proton pump* which allows *E. faecalis* to survive *Ca(OH)2* treatment
  - Evans et al. 2002
- Expression of *virulence factors*
- An *efflux pump* that may render *E. faecalis* biofilms more susceptible to antimicrobials
  - Upadya et al. 2011
**E. faecalis** can survive for extended periods in obturated root canals *ex vivo*

- Viable *E. faecalis* were recovered from teeth for 2 years after obturation

Sedgley et al. 2005, 2014

---

**E. faecalis** in secondary endodontic infections

**Bottom line**

- They survive
  - obturation for at least 2 years
  - and have the potential to be virulent

- Is there proof that residual *E. faecalis* cause endodontic treatment failure?
  - No

- Are they harmless hitchhikers?
- Are they “persisters” in biofilms?

---

**Comparison of Bacterial Community Composition of Primary and Persistent Endodontic Infections Using Pyrosequencing**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Primary Infection</th>
<th>Persistent Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Enterococcus</td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Lactobacillus</td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Eubacterium</td>
<td></td>
</tr>
</tbody>
</table>

Firmicutes phylum e.g. Enterococcus, Lactobacillus, Eubacterium

Tzanetakis et al. 2015

---

**Summary – Secondary endodontic infections**

**Culture-based studies**

- Fewer species and reduced microbial load compared with primary infection
- Polymicrobial
- *E. faecalis* frequently recovered, but not exclusively

---

**Summary – Secondary endodontic infections**

**Molecular-based studies**

- Multiple species (highly “diverse”)
- Less predominance of *E. faecalis* than in culture studies
- Many species as yet unidentified and unculturable

Chugal et al. 2011
Anderson et al. 2012
Tzanetakis et al. 2015
Siqura et al. 2016
and others...

Siqura et al. 2016

---

**Overview 5**

- Part 1: Basic microbiology update
- Part 2: Methods used for evaluating endodontic microflora
- Part 3: Microorganisms in primary endodontic infections
- Part 4: Microorganisms and unsuccessful endodontic treatment
- Part 5: Microorganisms in periapical lesions
Ricucci et al. 2015

Extraradicular infection as the Cause of Persistent Symptoms: A Case Series

Ricucci et al. 2015

Periapical lesions
Histology study - humans

Not detected in granulomas

Bacteria only detected in abscesses or cysts

Ricucci et al. 2006

Tronstad et al. 1987

“Anaerobic bacteria are able to survive and maintain an infectious disease process in periapical tissues”

Microorganisms in asymptomatic periapical lesions

8 PA lesions
Culture study

Sunde et al. 2000, Gatti et al. 2000

Microorganisms in periapical lesions
Sampling

Submarginal incision better than marginal incision to avoid contamination of surgery site

Sunde et al. 2000, Gatti et al. 2000

However…..
Disinfection procedures do not “destroy” the DNA

Sunde et al. 2003

Evaluated 39 PA lesions (root-filled teeth with asymptomatic apical periodontitis)

Sunde et al. 2003

Examined 34 apicoectomy samples for bacterial DNA in root end and soft tissue (including one “control” sample which was neg for DNA)

Subramanian and Mickel 2009
Sampling challenges
- Polymicrobial
- Predominantly Gram positive and Gram negative anaerobes

Culture-based studies
- Facultative anaerobes
  - Streptococcus
  - Micromonas (formerly Peptostreptococcus)
  - Enterococcus
  - Gemella
  - Staphylococcus
- Gram negative cocci
  - Veillonella
- Gram positive rods
  - Lactobacillus
  - Actinomyces
  - Bacillus
  - Eubacterium
  - Propionibacterium (formerly Arachnia)
- Gram negative rods
  - Enterobacter
  - Porphyromonas
  - Pseudomonas
  - Prevotella
  - Vibrio
  - Fusobacterium
  - Capnocytophaga
  - Bacteroides
  - Tannerella

Fungi
- Aspergillus
- Candida

Molecular-based studies
- Sampling challenges
- Evidence of bacterial DNA
- Multiple species ("polymicrobial")
- Many species as yet unidentified and unculturable
- Viruses

Sunde et al. 2003

Overview 6
Part 1: Basic microbiology update
Part 2: Methods used for evaluating endodontic microflora
Part 3: Microorganisms in primary endodontic infections
Part 4: Microorganisms and unsuccessful endodontic treatment
Part 5: Microorganisms and periapical lesions
Part 6: Microorganisms and regenerative endodontics

Verma et al. 2017

"Residual bacteria have a critical negative effect on the outcome of regenerative endodontic procedures."

Verma et al. 2017

Vishwanat et al. 2017

Residual biofilm promoted osteoblastic (BSP) versus dentinogenic (DSPP) gene expression of SCAP
- But, E. faecalis antigens inhibited SCAP osteogenic differentiation
Clinical management of infected root canals

Aiming to remove:
- Microorganisms
  - in biofilm and planktonic state
  - dead and alive
  - whole cells and parts of cells
- Debris from instrumentation, smear layer
- Pulpal remnants - cellular, fibrous
- Metabolic by-products
- Previous root filling material

Biofilms grow on root canals walls and in dentinal tubules

Biofilms are involved in all stages of root canal infection

Microbe tactics and strategies
Why it’s hard to get rid of biofilms
**Microbe tactics and strategies**

**Winners**
- Virulence factors
- Biofilm interactions
- Resistance

**Losers**
- Accessible?
- **Disruptible**
- **Antimicrobials**

---

**Clinical management of infected root canals**

**Antimicrobials**

**Mechanical disruption**

**Mechanical removal**

---

**Clinical management of infected root canals**

**Antimicrobials**

**Mechanical disruption**

**Mechanical removal**

---

**Clinician tactics and strategies**

**Conventional irrigation of root canals**

Do irrigants reach apical part of the canal?

- There is little fluid exchange and displacement of particles beyond the tip of the needle

  Choi 1983

---

**Apical pressure varies according to needle type**

Boutsikakis et al. 2010
What influences irrigant flow?

- Increased canal taper and apical size improves irrigant replacement and wall shear stress and reduces irrigant extrusion
  - Albrecht et al. 2004, Falk and Sedgley 2005, Boutsioukis et al. 2010
- Needle depth placement influences irrigation efficacy
  - Sedgley et al. 2005, Boutsioukis et al. 2010
- Irrigation significantly less effective in curved compared to straight canals
  - Njaj and Sedgley 2005

Mechanical disruption

- Conventional irrigation
- Activation of irrigant
- Different file systems

Sonic activation

- Lower frequency than ultrasonic
  - EndoActivator™
  - Sonicare CanalBrush™
  - Vbringe™
  - GentleWave™
- All remove debris/smear layer in vitro
- Need independent data on antimicrobial activity and biofilm removal in clinical situations

Photodynamic therapy

- Uses photosensitizer solutions (e.g., methylene blue, toluidine blue) and low-power laser light
  - Solution binds to microbial cell
  - Laser light applied (via plastic flexible fiber) activates dye
  - Free radicals produced destroy cell

Photodynamic therapy (PDT) reduced biofilms in root canals

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

- Viable biofilms (arrows)
- After PDT: destruction of biofilms, with some foci of live bacteria (arrow)

Photon-Initiated Photoacoustic Streaming (PIPS™)

- Uses pulsed erbium:YAG laser to create photon-initiated photoacoustic streaming
- In vitro study – culture of samples infected with oral flora
  - Extracted premolars prepared to apical #20.07
  - PIPS™ generated more negative bacterial samples than conventional and ultrasonic

- Photodynamic therapy: reduced biofilms in root canals
3 clinical studies reviewed
- All showed PDT had a “positive effect” in private office clinical setting
- Review authors conclusions:
  - Limited clinical information available on the use of PDT in root canal disinfection
  - If supported by future clinical research, PDT may have efficacy for additional root canal disinfection, especially in the presence of multi–drug-resistant bacteria

Chrepa et al. 2014

**In vitro studies comparing different file systems to reduce microbial load**

- No difference between:
  - SAF, Twisted File and Reciproc
    - Siqueira et al. 2013
  - WaveOne and One Shape
    - Nabeshima et al. 2014
  - Hand (K-file) and ProTaper
    - Nakamura et al. 2013
  - Single Reciproc file and BioRaCe series
    - Alves et al. 2012
  - WaveOne and ProTaper
    - Pinheiro et al. 2016

**Clinical studies comparing different file systems to reduce microbial load**

- No difference between:
  - Hand NiTi using alternated rotation motion or BioRace
    - Rôças and Siqueira 2013
  - Self-Adjusting File and Twisted File Adaptive for retreatment cases
    - Rodrigues et al. 2015
  - Single Reciproc file and BioRaCe series
    - Neves et al. 2016

Studies used real-time qPCR

**Clinician challenges**

- Uninstrumented niches for biofilms

**Clinical management of infected root canals**

- Antimicrobials
- Mechanical disruption

**Mechanical removal**
Clinician tactics and strategies

**Antimicrobials**
- Sodium hypochlorite
- Chlorhexidine
- EDTA
- Calcium hydroxide
- Antibiotics

**Sodium hypochlorite (NaOCl)**

Is sodium hypochlorite an effective antimicrobial agent?

**YES**

NaOCl inactivates the lipid moiety of lipotechoic acid through deacylation of the lipid moiety

Byström and Sundqvist 1983

**Sodium hypochlorite**

Classic clinical studies using culture-based techniques

- 0.5% NaOCl better than saline
  Byström and Sundqvist 1983

- No sig difference between 0.5% & 5% NaOCl
  Byström and Sundqvist 1985

- 5% NaOCl effects enhanced by EDTA
  Byström and Sundqvist 1985

**Chlorhexidine**

Is CHX an effective antimicrobial agent?

- Yes, but not as effective as NaOCl
- Does not have tissue solvent capacity

**Chlorhexidine**

**Antimicrobial spectrum**

- Bacteriostatic or bacteriocidal (depending on concentration used) for a wide range of Gram positive and Gram negative bacteria. Disrupts cell membrane

- CHX has limited activity against non-enveloped viruses and some bacterial and fungal spores
Bacteria in mature biofilms and nutrient-limited biofilms are more resistant to CHX killing than bacteria in young biofilms.

Shen et al. 2011

6% NaOCl rendered bacteria nonviable and physically removed polymicrobial biofilms from root segments (EDTA, MTAD, CHX did not)

Clegg et al. 2006

2% NaOCl was more effective against multispecies biofilms in dentin than 2% CHX

Yang et al. 2016

Clinical studies using molecular methods

Canals with necrotic pulp - 2.5% NaOCl killed more MOs than 2% CHX gel and removed more cells

Vianna et al. 2006

Teeth with AsAP - no difference between 0.12% CHX and 2.5% NaOCl

Rôças and Siqueira 2011

CONSORT trial - Retreatment cases: no difference between 2% CHX and 1% NaOCl

Zandi et al. 2016

NaOCl versus CHX precipitates when combined

Basrani et al. 2007

CHX and NaOCl precipitates when combined blocks dentinal tubules

Bui et al. 2008

CHX and NaOCl precipitates

Other chlorhexidine interactions

CHX and EDTA precipitates when combined

Rasmick et al. 2008

CHX and Ca(OH)₂ results in immediate degradation of CHX

Barbin et al. 2008

Clinician tactics and strategies

Antimicrobials

Sodium hypochlorite
Chlorhexidine
EDTA
Calcium hydroxide
Antibiotics
EDTA

- Introduced to endo as a chelating agent
  Nygaard-Ostby 1957
- In vitro: EDTA can act as an antibiofilm acid
  for limiting S. aureus biofilm attachment by decreasing iron availability
  Al-Azemi et al. 2011
- Destabilizes biofilms by sequestering calcium, magnesium, zinc, and iron
  Finnegan and Percival 2015

Alternate irrigation with EDTA and NaOCl

- Biofilm removal enhanced by alternate irrigation with NaOCl and EDTA rather than using EDTA all at once as a final rinse after NaOCl

<table>
<thead>
<tr>
<th>Group</th>
<th>S1</th>
<th>S2</th>
<th>S16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.111</td>
<td>0.114</td>
<td>0.154</td>
</tr>
<tr>
<td>Ca(OH)₂ group</td>
<td>0.114</td>
<td>0.154</td>
<td>0.185</td>
</tr>
</tbody>
</table>

Soares et al. 2010

Calcium hydroxide

Can calcium hydroxide kill endodontic microflora?

YES ….. but not always

Clinician tactics and strategies

- Antimicrobials
  - Sodium hypochlorite
  - Chlorhexidine
  - EDTA
  - Calcium hydroxide
- Antibiotics

Calcium hydroxide

Effect on biofilms in dentinal tubules

- Canals infected with E. faecalis
- Tooth slices (1mm) obtained

Parmar et al. 2011
Calcium hydroxide

**Antimicrobial effectiveness**

Clinical study - culture
- 7 day intracanal application effective in eliminating bacteria
- 10 minute application ineffective
- Intracanal Ca(OH)₂ for at least 1 wk rendered 92.5% of canals bacteria-free

*Sjögren et al. 1991, Shuping et al. 2000*

Intracanal medication for 4 weeks with Ca(OH)₂ limited but did not prevent regrowth of endodontic bacteria

*Peters et al. 2002*

Calcium hydroxide versus CHX medicament

**Antimicrobial effectiveness**

Prospective randomized clinical trial
- 69 single rooted adult teeth
  - Used real-time qPCR and viable counts to compare antimicrobial effectiveness of intracanal 2% CHX gel and Ca(OH)₂ paste
  - 14 day dressing with Ca(OH)₂ paste was significantly more effective, particularly in cases with apical periodontitis

*Teles et al. 2014*

Calcium hydroxide versus CHX medicament

**Healing outcome**

Clinical study (case series, retrospective)
- 2-4 yr follow-up on previous study of 22 teeth with apical periodontitis medicated with CHX
- No sig difference in healing outcome between 2% CHX liquid (94%) and Ca(OH)₂ (90%)(historical control)

*Solana et al. 2017*

Other recent antimicrobial approaches....

- Epigallocatechin: Kwon et al. 2017
- High-purity nisin: Kajwadkar et al. 2017
- Chitosan nanoparticles abd propolis: Carpio-Perochena et al. 2017
- Synthetic human beta-defensin-3-C15: Yoo et al. 2017
- Clindamycin-modified TAP nanofibers: Karczewski et al. 2017
- Mixed alkaline EDTA/NaOCL: Solana et al. 2017
- Peptide LL-37: Milhan et al. 2017
- 2-Hydroxyisocaproic acid: Sakko et al. 2017

In vitro studies

Clinician tactics and strategies

**Antimicrobials**
- Sodium hypochlorite
- Chlorhexidine
- EDTA
- Calcium hydroxide
- Antibiotics

Antibiotic resistance in endodontic microflora

A clinical problem?
Biofilms and resistance

Gene transfer occurs in biofilms

Antibiotic resistance in endodontic microflora: A clinical problem?

Resistance phenotype (Culture, MICs) to:
- Metronidazole, beta-lactams
  Khemaleelakul et al. 2002, Baumgartner and Xia 2003

Genes (PCR) associated with resistance to:
- Tetracycline, erythromycin, beta-lactams
  Jungermann et al. 2011, Rôças and Siqueira 2013

Proteins (enzymes) related to resistance:
- TetR, beta-lactamase, MarR regulator, efflux pump
  Provenzano et al. 2013, 2016

Antibiotic resistance in endodontic microflora: A clinical problem?

Reasons for prescribing antibiotics that are not necessary

- Aseptic technique
- Effective debridement
- Local antimicrobials
- Systemic antibiotics only if indicated
- Apical and coronal seal

Sample question.....
How do you control microbes in endo Rx?

Sample question....
What are the main developments in endo micro?

1960s-1970s
- Causative role of microorganisms in endo infections
- Aseptic sampling, controls, anaerobes

1970s-1980s
- Anaerobic species, symptom correlations

1990s
- Microflora of “failed” endodontic treatment

2000s
- Non-culturable species using molecular methods

Biofilms - observation and clinical management

Current and Future???
- Bioinformatics
- Targeting inaccessible root canal biofilms

Bottom line....the present
Thank you!!