ENDODONTIC MICROBIOLOGY

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Part 1

Definitions and basic microbiology update

1.1 Definitions

Biofilm: Complex aggregation of microorganisms attached to surface

Commensal: Microorganism living on or in a host but causing the host no harm

Genotype: Genetic composition of an organism

Infection: Invasion by and multiplication of microorganisms in body tissue resulting in disease. The presence of living infectious agents on exterior surfaces of the body, or on objects, is not infection, but represents **contamination** of such surfaces

Opportunistic infection: An infection caused by an organism capable of causing disease only in individuals whose resistance to infection is lowered

Pathogen: Any disease-producing microorganism

Phenotype: Observable characteristics of an organism

Virulence: Degree of pathogenicity or disease-producing ability of a microorganism

1.2 Basic microbiology update

Prokaryotes e.g. bacteria

- Chromosomes are NOT separated from the cytoplasm by a specialized membrane
- No mitochondria (usually)
- Cell wall usually contains peptidoglycan

Eukaryotes e.g. fungi, algae, lichens, animals

- Chromosomes separated from cytoplasm by a specialized membrane (i.e. forming nucleus)
- Mitochondria (usually)
- Cell wall, if present, never contains peptidoglycan

Classification of microorganisms

Taxon - a group having an identity distinct from that of any other category
- can be a group of strains, species, genera etc.

Ranked according to natural affinities or similarities:

Phylum (phila, plural) – rank below Kingdom

e.g. **Proteobacteria**, Spirochaetes, Bacteroidetes, Fusobacteria

Class: e.g. **Gammaproteobacteria**

Order: e.g. **Enterobacteriales**

Family e.g. Enterobacteriaceae

Genus (genera, plural) – one or more genera constitute a family

e.g. *Enterobacter*

Species – one or more species constitute a genus

e.g. *Enterobacter cloacae, Enterobacter aerogenes*

Strain – one or more strains constitute a species

Other means of classifying microorganisms

1. Classification according to morphology or shape

Cocci
- Spherical
- Can form:
  - pairs (diplococci, e.g. *Neisseria*)
  - clusters (*Staphylococcus*)
  - chains (*Streptococcus*)

Bacilli
- Rods
- Short bacilli are called coccobacilli

Spiral forms
- Comma-shaped
- S-shaped, spiral-shaped (e.g. *Treponema*) "spirochetes"

Pleomorphic
- Exhibiting variations in shape

2. Classification according to cell wall characteristics

Gram positive - thick wall, mainly peptidoglycan

Gram negative - thinner wall and have outer membrane; have lipopolysaccharide (LPS), an established pathogenic factor

3. Classification according to oxygen tolerance

Facultative anaerobes

e.g. some *Streptococcus*, *Enterococcus*, *Enterobacter*

- Prefer aerobic conditions but can grow in the absence of oxygen
- Have the FACULTY to be anaerobic by using fermentation for energy (like human muscle cells switching to anaerobic glycolysis during sprinting)
- Have catalase and superoxide dismutase to break down oxygen radicals

Microaerophilic (require oxygen but can only use at low concentrations)

e.g. spirochetes, some *Streptococci*

- Use fermentation for energy and have no electron transport system
- Have superoxide dismutase so can tolerate low amounts of oxygen

Obligate anaerobes

e.g. *Porphyromonas, Prevotella, Fusobacterium, Bacteroides*

- Have no enzymes to defend against oxygen
- **Obligate anaerobes are the most frequent species found in endodontic infections**

4. Classification according to metabolic end products

- End products resulting from
  - How the organism deals with oxygen
  - What the organism uses as a carbon and energy source
    - Asaccharolytic – do not convert sugars to acidic fermentation products
    - Proteolytic – growth depends on ability to metabolize peptides or proteins
- Different metabolic end products that bacteria produce such as acid and gas
- Fermentation end products – glucose is broken down to pyruvic acid, yielding ATP– i.e. glycolysis
  - When pyruvate is broken down different end products are formed and these can be used to classify bacteria, e.g. Lactic acid, ethanol, propionic acid, butyric acid, acetone

Part 2

Biofilms and virulence in endodontic infections

Biofilms: aggregates of microbial cells enclosed in a self-produced matrix adherent to a surface

- Microorganisms in biofilms live in a self-produced matrix of hydrated extracellular polymeric substances (EPS) (Flemming and Wingender 2010)
- Have structural heterogeneity, genetic diversity, complex community interactions
- Have channels that contribute to distribution of nutrients and signaling molecules

2010	Flemming HC, Wingender J	The biofilm matrix	Nat Rev Microbiol 2010:Aug	Extracellular polymeric substances (EPS) are mainly polysaccharides, proteins, nucleic acids and lipids; providing the mechanical stability of biofilms, mediating their adhesion to surfaces and forming a cohesive, three-dimensional polymer network that interconnects and transiently immobilizes biofilm cells.

Biofilm versus planktonic:

- Planktonic microorganisms are in suspension (e.g. cultures in broth)
- Biofilms are attached to a surface (e.g. root canal wall)
- Cells and clusters of cells in biofilms can detach into the surrounding fluid

Biofilms and antimicrobial resistance:

- Biofilm microorganisms are more resistant to antimicrobial agents than planktonic bacteria
- Microbes in biofilms can more easily interact with each other and exchange genes encoding:
    - Virulence factors
    - Antibiotic resistance

2.1 Variable physico-chemical factors in root canals

Microorganisms entering the pulp and periapical tissues must be able to :

Colonize

- Survive in pulpal environment
- Propagate: tissue penetration/invasion

Evade host defense mechanisms

- Such as neutrophils, complement and antibodies

Initiate tissue destruction

- Direct: enzymes, LPS, toxins
- Indirect: induction of host-mediated processes

Deal with variable conditions within the root canal system that include:

- Redox potential/anaerobiosis
- pH
- Nutrient availability
- Exogenous nutrients
- Endogenous nutrients
- Surfaces available for adherence
- Surface characteristics: dentinal, dentinal tubules, smear layer, existing materials

2.2 Microbial interactions occurring in biofilms

Antagonistic	**Beneficial**
Bacteriocins	Enzyme complementation
Hydrogen peroxide	Food chains (webs)
Organic acids (e.g. lactic)	Quorum sensing
Nutrient competition	Coaggregation

Quorum sensing: A means of regulating gene expression and diverse physiological activities within community

Coaggregation: Recognition and adhesion between genetically distinct bacteria. Involves specific adhesins and complementary receptor molecules

2.3 Root canal biofilms

Complex polymicrobal structures adherent to the root canal surface and formed by microorganisms that have invaded the pulpal space.

- Early histology study. Described clusters of "self-aggregating" colonies of one distinct type or "coaggregating" communities of several types in apical root canals of therapy resistant teeth observed using TEM (Nair et al. 1990).
- First in depth review to present how the biofilm concept may apply to endodontic infections - Svensater and Bergenholtz (2005).
- Conditions under which biofilms develop in root canals not well understood. Histological observations show biofilm morphology varies between cases (Nair et al. 2005, Chavez de Paz 2007, Carr et al. 2009, Ricucci and Siqueira 2010)
- "Apical periodontitis" should be viewed as a "biofilm-induced disease" (Ricucci and Siqueira 2010)

- Bacteria isolated from infected roots canals resisted alkaline stress better in biofilms than in planktonic cultures (Chavez de Paz et al. 2007)
- Mixed root canal microbial communities are variably "resistant" to disturbances, as measured by viability of the biofilm cells (Chavez de Paz 2012; Stojicic et al. 2013; Shrestha and Kishen 2014)
- Older biofilms are more resistant than younger biofilms to antimicrobials (Stojicic et al. 2013)
- Strains of *Enterococcus faecalis* differ in their ability to coexist in biofilms with other root canal bacteria (Chavez de Paz et al. 2015)
- Antibiotic resistance genes can transfer between *E. faecalis* and *S. gordonii* in root canal biofilms *ex vivo* (Sedgley et al. 2008)

1990	Nair PN, Sjogren U, Krey G, Kahnberg KE, Sundqvist G	Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study	J Endod 1990;16:580-8	Used light and transmission electron microscopy to study the apical root canal of teeth with asymptomatics periapical lesions. Described clusters of "self-aggregating" colonies of one distinct type or "coaggregating" communities of several types
2005	Nair PN, Henry S, Cano V, Vera J	Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment	OOOOE 2005;99:231-52	Used light and transmission electron microscopy to study mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Concluded that inaccessible biofilms in complex root canal systems of mandibular first molar roots cannot be removed by contemporary instruments and irrigation alone in one-visit treatment
2007	Chavez de Paz L	Redefining the persistent infection in root canals: possible role of biofilm communities	J Endod 2007;33:652-62	Review. Presents evidence that the nature of persisting endodontic infections depends not on the robustness of the organisms in the infected site, but on their capability of adapting their physiology to the new environmental conditions set by the treatment.
2007	Chavez de Paz LE, Bergenholtz G, Dahlen G, Svensater G	Response to alkaline stress by root canal bacteria in biofilms	Int Endod J 2007;40:344-55	*In vitro*: *E. faecalis*, *L. paracasei*, *O. uli* and *S. gordonii* isolated from infected roots canals resisted alkaline stress better in biofilms than in planktonic cultures. Planktonic cells use aggregation and the extracellular transport of specific proteins as survival mechanisms.
2008	Sedgley CM, Lee EH, Martin MJ, Flannagan SE	Antibiotic resistance gene transfer between *Streptococcus gordonii* and *E. faecalis* in root canals of teeth *ex vivo*	J Endod 2008;34:570-4	*Ex vivo*. Antibiotic resistance gene transferred between different species in root canals. Findings demonstrate that horizontal genetic exchange in endodontic infections might facilitate adoption of an optimal genetic profile for survival.
2009	Carr GB, Schwartz RS, Schaudinn C, Gorur A, Costerton JW	Ultrastructural examination of failed molar retreatment with secondary apical period-ontitis: an examination of endodontic biofilms in an endodontic retreatment failure	J Endod 2009;35(9):1303-9	Case report. LM and TEM examination of resected root tip of a failing endodontically re-treated lower molar.. Observed a complex, variable, multispecies biofilm was present the entire length of the specimen

2010	Ricucci D, Siqueira JF Jr	Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings	J Endod 2010;36:12 77-88	Histology study. Case series. 106 root apices sectioned, stained Brown & Brenn and examined under LM. Observed highly variable morphological appearance of biofilms on root canal walls. In 71 cases where Xrays available, associated increased likelihood of biofilm with larger PARL and presence of epithelialized lesion. Proposed that apical periodontitis be included in the set of "biofilm-induced" diseases
2012	Chavez de Paz LE	Development of a multispecies biofilm community by four root canal bacteria	J Endod 2012;38:31 8–23	Grew *L. salivarius, S. gordonii. E. faecalis* and *A. naeslundii* biofilms. Biofilm architecture varied over time (viewed by CLSM). Metabolic activity rates in young biofilms increased in presence of glucose .
2013	Stojicic S, Shen Y, Haapasalo M	Effect of the source of biofilm bacteria, level of biofilm maturation, and type of disinfecting agent on the susceptibility of biofilm bacteria to antibacterial agents	J Endod 2013:39:47 3–7	Showed that the source of biofilm bacteria, level of biofilm maturation, and type of disinfecting agent affect the susceptibility of multispecies biofilm bacteria to antibacterial agents. The change of biofilm bacteria from sensitive to resistant against disinfecting agents occurred between 2 and 3 weeks of biofilm maturation
2014	Shrestha A, Kishen A	Antibiofilm efficacy of photosensitizer-functionalized bioactive nanoparticles on multispecies biofilm	J Endod 2014;40:16 04–10	Multispecies biofilms of *Streptococcus oralis*, *Prevotella intermedia*, and *Actinomyces naeslundii* grown on dentin sections for 21 days were disruptable by photodynamic therapy and rose bengal-functionalized chitosan nanoparticles.
2015	Chavez de Paz LE, Davies JR, Bergenholtz G, Svensater G	Strains of *Enterococcus faecalis* differ in their ability to coexist in biofilms with other root canal bacteria	Int Endod J 2015;48:91 6-25	*L. salivarius, S. gordonii* and *A. naeslundii* formed mutualistic biofilm communities with strain *E. faecalis* GUL1. In contradistinction, *L. salivarius* and *S. gordonii* were outcompeted when *E. faecalis* OG1RF was included.

2.4 Virulence of endodontic microorganisms

Virulence: degree of pathogenicity or disease-producing ability of a microorganism

Virulence factors: characteristics of microorganism that enable it to induce disease

Factors	Effects
Endotoxin (LPS)	Fever, bone resorption, pain, vasomotor shock Recognized by TLR4
Enzymes	
Collagenase	Destroys collagen
Chondroitin sulfatase	Digests ground substance
Hyaluronidase	Digests ground substance
Kinases	Fibrinolysin
Gelatinase	Proteolytic
Protease	Proteolytic
Coagulase	Activates fibrin clot
Hemolysins	Destroys erythrocytes
Leukocidin	Destroys leukocytes
DNAse	Destroys nucleic acids
Metabolic factors	
Acids	Denatures protein
Alcohols	Denatures protein, solvent effect

2.5 Endotoxin and endodontic infections

In endodontic literature, the terms "endotoxin" and "LPS" have been used interchangeably
Presence of endotoxin is associated with gram-negative bacteria:

- Pulpal pain and periapical inflammation (Schein and Schilder 1975, Schonfeld et al. 1982, Horiba et al. 1991, Jacinto et al. 2005)
- Bone destruction (Dahlén et al. 1981, Dwyer and Torabinejad 1981)
- 1° infections had higher contents of endotoxins and more complex gram-negative bacterial community than teeth with 2° infections. Levels of endotoxins were related to the severity of bone destruction in periapical tissues (Gomes et al. 2012)

1975	Schein B, Schilder H	Endotoxin content in endodontically involved teeth	J Endod 1975;1:19-21	Found teeth that were pulpless, painful, and had radiolucencies contained more endotoxin. Classic article that relates endotoxin with endodontic symptoms
1980	Dahlén G, Bergenholtz G	Endotoxic activity in teeth with necrotic pulps	J Dent Res 1980;59:1033-40	LPS present in canals with infected necrotic pulps Samples from 13 teeth with necrotic pulps analyzed using limulus lysate technique. Endotoxic activity of the root canal samples was correlated with the presence and the number of Gram-negative bacteria in the root canal
1981	Dwyer TG, Torabinejad M	Radiographic and histologic evaluation of the effect of endotoxin on the periapical tissues of the cat	J Endod 1981;7:31-5	Animal study. Endotoxin placed in vital canals in cats caused severe pulpal and periradicular reaction. Endotoxin plays a significant role in periradicular inflammatory disease.
1981	Dahlén G, Magnuson BC, Möller AJ	Histological and histochemical study of the influence of LPS	Arch Oral Biol 1981;26:59	Studied the influence on the periapical tissues of LPS extracted and purified from a strain of *F. nucleatum* originally isolated from an infected root canal in a

		extracted from *F. nucleatum on* the periapical tissues in the monkey *Macaca fascicularis*	1-8	monkey. Found that LPS can be retained within the root canal as well as incorporated into the cementum for long periods of time. LPS can induce bone destruction *in vivo*
1982	Schonfeld SE, Greening AB, Glick DH, Frank AL, Simon JH, Herles SM	Endotoxic activity in periapical lesions	OOO 1982;53:82-7	Examined 30 human tissue samples histologically and classified as inflamed (apical granulomas) or non-inflamed (scars or noninflamed cysts). Presence of endotoxin in samples measured by limulus assay; 75% of inflamed tissues positive for endotoxin, while 20% of noninflamed tissues contained endotoxin ($p = 0.015$).
1991	Horiba N, Maekawa Y, Abe Y, Ito M, Matsumoto T, Nakamura H	Correlations between endotoxin and clinical symptoms or radiolucent areas in infected root canals	OOO 1991;71:492-5	Samples were collected from the root canals of 30 teeth of patients with apical periodontitis and assayed for endotoxin content. Endotoxin and endotoxin content were higher in symptomatic teeth, teeth with radiolucent areas, and teeth with exudation.
2005	Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ	Quantification of endotoxins in necrotic root canals from symptomatic and asymptomatic teeth	J Med Microbiol 2005;54:777-83	Samples collected from 50 patients with necrotic root canals. Positive association found between endotoxin and symptomatic cases (e.g. spontaneous pain, tenderness to percussion, pain on palpation, swelling and purulent exudates). Endotoxin was present in high concentrations in root canals of symptomatic teeth.
2012	Gomes BP, Endo MS, Martinho FC	Comparison of endotoxin levels found in primary and secondary endodontic infections	J Endod 2012;38:1082-6	Clinical study. Samples taken from 15 canals with 1° infections and 15 with 2° infections. 1° infections had higher contents of endotoxins and more complex gram-negative bacterial community than teeth with 2° infections. Levels of endotoxins were related to the severity of bone destruction in periapical tissues

2.6 Other virulence factors associated with endodontic microflora

- Increased collagenase production associated with larger periapical lesions (Hashioki et al. 1994)
- Fimbriae enhancing adhesion (Leung et al. 1996, Figdor and Davies 1997, Rôças and Siqueira 2010)
- *P. gingivalis* types II, IV, and Ib fimA (fimbriae) were associated with greater risk of clinical signs (swelling, sinus tract, or intracanal exudates) than type I (Wang et al. 2010)
- *Fusobacterium nucleatum* ATCC 10953 produces four types of proteases: serine, cysteine, aspartic and metallo-proteases (Ogawa et al. 2006)
- *F. nucleatum* pathogenesis may be associated with its ability to induce aggregation and apoptosis of immune cells (Huynh et al. 2011)
- Production of gelatinase and response to pheromones by root canal *E. faecalis* isolates (Sedgley et al. 2005)
- Gelatinase activity may play a role in long-term survival of *E. faecalis* in obturated root canals (Sedgley 2007)
- Sonicated bacterial extracts from *P. gingivalis* activated PDL cell matrix metalloproteinase MMP-2, suggesting a potential role in connective tissue destruction (Sato et al. 2009)

- *P. nigrescens* might be able to activate proMMP-2 and proMMP-9 *in vivo* and this activation might be related to the destruction of periapical tissues (Itoh et al. 2009)
- Protein production by *S. gordonii*, *S. anginosus*, and *S. oralis* may be of pathogenic significance in posttreatment apical periodontitis (Chavez de Paz et al. 2005)
- Bacterial proteins in root canal samples included : adhesins, autolysins, proteases, virulence factors, and antibiotic-resistance proteins (Nandakumar et al. 2009)
- Butyrate, a short chain fatty acid metabolic byproduct is cytotoxic to human pulp fibroblasts (Ho and Chang 2007)
- Short chain fatty acids were identified in samples from 18 cases with infected root canals (Provenzano et al. 2015)

1994	Hashioka K, Suzuki K, Yoshida T, Nakane A, Horiba N, Nakamura H	Relationship between clinical symptoms and enzyme-producing bacteria isolated from infected root canals	J Endod 1994;20:75-7	28 teeth from 25 patients with apical periodontitis. Bacteria producing collagenase or chondroitinase and hyaluronidase were found to be significantly related to subacute clinical symptoms involving percussion pain. Increased collagenase was associated with larger periapical lesions.
1996	Leung KP, Fukushima H, Nesbitt WE, Clark WB	*Prevotella intermedia* fimbriae mediate hemagglutination	Oral Microbiol Immunol 1996;11:42-50	These bacteria have the ability to agglutinate selected mammalian erythrocytes
1997	Figdor D, Davies J	Cell surface structures of *Actinomyces israelii*	Aust Dent J 1997;42:125-8	Seen under EM, some species of *A. israelii* had hairlike fimbriae protruding through a thick surface coat, structures perhaps able to contribute to pathogenicity.
2005	Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, Dahlén G	Virulence, phenotype and genotype characteristics of endodontic *Enterococcus* spp.	Oral Microbiol Immunol 2005;20:10-9	*In vitro*. Investigated the virulence, phenotype and genotype of 33 endodontic enterococcal isolates. Phenotypic and genotypic evidence of potential virulence factors were identified in endodontic *Enterococcus* spp., specifically production of gelatinase and response to pheromones.
2005	Chavez de Paz L, Svensater G, Dahlén G, Bergenholtz G	*Streptococci* from root canals in teeth with apical periodontitis receiving endodontic treatment	OOOOE 2005;100:232-41	Root canal isolates of *S. gordonii*, *S. anginosus*, and *S. oralis* were shown to be strong producers of extracellular proteins. This may be of pathogenic significance in posttreatment apical periodontitis
2006	Ogawa AT, Brasil de Souza Tde A, de Uzeda M, etal	Characterization of proteolytic activities of *Fusobacterium nucleatum*	J Endod 2006;32:521-3	*In vitro*: Showed that *Fusobacterium nucleatum* ATCC 10953 produced four types of proteases: serine, cysteine, aspartic and metallo-proteases
2007	Ho YC, Chang YC	Effects of a bacterial lipid byproduct on human pulp fibroblasts *in vitro*	J Endod 2007;33:437-41	*In vitro*. Butyrate, a short chain fatty acid, is a metabolic lipid byproduct of various root canal pathogens, such as *P. endodontalis*. Butyrate is cytotoxic to human pulp fibroblasts by inhibiting cell growth, cell-cycle kinetics, and protein synthesis.

2007	Sedgley CM	Influence of root canal sealer and gelatinase activity on extended survival of *E. faecalis* in obturated root canals *in vitro*	J Endod 2007;33:561-6	*In vitro*. Gelatinase activity may play a role in long-term survival of *E. faecalis* in obturated root canals
2008	Baik JE, Ryu YH, Han JY, Im J, Kum KY, Yun CH, Lee K, Han SH	Lipoteichoic acid partially contributes to the inflammatory responses to *Enterococcus faecalis*	J Endod 2008;34:975-82	*In vitro*. Lipoteichoic acid derived from *E. faecalis* upregulated mouse macrophage inflammatory cytokines.
2009	Nandakumar R, Madayiputhiya N, Fouad AF	Proteomic analysis of endodontic infections by liquid chromatography-tandem mass spectrometry	Oral Microbiol Immunol 2009 Aug;24:347-52.	In clinical samples from 7 infected root canals, identified proteins from bacteria (especially *E. faecalis, E. faecium, P.gingivalis, F. nucleatum*, and *T. denticola*) were identified from all the samples tested. Identified proteins included adhesins, autolysins, proteases, virulence factors, and antibiotic-resistance proteins.
2009	Sato Y, Kishi J, Suzuki K, Nakamura H, Hayakawa T	Sonic extracts from a bacterium related to periapical disease activate gelatinase A and inactivate tissue inhibitor of metalloproteinases TIMP-1 and TIMP-2	Int Endod J 2009 Dec;42:1104-11	*In vitro.* In PDL cell culture supernatants exposed to *P. gingivalis* SBE, matrix metalloproteinase MMP-2 was ACTIVATED and tissue inhibitors of metalloproteinase (TIMP-1 and TIMP-2) were INACTIVATED, suggesting a role for *P. gingivalis* in connective tissue destruction.
2009	Itoh T, Nakamura H, Kishi J, Hayakawa T	The activation of matrix metalloproteinases by a whole-cell extract from *Prevotella nigrescens*	J Endod 2009;35:55-9	*In vitro*. *P. nigrescens* whole cell extract was mixed with proMMP-2 or -9, and the activation of the latter was determined by gelatin zymography. Concluded that *P. nigrescens* might be able to activate proMMP-2 and proMMP-9 *in vivo* and that this activation might be related to the destruction of periapical tissues.
2010	Rôças IN, Siqueira JF Jr	Distribution of *Porphyromonas gingivalis fimA* genotypes in primary endodontic infections	OOOOE 2010 Mar;109:474-8	Long fimbriae (FimA) are virulence factors of *P. gingivalis*. Isolated *P. gingivalis* in 9/25 root canal samples. Four of the six known fimA variants were detected in PCR assays of genomic DNA from the 9 samples.
2010	Wang Q, Zhou XD, Zheng QH, Wang Y, Tang L, Huang DM	Distribution of *Porphyromonas gingivalis* fimA genotypes in chronic apical periodontitis associated with symptoms	J Endod. 2010 Nov;36(11):1790-5	Root canal samples were obtained from 158 infected root canals with apical periodontitis. *P. gingivalis* was detected in 39.9% samples. The occurrences of types II, IV, and Ib fimA were associated with greater risk of clinical signs (swelling, sinus tract, or intracanal exudates) than type I.
2015	Provenzano JC, Rôças IN, Tavares LF, Neves BC, Siqueira JF, Jr	Short-chain fatty acids in infected root canals of teeth with apical periodontitis before and after treatment	J Endod 2015;41:831-5	Clinical study. SCFAs are implicated as potential virulence factors in perio disease. This study identified SCFAs in samples from 18 cases with infected root canals. Butyric acid most common, then propionic acid.

Part 3

Methods used to evaluate endodontic microflora

- **Histology**
- **Culturing and biochemical tests**
- **Microbial bioinformatics**
- **Assessing bacterial viability**

3.1 Histology

Primarily limited to observing morphology

- Light microscopy (LM) (Ricucci and Bergenholtz 2003, Nair et al. 2005, Ricucci et al. 2015)
- Transmission electron microscopy (TEM) (e.g. Nair et al. 2005)
- Scanning electron microscopy (SEM) (e.g. Leonardo et al. 2002)
- Environmental SEM (Bergmans et al. 2005)
- Combination of LM, TEM, SEM offers complementary information (Richardson et al. 2009)
- Confocal laser SEM (Zapata et al. 2008, Parmar et al. 2011)

2002	Leonardo MR, Rossi MA, Silva LA, Ito IY, Bonifacio KC	SEM evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth	J Endod 2002;28:815-8	SEM examination of apices of eight teeth with pulp necrosis and periapical lesions. Reported detection of biofilm on external surfaces of apices of teeth
2003	Ricucci D, Bergenholtz G	Bacterial status in root-filled teeth exposed to the oral environment by loss of restoration and fracture or caries – a histobacteriological study of treated cases	Int Endod J 2003;36:787-802	Longitudinal tissue sections of 39 roots. Observed presence of stainable bacteria in abundance at canal entrance and in dentinal tubules but absent mid-root and apically in all but two specimens. Concluded that well-prepared and filled root canals resist bacterial penetration even upon frank and long-standing oral exposure by caries, fracture or loss of restoration.
2005	Nair PN, Henry S, Cano V, Vera J	Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment	OOOOE 2005;99:231-52	Used light and transmission electron microscopy to study mandibular first molars with apical periodontitis after "one-visit" endodontic treatment.
2005	Bergmans L, Moisiadis P, Van Meerbeek B, Quirynen M, Lambrechts P	Microscopic observation of bacteria: review highlighting the use of environmental SEM	Int Endod J 2005;38:775-88	SEM that can examine under environmental conditions *in situ* (without need for critical point drying and sputter-coating). May allow observation of root canal bacteria in their native state without prior preparation.

2008	Zapata RO, Bramante CM, de Moraes IG, Bernardineli N, Gasparoto TH, Graeff MS, Campanelli AP, Garcia RB	Confocal laser scanning microscopy is appropriate to detect viability of *Enterococcus faecalis* in infected dentin	J Endod 2008;34:1198-201	CLSM analysis shows that the discrimination between viable (green) and dead (red) bacteria in infected dentinal tubules could be observed after staining
2009	Richardson N, Mordan NJ, Figueiredo JA, Ng YL, Gulabivala K.	Microflora in teeth associated with apical periodontitis: a methodological observational study comparing two protocols and three microscopy techniques	Int Endod J 2009;42:908-21	Studied 9 root samples of 7 teeth with acute epsidoes of chronic apical periodontitis. Concluded that a combination of techniques (light microscopy, transmission electron microscopy and SEM) offered complementary views. Also noted that PMNs may be found in the coronal third of root canals containing necrotic pulp tissue.
2011	Parmar D, Hauman CH, Leichter JW, McNaughton A, Tompkins GR	Bacterial localization and viability assessment in human *ex vivo* dentinal tubules by fluorescence confocal laser scanning microscopy	Int Endod J. 2011 Jul;44(7):644-51	*Ex vivo*. Obtained root slices from 12 teeth infected with *E. faecalis*, and bacterial evaluated viability using live/dead stains and CLSM. Percentage survival of bacteria was sig less in teeth (n=6) treated with CaOH2 (29%-50%) compared to untreated control teeth (n=6) (83%-96%). Concluded that fluorescent viability staining is a convenient, accurate and reproducible method for localizing and quantifying live and dead bacteria in human ex vivo mineralized dentinal tubules.
2015	Ricucci D, Siqueira JF, Jr., Lopes WS, Vieira AR, Rocas IN J Endod 2015;41:265-73	Extraradicular infection as the cause of persistent symptoms: a case series	J Endod 2015;41:265-73	3 cases. Different forms of extraradicular infection were associated with three symptomatic cases. Observed (1) necrotic debris, heavily colonized by ramifying bacteria, in cyst lumen, (2) numerous bacterial aggregations through the inflammatory tissue in granuloma, (3) bacterial biofilms on external apical root surface, filling large lateral canal and other apical ramifications, and between layers of cementum detached from root surface

3.2 Culturing and biochemical tests to identify endodontic microflora

Traditional methods providing identification based on PHENOTYPE

- **Advantage of culturing**
  - Able to keep the microorganism for further analyses
- **Disadvantages of culturing**
  - Can take a long time to grow, e.g. spirochetes
  - Fast growing bacteria can distort results
  - Not all species can be cultured routinely

•Minor differences in culturing and examination procedures can greatly influence results, e.g. degree of anaerobiosis within the media, size and age of the inoculum

•Culturing has been used to study the microflora of:

•Caries (e.g. Hahn et al. 1991)
•Necrotic pulps (e.g. Kantz and Henry 1974)
•Dentinal tubules (e.g. Peters et al. 2001)
•Exudates from periapical acute infections (e.g. Noda et al. 1999)
•Periapical lesions (e.g. Sunde et al. 2000)

1974	Kantz WE, Henry CA	Isolation and classification of anaerobic bacteria from intact chambers of non-vital teeth in man	Arch Oral Biol 1974;19:91-6.	Utilized an anaerobic glove box which contained 85% nitrogen, 10% carbon dioxide and 5% hydrogen. Anaerobes alone were isolated from 3/24 teeth, facultative microbes alone from 4/24 teeth, anaerobes mixed with facultative anaerobes in 9/24 teeth
1991	Hahn CL, Falkler WA Jr, Minah GE	Microbiological studies of carious dentine from human teeth with irreversible pulpitis	Arch Oral Biol 1991;36:147-53	In 15 lesions lactobacilli constituted 91.9% of total flora at pulpal site and gradually decreased in number as moved away from pulp. In 14 lesions with low numbers of lactobacilli, Gram positive cocci, anaerobic Gram positive rods and/or *Bacteroides* were the main isolates.
1999	Noda M, Inoue S, Komatsu H	A comparison of methods for detecting bacteria in root canal exudates	J Endod 1999;25:187-9	Sampled 22 root canal exudates. 6/22 positive samples using conventional anaerobic sampling compared to 18/22 using a preculture sampling method. Suggested using a preculture method to detect bacteria in root canal samples. Gram positive cocci were the main bacteria recovered.
2000	Sunde PT, Olsen I, Lind PO, Tronstad L	Extraradicular infection: a methodological study	Endod Dent Traumatol 2000;16:84-90	Sampled 30 patients with root-filled teeth and periapical radiolucencies. Predominant cultivable bacteria were anaerobic. Also compared marginal and submarginal incisions. Phenotypic profiling indicated that following marginal incision, bacteria from the periodontal pocket might have reached the underlying tissues by surgeon-released bacteremia or direct translocation.
2001	Peters LB, Wesselink PR, Buijs JF, van Winkelhoff AJ	Viable bacteria in root dentinal tubules of teeth with apical periodontitis	J Endod 2001;27:76-81	Root dentin of 20 teeth was cultured from three locations between pulp and cementum (A, B, and C). At greater distance, in layer C, from the pulp bacteria were found in 62% (13 of 21) of the dentin samples. In layers closer to the pulp higher numbers of anaerobic bacteria and gram-positive rods were found, as well as a larger number of bacterial species.

3.3 Microbial bioinformatics for identification of endodontic microflora

2.3.1. Identification based on GENOTYPE (DNA-based identification)

- **Molecular methods most commonly used in endodontic microbiology are**:

Polymerase chain reaction (PCR)

- Introduced in the mid-80s
- A rapid, sensitive and specific molecular diagnostic tool for the analysis of microorganisms in clinical, environmental and food samples.
- Process involves enzymatic **amplification** of nucleic acid sequences via repeated cycles of denaturation, oligonucleotide annealing, and DNA polymerase extension
- Extremely effective with **pure** solutions of nucleic acids
- BUT sensitivity may be reduced dramatically when applied directly to biological samples due to PCR inhibitors in biological samples

DNA-DNA checkerboard hybridization

- Developed by Socransky et al. (1994) to study periodontal microflora
- **Hybridizes** large numbers of DNA samples against large numbers of DNA **probes** on a single support membrane
- Since there is no amplification of the bacteria (as in PCR) then minor contaminants are not likely to be detected, but this means the method is unable to detect species present in low numbers

Nested-PCR

- A variant form of PCR carried out in 2 stages. 1st stage – standard PCR, 2nd stage – uses 1st product as a template for another phase of PCR using different primers to detect a PCR product within the 1st sample. Useful in cases where the sample is limited in quantity, e.g. root canal sample.

Multiplex-PCR

- A variant form of PCR that allows detection of more than one PCR product by including additional primers. Useful if looking for more than one type of microorganism in a sample.

PCR-denaturing gradient gel electrophoresis (PCR-DGGE)

- A variant form of PCR. DNA is extracted from the sample, and part of the 16S rDNA of all bacteria is amplified by PCR. These PCR products are separated by DGGE, generating banding patterns that provide a "fingerprint" of the sample

Pulsed field gel electrophoresis (PFGE)

- A technique used to separate long strands of DNA by length in order to tell differences among samples. It operates by alternating electric fields to run DNA through a flat gel. Provides a "fingerprint".

Reverse transcriptase PCR (RT-PCR)

- Allows DNA copies to be manufactured from messenger RNA

Quantitative real-time PCR (QRT-PCR, also called "real-time PCR", qPCR)

- Enables both detection and quantification of a specific DNA sequence in a sample

Pyrosequencing - analysis of 16S rRNA gene sequences

- Enables more comprehensive analysis than traditional sequencing

- **Advantages of DNA- based molecular methods over other identification methods**
  - Allows relatively rapid screening of an endodontic sample for selected microbes of interest
  - Can identify microbes that are not culturable

- **Disadvantages of PCR-based molecular methods**
  - Not distinguishing between DNA from live or dead microorganisms (Young et al. 2007)
  - Researcher selects microorganism to search for in sample. Operator bias?
  - Don't get to keep the microorganism for further phenotypic analyses
  - Many studies have not included sterility sampling "controls", therefore the DNA being "identified" may actually be contaminants from sampling site
  - Free DNA in samples undergoes spontaneous decomposition (Brundin et al. 2010)
  - The strong binding affinity between DNA and dentin may confound molecular analysis of the endodontic microbiota (Brundin et al. 2014)

- **DNA-based molecular methods have been used to study the microflora of:**
  - Canal contents
    - DNA-DNA hybridization (e.g. de Souza et al. 2005)
    - PCR (e.g. Fouad et al. 2002)
    - Nested-PCR (Siqueira et al. 2004)
    - Multiplex-PCR (Siqueira and Rôças 2004)
    - QRT-PCR (Sedgley et al. 2006)
    - Analysis of 16S rRNA gene sequences using pyrosequencing (Li et al. 2010, Siqueira et al. 2011)
  - PA tissues
    - DNA-DNA hybridization (e.g. Gatti et al. 2000)
    - PCR (e.g. Elkins et al. 1994)

- **Are DNA-based molecular methods better than traditional culturing methods?**
  - Molecular methods more sensitive than culturing (Rolph et al. 2001, Sedgley et al. 2006)
  - Molecular methods no better than culturing for *F. nucleatum* in clinical samples (Moraes et al. 2002)
  - Some molecular procedures require large quantities of DNA or RNA that need to be extracted from large numbers of bacteria which are grown via traditional culturing

- **Pyrosequencing is presently the most sensitive DNA-based identification method for analysing endodontic infections**
  - Evaluates the presence of 16S rRNA gene sequences
  - In 7 infected root canal samples: 200,129 sequences belonging to 97 families phylotypes, 179 genera and 13 phyla (Li et al. 2010)
  - In 10 infected apical root segments: 9,818 gene sequences belonging to 187 phylotypes, 84 genera and 10 phyla (Siqueira et al. 2011)
  - In 13 PA lesions from symptomatic teeth: 35,731 gene sequences belonging to 73 bacterial genera and 10 phyla (Saber et al. 2012)
  - Comparison of 24 primary and 24 persistent root canal infections - persistent infections may have more diverse bacterial communities than primary infections (Tzanetakis et al. 2015)

1994	Elkins DA, Torabinejad M, Schmidt RE, Rossi JJ, Kettering JD	Polymerase chain reaction detection of HIV DNA in human periradicular lesions	J Endod 1994;20:386-8	Used PCR to determine that periradicular lesion from an HIV-positive patient contained HIV DNA
2000	Gatti JJ, Dobeck JM, Smith C, Socransky SS, Skobe Z	Bacteria of asymptomatic periradicular endodontic lesions identified by DNA-DNA hybridization	Endod Dent Traumatol 2000;16:197-204	Used Checkerboard DNA-DNA hybridization to detect bacterial DNA in 36 lesions from patients with asymptomatic, chronic suppurative periodontitis (all previously root filled). Submarginal incision better than intrasulcular in avoiding sample contamination. Bacterial DNA was found in all lesions. *Bacteroides forsythus* and *Actinomyces naeslundii* predominant.
2001	Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie. D, Coldero L, Bagg J	Molecular identification of microorganisms from endodontic infections	J Clin Microbiol 2001;39:3282-9	41 clinical samples from 15 *de novo* and 26 refractory cases of endodontic infections assessed using culturing and PCR: 44% of samples positive by culture compared to 68% positive by PCR. Molecular techniques can detect the presence of bacteria in endodontic infections when cultures are negative, and can be used to identify previously unidentified/unculturable bacteria.
2002	Moraes SR, Siqueira JF, Colombo AP, Rôças IN, Ferreira M, Domingues R	Comparison of the effectiveness of bacterial culture, 16S rDNA directed PCR, and checkerboard DNA-DNA hybridization for detection of *F. nucleatum* in endodontic infections	J Endod 2002;28:86-9	Compared bacterial culture, 16S rDNA directed polymerase chain reaction, and checkerboard DNA-DNA hybridization. No method could be considered more sensitive for detecting *F. nucleatum* directly from clinical samples. Taken together, *F. nucleatum* was present in 31% (4/13) of specimens.
2002	Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R, Hazlett K, Radolf JD	PCR-based identification of bacteria associated with endodontic infections	J Clin Microbiol 2002;40:3223-31	Used PCR primers that targeted the bacterial 16S rRNA genes of ten pathogens. 24 teeth with necrotic pulps. Found that preoperative symptoms were significantly associated with the presence of *Streptococcus* spp. Black pigmented Gram negative anaerobes were not associated with symptoms.

2004	Siqueira JF Jr, Rôças IN, Alves FR, Santos KR	Selected endodontic pathogens in the apical third of infected root canals: a molecular investigation	J Endod 2004;30:638-43	Apical root portion of 23 extracted teeth sectioned, and root canals were sampled. DNA extracted from the samples. Nested PCR methods identified *P. alactolyticus* in 10 cases (44%), *T. denticola* in 6 (26%), *F. nucleatum* in 6 (26%), *Porphyromonas endodontalis* in 4 (17%), *Filifactor alocis* in 2 (9%), *Dialister pneumosintes* in 1 (4%), *Porphyromonas gingivalis* in 1 (4%), and *Tannerella forsythensis* in 1 (4%). Overall, 17/23 samples were positive for at least 1 target species.
2004	Siqueira Junior JF, Rôças IN	Simultaneous detection of *Dialister pneumosintes* and *Filifactor alocis* in endodontic infections by 16S rDNA-directed multiplex PCR	J Endod 2004;30:851-4	Identified *D. pneumosintes* and *F. alocis* in the one PCR reaction.
2005	de Souza CA, Teles RP, Souto R, Chaves MA, Colombo AP	Endodontic therapy associated with calcium hydroxide as an intracanal dressing: microbiologic evaluation by the checkerboard DNA-DNA hybridization technique	J Endod 2005;31:79-83	Evaluated the predominant microbiota of 12 infected necrotic pulps and the effects of calcium hydroxide therapy on these microorganisms by the checkerboard DNA-DNA hybridization. Results indicated that conventional endodontic therapy with calcium hydroxide reduced pathogenic species but did not eliminate the whole spectrum of microorganisms
2006	Sedgley CM, Nagel AC, Dahlén G, Reit C, Molander A	Real-time quantitative PCR and culture analysis of *Enterococcus faecalis* in root canals	J Endod 2006;32: March	Consecutive root canal samples obtained from 40 primary infection and 48 retreatment cases were divided into two equal aliquots that were independently analyzed using culture and qPCR by investigators blinded to the analysis results of the other sample. Bacteria were detected in 48/88 (54.5%) endodontic samples by culture compared to 88/88 (100%) by qPCR
2007	Young G, Turner S, Davies JK, Sundqvist G, Figdor D	Bacterial DNA persists for extended periods after cell death	J Endod 2007;33:1417-20	*In vitro*. Studied fate of bacterial DNA 1 year after cell death using *E. faecalis* broth cultures. A year after cell death DNA could still be detected by PCR, although there was a 1000-fold decay. Implications: questions the validity of PCR-based identification methods for RCT sampling, as detected DNA may be from dead cells and not viable cells. Investigators also noted that the PCR reactions can be inhibited by thiosulfate-inactivated NaOCl (used to disinfect tooth prior to sampling).
2010	Brundin M, Figdor D, Roth C, Davies JK, Sundqvist G, Sjögren U	Persistence of dead-cell bacterial DNA in ex vivo root canals and influence of nucleases on DNA decay in vitro	OOOOE 2010;110:789-94	Assessed the recovery of PCR-detectable DNA in root canals of human extracted teeth. Amplifiable DNA is preserved after cell death, but the critical determinant is the form of DNA. *E. faecalis* free DNA undergoes spontaneous and enzymatic decomposition, whereas cell-bound DNA persists for long periods.
2010	Li L, Hsiao WW, Nanda-	Analyzing endodontic infections by deep	J Dent Res. 2010;89:98	Pyrosequencing technology should allow for more comprehensive analysis than traditional Sanger

	kumar R, Barbuto SM, Mongodin EF, Paster BJ, Fraser-Liggett CM, Fouad AF	coverage pyrosequencing	0-4	sequencing. Analysis of 7 root canal samples (1 retreatment) showed 47 vs. 28,590 sequences obtained per sample for Sanger sequencing vs. pyrosequencing, i.e. representing a 600-fold difference in "depth-of-coverage". Bottom line: bacterial communities in endodontic infections are more diverse than previously demonstrated.
2011	Siqueira JF Jr, Alves FR, Rôças IN	Pyrosequencing analysis of the apical rootcanal microbiota	J Endod. 2011 Nov; 37(11):1499-503	Used pyrosequencing to evaluate the microflora of 10 cryopulverized apical root segments of 10 extracted teeth. Generated 9,818 partial 16S rRNA gene sequences. Identified sequences belonging to 187 phylotypes, 84 genera and 10 phyla.
2012	Saber MH, Schwarzberg K, Alonaizan FA, Kelley ST et al	Bacterial flora of dental periradicular lesions analyzed by the 454-pyrosequencing technology	J Endod 2012;38:1484-8	Used pyrosequencing to evaluate 16S rRNA genes in 13 periapical lesion samples from symptomatic teeth. Generated 35,731 high-quality sequences belonging to 10 bacterial phyla and 73 bacterial genera.
2014	Brundin M, Figdor D, Johansson A, Sjogren U	Preservation of bacterial DNA by human dentin	J Endod 2014;40:241-5	*In vitro*. Showed there is a strong binding affinity between DNA and dentin, and between DNA and serum proteins or collagen. These substrates preserve DNA against natural decomposition and protect DNA from nuclease activity, factors that may confound molecular analysis of the endodontic microbiota.
2015	Tzanetakis GN, Azcarate-Peril MA, Zachaki S, Panopoulos P, Kontakiotis EG, Madianos PN, Divaris K	Comparison of Bacterial Community Composition of Primary and Persistent Endodontic Infections Using Pyrosequencing	J Endod 2015;41:1226-33	Used pyrosequencing to evaluate 16S rRNA gene sequences in samples from 24 primary and 24 persistent root canal infections. Persistent infections significantly enriched for Proteobacteria and Tenericutes compared with 1° ones; increased enrichment of persistent infections for *Lactobacillus*, *Streptococcus*, and *Sphingomonas*. Persistent infections may have more diverse bacterial communities than primary infections.

3.3.2. PROTEOMIC analysis of endodontic infections

Proteome: the entire set of proteins expressed by a genome, cell, tissue or organism at a certain time

"Proteomic analysis of endodontic infections can provide global insights into the invasion, pathogenicity mechanisms, and multifactorial interactions existing between root canal bacteria and the host in the initiation and progression of apical periodontitis." (Nandakumar et al. 2009)

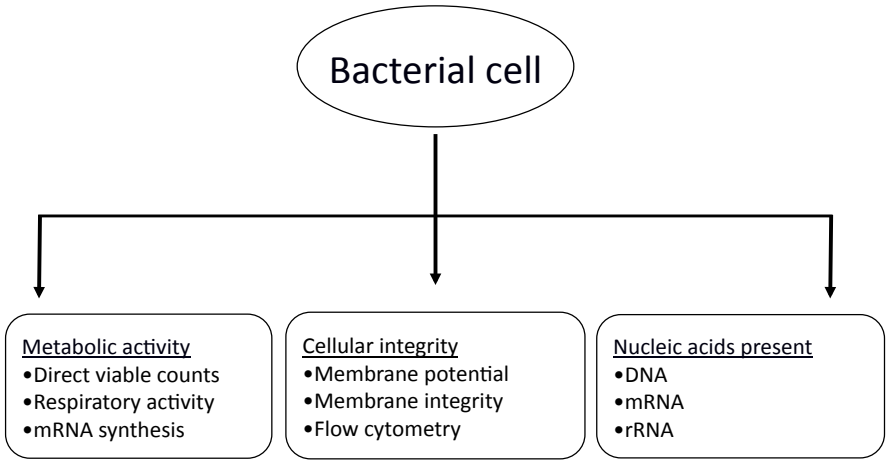
- Used mass spectrometry. Proteins identified in 7 root canal samples included adhesins, autolysins, proteases, virulence factors, and antibiotic-resistance proteins (Nandakumar et al. 2009)

"Interrogation of the metaproteome of endodontic microbial communities provides information on the physiology and pathogenicity of the community at the time of sampling" (Provenzano et al. 2013)

- Used mass spectrometry. Qualitatively described the proteins of microbial and human origin in 12 cases of asymptomatic apical periodontitis and 2 acute apical abscess (Provenzano et al. 2013)

2009	Nandakumar R, Madayiputhiya N, Fouad AF	Proteomic analysis of endodontic infections by liquid chromatography-tandem mass spectrometry	Oral Microbiol Immunol 2009 Aug;24:347-52.	In clinical samples from 7 infected root canals, identified proteins from bacteria (especially *E. faecalis, E. faecium, P.gingivalis, F. nucleatum*, and *T. denticola*) were identified from all the samples tested. Identified proteins included adhesins, autolysins, proteases, virulence factors, and antibiotic-resistance proteins.
2013	Provenzano JC, Siqueira JF, Rôças IN, Domingues RR, Paes Leme AF, Silva MR	Metaproteome analysis of endodontic infections in association with different clinical conditions	PLoS One 2013;8:e76108	Samples from 12 teeth with asymptomatic apical periodontitis and 2 acute apical abscess pus aspirants. Most microbial proteins were related to metabolic and housekeeping processes (protein synthesis, energy metabolism and DNA processes). Other proteins related to pathogenicity and resistance/survival were found [adhesion, biofilm formation, antibiotic resistance, stress proteins, exotoxins, invasins, proteases and endopeptidases (mostly in abscesses)]. Majority of human proteins detected were related to cellular processes and metabolism, as well as immune defense. The number of proteins in abscesses was higher than in asymptomatic cases.

3.3.3 Assessing bacterial viability



Adapted from Chavez 2007

2007	Chavez de Paz L	Redefining the persistent infection in root canals: possible role of biofilm communities	J Endod 2007;33:652-62	Review. Presents evidence that the nature of persisting endodontic infections depends not on the robustness of the organisms in the infected site, but on their capability of adapting their physiology to the new environmental conditions set by the treatment.

Part 4

How do microorganisms reach the root canal?

- **Caries, fractures, exposed dentinal tubules**
- **Coronal leakage**
- **Via periodontium**
- **Anachoresis?**

4.1 Caries, fractures, exposed dentinal tubules

Bacteria in deep carious lesions

- *Lactobacillus*
- *Actinomyces*
- *Streptococcus mutans*
- *Bacteroides* family
- *Porphyromonas endodontalis*
- *Micromonas* (formerly *Peptostreptococcus*) *micros*
- *Atopobium genomospecies* C1, *Pseudoramibacter alactolyticus*, *Streptococcus* spp., *Parvimonas micra*, *F. nucleatum*, and *Veillonella* species, *Dialister invisus*

(Martin et al. 2002, Chhour *et al*. 2005, Rôças et al. 2015)

2002	Martin FE, Nadkarni MA, Jacques NA, Hunter N	Quantitative microbiological study of human carious dentine by culture and real-time PCR: association of anaerobes with histopathological changes in chronic pulpitis	J Clin Microbiol 2002;40:1698-704	Studied dental pulps of 65 teeth extracted from patients with advanced caries and pulpitis. Culture - predominance of Gram-positive microorganisms, particularly lactobacilli. Real-time PCR data - high levels of *Micromonas* (formerly *Peptostreptococcus*) *micros* and *P. endodontalis* in carious lesions may be indicative of irreversible pulpal pathology
2005	Chhour KL, Nadkarni MA, Byun R, Martin FE, Jacques NA, Hunter N	Molecular analysis of microbial diversity in advanced caries	J Clin Microbiol 2005;43:843-9	Studied 10 advanced caries lesions. Real-time PCR. Lactobacilli comprised 50% of species, with prevotellae also abundant, comprising 15% of the species. Other taxa present: *Selenomonas* spp., *Dialister* spp., *F. nucleatum*, *Eubacterium* spp., *Lachnospiraceae*, *Olsenella* spp., *Bifidobacterium* spp., *Propionibacterium* sp., and *Pseudoramibacter alactolyticus*.
2015	Rôças IN, Lima KC, Assuncao IV,	Advanced Caries Microbiota in Teeth with Irreversible	J Endod 2015;41:1450-5	Clinical study. Sampled deep caries in 30 patients. Used reverse-capture checkerboard hybridization and real-time qPCR. Detected taxa: *Atopobium*

	Gomes PN, Bracks IV, Siqueira JF, Jr	Pulpitis		*genomospecies* C1, *Pseudoramibacter alactolyticus*, *Streptococcus* spp, *S. mutans*, *Parvimonas micra*, *F. nucleatum* and *Veillonella* species. *Streptococcus* spp, *Dialister invisus* and *P. micra* sig associated with throbbing pain, *S. mutans* with pain to percussion and *Lactobacillus* with continuous pain.

4.2 Coronal leakage

Correlation between results of leakage studies and clinical outcomes has not been established

- In endodontic literature usually refers to microbial ingress to the root canal system after obturation
- Leakage of *Fusobacterium nucleatum* and *Campylobacter rectus* by 90 days after obturation and post preparation (Barrieshi et al. 1997)
- Endotoxin penetrated along obturation material (Alves et al. 1998)
- Coronal seal delayed but did not prevent leakage of microorganisms (Gomes et al. 2003).
- Healing of clinical cases can occur in presence of coronal leakage (Ricucci et al. 2000, 2003).
- Leakage of *E. faecalis* by 65 days after obturation with ActiV GP/glass ionomer sealer, Resilon/Epiphany and gutta-percha/AH Plus (Fransen et al. 2008)
- Reliability of the two-chamber model designed to evaluate microbial leakage through root fillings questioned (Rechenberg et al. 2011)

1997	Barrieshi KM, Walton RE, Johnson WT, Drake DR	Coronal leakage of mixed anaerobic bacteria after obturation and post space preparation	OOOOE 1997;84:310-4	*In vitro,* 80% of 40 teeth demonstrated coronal leakage of *F. nucleatum* and *C. rectus* by 90 days. SEM examination showed a heterogeneous biofilm of coccal and bacillary species colonizing the apical portion of the canal wall
1998	Alves J, Walton R, Drake D	Coronal leakage: endotoxin penetration from mixed bacterial communities through obturated, post-prepared root canals	J Endod 1998;24:587-91	Endotoxin penetration along the obturating materials in post-prepared canals was faster than bacterial penetration *in vitro*. Recommended placement of permanent restoration as soon as possible after completion of endodontic treatment.
2000	Ricucci D, Grondahl K, Bergenholtz G	Periapical status of root-filled teeth exposed to the oral environment by loss of restoration or caries	OOOOE 2000;90:354-9	Retrospective analysis of 55 root fillings exposed to oral environment because of caries or no restoration for 3+ yrs. In many cases lesions did not worsen. Authors question role of coronal leakage in endodontic failures
2003	Gomes BP, Sato E, Ferraz CC, Teixeira FB, Zaia AA, Souza-Filho	Evaluation of time required for recontamination of coronally sealed canals medicated with	Int Endod J 2003;36:604-9	*In vitro*. Using saliva, recontamination was detected after 3.7 days in unsealed canals medicated with CHX gel (CG), 1.8 days with $Ca(OH)_2$ and 2.6 days with $Ca(OH)_2$ + CG. IRM-sealed crowns: recontamination detected within 13.5 days in canals with CG, after 17.2 days in canals with

	FJ	calcium hydroxide and chlorhexidine		$Ca(OH)_2$ and after 11.9 days in canals with CG + $Ca(OH)_2$. The group with no medication, but sealed with IRM, showed recontamination after 8.7 days. The coronal seal delayed but did not prevent leakage of microbes.
2003	Ricucci D, Bergenholtz G	Bacterial status in root-filled teeth exposed to the oral environment by loss of restoration and fracture or caries – a histobacteriological study of treated cases	Int Endod J 2003;36:787-802	Longitudinal tissue sections of 39 roots. Observed presence of stainable bacteria in abundance at canal entrance and in dentinal tubules but absent mid-root and apically in all but two specimens. Concluded that well-prepared and filled root canals resist bacterial penetration even upon frank and long-standing oral exposure by caries, fracture or loss of restoration.
2008	Fransen JN, He J, Glickman GN, Rios A, Shulman JD, Honeyman A	Comparative assessment of ActiV GP/glass ionomer sealer, Resilon/ Epiphany, and gutta-percha /AH plus obturation: a bacterial leakage study	J Endod 2008;34:725-7	*In vitro*. In extracted teeth, observed leakage of *E. faecalis* by 65 days after obturation with ActiV GP/glass ionomer sealer, Resilon/Epiphany and gutta-percha/AH Plus. No sig diff between groups.
2011	Rechenberg DK, Thurnheer T, Zehnder M	Potential systematic error in laboratory experiments on microbial leakage through filled root canals: an experimental study	Int Endod J. 2011 Sep;44(9):827-35	*In vitro*. Assessed routes of bacterial leakage in a commonly used two-chamber model designed to evaluate root fillings. Concluded: Sticky wax does not provide a suitable seal, leakage occurred through tubular dentin or at the interface b/n root filling and tubular dentin. GP and AH-Plus root fillings are likely more bacteria-tight than previously thought.

4.3 Via periodontium

- Langeland 1974 – classic paper for microbial access to the dental pulp by periodontal disease
- Similarities in microflora in periodontal pockets and root canals
    - *Wolinella recta* found in both periodontal pockets and root canals (Tanner et al. 1982)
    - *Bacteroides, Fusobacterium, Eubacterium, Wolinella,* spirochetes, *Selenomonas, Campylobacter,* and *Peptostreptococcus* are bacteria likely to be involved in cross-infection between sites (Kobayashi et al. 1990)
    - *Prevotella intermedia* associated with both endodontic and chronic periodontal disease (Baumgartner et al. 1992)
    - Using PCR, similarities in microflora found between "chronic apical periodontitis" and "chronic adult periodontitis" (Rupf et al. 2000)
- Differences in microflora between the two sites:
    - Aerobe/anaerobe ratio in the periodontal pocket was 0.23, compared to 0.0022 in the root canal (Kobayashi et al. 1990)
    - Spirochetes found in 0-10% of flora in endodontic abscesses, vs. 30-58% in periodontal infections (Dahle et al. 1993)
    - *Prevotella intermedia* associated with periodontal pockets and *Prevotella nigrescens* with

root canals (Gharbia et al. 1994)

- *F. nucleatum, P. micra* and *C. sputigena* may play a role in the pathogenesis of endo-periodontal lesions (Didilescu et al. 2012)

1974	Langeland K, Rodrigues H, Dowden W	Periodontal disease, bacteria, and pulpal histopathology	OOO 1974;37:257-70	Classic paper discussing microbial access to the dental pulp by periodontal disease
1982	Tanner ACR, Visconti RA, Holdeman LV, Sundqvist G, Socransky SS	Similarity of *Wolinella recta* strains isolated from periodontal pockets and root canals	J Endod 1982;8:294-300	Cultured periodontal pockets and root canals and performed DNA analysis for characterization of the species. Found *W. recta* in both the periodontal pockets and the canals. Suggested that the periodontal pocket may be a source of canal infection.
1990	Kobayashi T, Hayashi A, Yoshikawa R, Okuda K, Hara K	The microbial flora from root canals and periodontal pockets of non-vital teeth associated with advanced periodontitis	Int Endod J 1990;23:100-6	Aerobe/anaerobe ratio in the periodontal pocket was 0.23, compared to 0.0022 in the root canal. The predominant bacterial species common to both regions were *Streptococcus*, *Peptostreptococcus*, *Eubacterium*, *Bacteroides*, and *Fusobacterium*
1992	Baumgartner JC, Falkler WA JR, Bernie. RS, Suzuki JB	Serum IgG reactive with oral anaerobic microorganisms associated with infections of endodontic origin	Oral Microbiol Immunol 1992;7:106-10	Compared serum IgG levels of 10 oral anaerobic microorganisms. Associated *P. intermedia* with both endodontic disease and chronic periodontal disease. Also implicated *P. gingivalis* as a periodontal pathogen.
1993	Dahle UR, Tronstad L, Olsen I	Spirochaetes in oral infections	Endod Dent Traumatol 1993;9:87-94	Oral spirochaetes include species of the genus *Treponema*, many of which have not yet been cultured. Found in 0-10% of flora in endodontic abscesses, vs. 30-58% in periodontal infections
1994	Gharbia SE, Haapasalo M, Shah HN, Kotiranto A, Lounatmaa K, Pearce MA, Devine DA	Characterization of *Prevotella intermedia* and *Prevotella nigrescens* isolates from periodontic and endodontic infections	J Periodontol 1994;65:56-61	*P. intermedia* and *P. nigrescens* are site specific. *P. intermedia* is associated with periodontal pockets and *P. nigrescens* associated with root canals.
2000	Rupf S, Kannengiesser S, Merte K, Pfister W, Sigusch B, Eschrich K	Comparison of profiles of key periodontal pathogens in periodontium and endodontium	Endod Dent Traumatol 2000;16:269-75	Assessed profiles of periodontal pathogens in pulpal and periodontal diseases affecting the same tooth using PCR. Similarities found between "chronic apical periodontitis" and "chronic adult periodontitis" pathogen profiles *(A. actinomycetemcomitans, B. forsythus, Eikenella corrodens, F.nucleatum, P. gingivalis, P. intermedia* and *Treponema denticola)*
2012	Didilescu AC, Rusu D, Anghel A, Nica L, Iliescu A, Greabu M et al.	Investigation of six selected bacterial species in endo-periodontal lesions	Int Endod J. 2012;45:282-93	Clinical. 46 patients presenting with different types of endo-periodontal lesions were investigated. Conclusions: *F. nucleatum, P. micra* and *C. sputigena* may play a role in the pathogenesis of endo-periodontal lesions

4.4 Bacteremias and anachoresis

- **Bacteremia:** Presence of viable organisms in blood
- **Anachoresis:**
    - Localization of blood-borne bacteria during bacteremia to a site of inflammation
    - Seeding of bacteria directly into the inflamed pulp via the pulpal blood supply during bacteremia

Bacteremias
Can endodontic microorganisms enter the systemic circulation?

Yes

- **Bacteremias** have been identified following
    - Nonsurgical endodontics (Baumgartner et al. 1976, Debelian et al. 1995; 1998)
    - Surgical endodontics (Baumgartner et al. 1977)
- **Fungemia** - *Saccharomyces cerevisiae* (i.e. fungi) isolated from an infected root canal and from the blood of a patient undergoing endodontic therapy (Debelian et al. 1997)
- Is bacterial endocarditis following endodontic treatment possible???
    - If bacteria in root canals possess FnBP or FgBP (genes encoding bacterial colonization of the endocardium), they may have the potential to cause infective endocarditis (Bate et al. 2000)

1976	Baumgartner JC, Heggers JP, Harrison JW	The incidence of bacteremias related to endodontic procedures I. Nonsurgical endodontics	J Endod 1976;2:135-40	Found very low incidence (3.3%) of bacteremia from nonsurgical endodontics regardless of the pulpal status. Same organisms were found in the blood and the canal. No bacteremia if instrumentation confined to the root canal
1977	Baumgartner JC, Heggers JP, Harrison JW	Incidence of bacteremias related to endodontic procedures II. Surgical endodontics	J Endod 1977;3:399-402	Found a high number of bacteremias from surgical endodontics, especially as result of flap reflection. extraction causes 100% bacteremias (cited), flap reflection causes >80% and curettage causes 33%.
1995	Debelian GJ, Olsen I, Tronstad L	Bacteremia in conjunction with endodontic therapy	Endod Dent Traumatol 1995;11:142-9	Bacteremia was demonstrated following endodontic treatment. Biochemical tests and antibiograms showed that the isolates from the root canal and blood had identical profiles within the same patient
1997	Debelian GJ, Olsen I, Tronstad L	Observation of *Saccharomyces cerevisiae* in blood of patient undergoing root canal treatment	Int Endod J 1997;30:313-7	*Saccharomyces cerevisiae* (i.e. fungi) isolated from an infected root canal and from the blood of a patient undergoing endodontic therapy of a tooth with asymptomatic apical periodontitis is reported.

1998	Debelian GJ, Olsen I, Tronstad L	Anaerobic bacteremia and fungemia in patients undergoing endodontic therapy: an overview	Ann Periodontol 1998;3:281-7	Used phenotypic and genetic methods to trace microorganisms in the blood back to their origin. Demonstrated that endodontic treatment can be the cause of anaerobic bacteremia and fungemia.
2000	Bate AL, Ma JK, Pitt Ford TR	Detection of bacterial virulence genes associated with infective endocarditis in infected root canals	Int Endod J 2000;33:194-203	If bacteria in root canals possess FnBP or FgBP (genes encoding bacterial colonization of the endocardium), they may have potential to cause infective endocarditis.

Does anachoresis occur?

- **"Yes"**
  - Historic papers (Robinson and Boling 1941, MacDonald et al. 1957, Wittgow and Sabiston 1975). Gier and Mitchell (1968) demonstrated anachoresis in traumatized pulps after IV injection of microbes.
  - Gram positive cocci in tissue sections of previously mechanically exposed pulps in dogs injected intravenously with streptococci (Tziafas 1989)
  - NB. These studies do not prove that the pulp subsequently became necrotic as a direct result of the bacteremia

- **Not in unfilled canals under experimental conditions**
  - In order for anachoresis to occur, need some tissue in canals; stagnant fluid in unfilled canals does not become infected from the bloodstream (Delivanis et al. 1981, Delivanis and Fan 1984)

1941	Robinson HBG, Boling LR	The anachoretic effect in pulpitis bacteriologic studies	JADA 1941;28:268-82	States that anachoresis occurs in presence of pulpal inflammation. There must be a transient bacteremia. Anachoretic pulpitis is termed.
1957	MacDonald JB, Hare GC, Wood AWS	The bacteriologic stat-us of the pulp chamb-ers in intact teeth found to be nonvital following trauma	OOO 1957;10:318-22	Looked at teeth that were intact and necrotic and found bacteria. Describes anachoresis
1968	Gier RE, Mitchell DF	Anachoretic effect of pulpitis	J Dent Res 1968;47:564-70	Demonstrated anachoresis in traumatized pulps after IV injection of microbes
1975	Wittgow WC, Sabistan CB Jr	Microorganisms from pulp chambers of intact teeth with necrotic pulps	J Endod 1975;1:168-71	Found bacteria in intact, nonvital teeth. Concluded that anachoresis occurs and should be considered when beginning these cases.
1981	Delivanis PD, Snowden RB, Doyle RJ	Localization of blood-borne bacteria in instrumented unfilled root canals	OOO 1981;52:430-2	Stagnant fluid in instrumented but unfilled root canals of 5 cats analyzed for possible localization and growth of IV injected bacteria. 48hr after injection, all samples from the canals were negative for the test organism.

1984	Delivanis PD, Fan VSC	The localization of blood-borne bacteria in instrumented unfilled and overinstrumented canals	J Endod 1984;10:521-4	In order for anachoresis to occur, need some tissue in canals. Unfilled canals did not become infected from the bloodstream.
1989	Tziafas D	Experimental bacterial anachoresis in dog dental pulps capped with calcium hydroxide	Endod 1989;15:591-5	Pulps of 36 dog teeth mechanically exposed and capped. IV injection of streptococci. 24 hrs later dogs destroyed. Found Gram positive cocci in tissue sections of previously mechanically exposed pulps. Controls (*n*=6): pulps mechanically exposed, teeth extracted prior to injection of streptococci – no bacteria found.

Part 5

Microorganisms in primary endodontic infections

Are microorganisms the cause of apical periodontitis?
Yes – proven in animal studies

5.1 Classic animal studies

Bacteria in the pulp are essential to the development of periapical disease

(Kakehashi et al. 1965, Möller et al. 1981, Fabricius et al. 1982a, 1982b)

1965	Kakehashi S, Stanley HR, Fitzgerald RJ	The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats	OOO 1965:20:340-9	In a germ-free rats, minimal inflammation (and no abscess) developed in the exposed pulpal tissues, and dentinal bridges formed over the exposed pulps. In conventional animals, exposed pulpal tissues developed chronic pulpal inflammation, and eventually granulomas and abscesses.
1981	Möller AJR, Fabricius L Dahlén G, Ohman Ae, Heydon G	Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys	Scand J Dent Res 1981:89:475-84	In monkeys, infection was a prerequisite for the development of apical periodontitis. Several indigenous oral bacteria have the ability to survive in the root canal and induce apical periodontitis
1982a	Fabricius L, Dahlén G, Ohman AE, Möller AJR	Predominant indigenous oral bacteria isolated from infected root canals after varied times of closure	Scand J Dent Res 1982;90:134-44	Monkey teeth were left open for 1 week followed by various periods of closure. Relative number of obligate anaerobes increased with time and at the apical portion of the canal. Over periods of 7 days, 3 months, 6 months and 3 years the relative proportion of obligate anaerobes in the sealed root canal increased over time, indicating that selective mechanisms exist which allow certain bacteria to survive and proliferate.
1982b	Fabricius L, Dahlén G, Holm S, Möller AJR	Influence of combinations of oral bacteria on periapical tissues of monkeys	Scand J Dent Res 1982;90:200-6	8 bacterial strains selected from autogenously infected root canals with radiographic apical periodontitis after 670 days. 75 root canals were inoculated with bacterial strains separately or in combination and sealed for 6 months. *Bacteroides* did not survive as a pure culture. Mixed infections showed the greatest capacity for inducing apical periodontitis in monkeys. Only *S. faecalis* subsp. *liquefaciens* [now known as *E. faecalis*] appeared at the end of the observation period in all canals inoculated with a separate strain but produced weak periapical reaction.

5.2 Early clinical studies on endodontic microflora

- Sampling and culturing methods encouraged growth of facultative anaerobes (e.g. Winkler and van Amerongen 1959)
- Distribution of bacteria in dentinal tubules examined using histological methods (Shovelton 1964)

1959	Winkler KC, van Amerongen J	Bacteriologic results from 4000 root canal cultures	OOO 1959; 12:857-75	Streptococci formed 61% of the isolated organisms. Study done before anaerobic culturing of sampling routine.
1964	Shovelton DS	The presence and distribution of microorganisms within non-vital teeth	Brit Dent J 1964;117:101-7	Histological study. Bacteria located in 79/97 teeth studied. Non-vital teeth, when infected, showed bacteria at all levels within tooth. Possibly fewer bacteria in apical third.

5.3 Sampling

- Moller 1966: Landmark publication in endodontic microbiology
- Introduced scientific basis for:
  - Aseptic endodontic sampling
  - Anaerobic transport and processing of samples to allow maintenance of viability and growth of anaerobes

1966	Möller AJ	Microbiological examination of root canals and periapical tissues of human teeth	Thesis Odontol Tidskr 1966; spec 74	Thesis quoted mainly for sampling and growth techniques resulting in more accurate microbiological examination of the root canal. Also provides detailed analyses of endodontic microflora from teeth with different clinical presentations

Measures	**Materials/methods**
Preparation of the operation field	Scaling of hard and soft deposits, gingivectomy or temporary restorations, rubber dam application
Sterilization of the operation field	Prewashing with 30% hydrogen peroxide, washing with 5% or 10% iodine tincture
Sterility control of the operation field	Charcoal-impregnated pellets, transport medium
Sampling from the root canal	Sampling fluid (VMG I), charcoal-impregnated paper points, transport medium, or semifluid culture medium
Transport medium	VMGA III
Culturing	Semi-liquid medium (eg Brewer thioglycollate medium with 10% horse serum or HCMG-Sula), enriched solid blood agar medium (eg Brucella agar medium)
Microbiological analysis	Growth-no growth determination; identification of isolated microorganisms based on colony morphology, micromorphology, physical and biochemical tests

Sampling decontamination procedures and molecular methods

- H_2O_2/NaOCl better than H_2O_2/iodine for tooth surface decontamination, but not 100% (Ng et al. 2003)
- H_2O_2/NaOCl tooth surface decontamination did not remove all DNA (Sedgley et al. 2006)
- PCR reactions can be inhibited by thiosulfate-inactivated NaOCl (Young et al. 2007)
- Strong binding affinity between DNA and dentin may influence sampling (Brundin et al. 2014)

2003	Ng YL, Spratt D, Sriskantharajh S, Gulabivala K.	Evaluation of protocols for field decontamination before bacterial sampling of root canals for contemporary microbiology techniques	J Endod 2003;29:317-29	Using PCR bacterial DNA could be detected significantly (p = 0.010) more frequently from the tooth surfaces (n=63 teeth) after 10% iodine (45%) compared with 2.5% NaOCl (13%) decontamination, although on the rubber dam or Oraseal surfaces there was no difference. Root canal sampling for polymerase chain reaction might be better preceded by NaOCl decontamination than by iodine, based on the findings.
2006	Sedgley C, Buck G, Appelbe O	Prevalence of *Enterococcus faecalis* at multiple oral sites in endodontic patients using culture and PCR	J Endod 2006;32:104-9	Clinical study. Bacterial DNA was detected in all access cavity samples using PCR and universal 16S rRNA gene primers designed specifically to target oral bacteria. Questioned whether studies using molecular methods to identify root canal flora should additionally determine if the assumed "root canal" microorganism is also in the corresponding access cavity control sample, and therefore potentially an oral flora contaminant introduced at the time of sampling.
2007	Young G, Turner S, Davies JK, Sundqvist G, Figdor D	Bacterial DNA persists for extended periods after cell death	J Endod 2007;33:1417-20	*In vitro*. Studied fate of bacterial DNA 1yr after cell death using broth cultures of *E. faecalis*. Results: DNA could still be detected by PCR, although there was a 1000-fold decay. Implications: this study questions validity of PCR-based identification methods for RCT sampling, as detected DNA may come from dead cells and not viable cells. Investigators also noted: PCR reactions can be inhibited by thiosulfate-inactivated NaOCl (used to disinfect tooth prior to sampling).
2014	Brundin M, Figdor D, Johansson A, Sjogren U	Preservation of bacterial DNA by human dentin	J Endod 2014;40:241-5	*In vitro*. Showed there is a strong binding affinity between DNA and dentin, and between DNA and serum proteins or collagen. These substrates preserve DNA against natural decomposition and protect DNA from nuclease activity, factors that may confound molecular analysis of the endodontic microbiota.

5.4 Early human clinical studies describing endodontic microflora using culture-based anaerobic methods

Identified a mixed flora dominated by anaerobes (Bergenholtz 1974, Sundqvist 1976)

1974	Bergenholtz G	Micro-organisms from necrotic pulp of traumatized teeth	Odont Revy 1974;25:347-58	Studied 84 teeth with previous traumatic injuries, intact pulp chambers, necrotic canals. Found 54/84 had microorganisms present, mostly mixed flora with anaerobes predominant. Aseptic necrosis found in the other 30 teeth.
1976	Sundqvist G	Bacteriological studies of necrotic dental pulps	UMEA 1976; dissertation #7	Samples obtained from infected human root canals of non-vital teeth with intact crowns for culture of anaerobic, facultatively anaerobic, and aerobic bacteria. Bacteria were isolated from the necrotic pulps of teeth only when periapical destruction was present. More than 90% of strains isolated were anaerobic. 88 strains were isolated from 18 pulp chambers. Concluded that acute periapical inflammation is induced by combinations of bacterial strains, and the presence of *Bacteroides melaninogenicus* was associated with acute periapical inflammation.

5.5 Distribution of microorganisms in the root canal

- Variations noted according to which part of the root canal system is being sampled:
  - Apical areas - dominated by slow growing obligate anaerobes
  - Coronal part of the canal - more rapidly growing facultative anaerobes (Fabricius et al. 1982a)
- Anaerobes in apical region (Baumgartner and Falkler 1991)
- Anaerobes were found in cementum in 2/10 samples (Kiryu et al. 1994)
- Bacterial infection of dentinal tubules may occur to a lesser extent in older patients compared to younger patients (Kakoli et al. 2009)

1982a	Fabricius L, Dahlén G, Ohman AE, Möller AJR	Predominant indigenous oral bacteria isolated from infected root canals after varied times of closure	Scand J Dent Res 1982;90:134-44	Monkey teeth were left open for 1 week followed by various periods of closure. Relative number of obligate anaerobes increased with time and at the apical portion of the canal. Over periods of 7 days, 3 months, 6 months and 3 years the relative proportion of obligate anaerobes in the sealed root canal increased over time, indicating that selective mechanisms exist which allow certain bacteria to survive and proliferate.
1991	Baumgartner JC, Falkler WA Jr	Bacteria in the apical 5 mm of infected root canals	J Endod 1991;17:380-3	Predominantly anaerobic bacteria (68%) in apical 5 mm of infected root canals in teeth (*n*=10) with carious pulpal exposures and PA lesions.
1994	Kiryu T, Hoshino E, Iwaku M	Bacteria invading periapical cementum	J Endod 1994;20:169-72	Looked for viable bacteria in the cementum of 10 endodontically treated teeth with persistent PA lesions. Bacteria, all anaerobes, cultured from 2/10 samples, found in "deep cementum".
2009	Kakoli P, Nandakumar R, Romberg	The effect of age on bacterial penetration of radicular dentin	J Endod 2009;35:78-81	Light microscopy. 56 single-rooted teeth : young (18-25y) and old (>or=60y) were instrumented and infected with *E. faecalis* for 20 days. "Young" teeth had sig

	E, Arola D, Fouad AF			higher number of tubules invaded by bacteria, and more deeply, compared with "old" teeth (p = 0.014). Concluded that bacterial infection of dentinal tubules may occur to a lesser extent in older patients.

5.6 "Black-pigmented bacteria", "Black pigmented *Bacteroides*" (BPB) "Black-pigmented Gram-negative anaerobes" "Dark-pigmenting anaerobic rods"

BPBs are Gram negative anaerobic rods, e.g.:

Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia Prevotella nigrescens, Prevotella denticola, Prevotella melaninogenicus, Tannerella forsythia

- Present in both symptomatic and asymptomatic endodontic infections (Haapasalo et al. 1986, Sundqvist et al. 1989, Siqueira et al. 2001)
- In Oregon study, *Prevotella nigrescens* was the most commonly isolated BPB in endodontic infections (Bae et al. 1997, Dougherty et al. 1998)
- In Brazil study, *P. endodontalis* most frequent BPB in endodontic infections (Siqueira et al. 2001)
- *P. gingivalis, P. endodontalis, P. intermedia, and P. nigrescens* identified more frequently in teeth with necrotic pulp than in teeth with failing endodontic treatment (Gomes et al. 2005)
- *P. endodontalis, P. gingivalis, Prevotella intermedia, P. nigrescens*, and *P. tannerae* have been detected in clinical samples using multiplex polymerase chain reactions (Soel et al. 2006)
- *Tannerella forsythia* found in combination with *P. gingivalis* and *T. denticola* (i.e. "Red Complex") (Gomes et al. 2007)
- *Prevotella* sp. was commonly found in infected deciduous teeth (Tavares et al. 2011)

1986	Haapasalo M, Ranta H, Ranta K, Shah H	Black-pigmented *Bacteroides* spp. in human apical periodontitis	Infect Immun 1986;53:149-53	Clinical study: 35 acute and 27 clinically asymptomatic cases of apical periodontitis in 57 adults. *Bacteroides* present in both symptomatic and asymptomatic infections. *B. gingivalis* and *B. endodontalis* present only in acute infections, *B. intermedius* found both in symptomatic and asymptomatic infections, and *B. denticola* occurred mostly in asymptomatic infections.
1989	Sundqvist G, Johansson E, Sjögren U	Prevalence of black-pigmented *Bacteroides* species in root canal infections	J Endod 1989;15:13-9	Examined the occurrence of BPB species in 72 human apical periodontitis cases. BPB present in both symptomatic and asymptomatic infections. 16/22 canals containing black-pigmented *Bacteroides* species were associated with acute apical abscesses and purulent drainage. 6/22 teeth with BPB were asymptomatic.
1997	Bae KS, Baumgartner JC, Shearer	Occurrence of *Prevotella nigrescens* and *Prevotella*	J Endod 1997;23:620-3	Culture and PCR. BPB from 40 intact teeth with necrotic pulps and apical periodontitis isolated in pure culture. PCR (16S rDNA) used to differentiate *P.*

	TR, David LL	*intermedia* in infections of endodontic origin		*nigrescens* from *P. intermedia*. 22/40 (55%) samples were positive for the growth of BPB. *P. nigrescens* more frequent isolate than *P. intermedia*.
1998	Dougherty WJ, Bae KS, Watkins BJ, Baumgartner JC	Black-pigmented bacteria in coronal and apical segments of infected root canals	J Endod 1998;24:356-8	*In vitro*. Culture. *Prevotella nigrescens* was the most frequent BPB isolate from both coronal and apical segments in 12 infected (extracted) teeth from which BPB were cultured.
2001	Siqueira JF Jr, Rôças IN, Oliveira JC, Santos KR	Molecular detection of black-pigmented bacteria in infections of endodontic origin	J Endod 2001;27:563-6	Used 16S rDNA PCR to assess BPB in samples from 54 infected teeth. 12 cases had >1 BPB species. *P. endodontalis* most frequent BPB in endodontic infections.
2005	Gomes BP, Jacinto RC, Pinheiro ET, Sousa EL, Zaia AA, Ferraz CC, Souza-Filho FJ	*Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia* and *Prevo-tella nigrescens* in endodontic lesions detected by culture and by PCR	Oral Microbiol Immunol 2005;20:211-5	Microbial samples were obtained from 50 teeth with untreated necrotic pulps (primary infection) and from 50 teeth with failing endodontic treatment (secondary infection). *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia* and *Prevotella nigrescens* were identified more frequently in teeth with necrotic pulp than in teeth with failing endodontic treatment, and by PCR than by culture.
2006	Seol JH, Cho BH, Chung CP, Bae KS	Multiplex polymerase chain reaction detection of black-pigmented bacteria in infections of endodontic origin	J Endod 2006;32:110-4	Microbial samples from 40 infected root canals and abscesses. Extracted DNA. Performed multiplex PCR. At least one of five species of black-pigmented bacteria (*Porphyromonas endodontalis, P. gingivalis, Prevotella intermedia, P. nigrescens*, and *P. tannerae*) detected in 65% (26/40) of samples using multiplex PCR, and in 15% (6/40) using the conventional culture procedures.
2007	Gomes BP, Montagner F, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ	Polymerase chain reaction of *P. gingivalis, Treponema denticola,* and *Tannerella forsythia* in primary endodontic infections	J Endod 2007;33:1049-52	Clinical study. These 3 species make up the "Red Complex" which has been associated with severity of periodontal disease. 50 cases with necrotic pulps. Used nested PCR. Found *P. gingivalis*, *T. denticola*, and *T. forsythia* in 22, 12 and 19 canals, respectively. The combination found in 7/50 cases.
2011	Tavares WL, Neves de Brito LC, Teles RP, Massara ML, Ribeiro Sobrinho AP, Haffajee AD, Socransky SS, Teles FR	Microbiota of deciduous endodontic infections analysed by MDA and Checkerboard DNA-DNA hybridization	Int Endod J. 2011 Mar;44(3):225-35	Analyzed 32 root canal samples obtained from deciduous teeth in children in Brazil by using checkerboard DNA-DNA hybridization. Used multiple displacement amplification (MDA) to enhance DNA amplification. Reported diverse bacterial population with *Prevotella* sp. commonly found (*P. intermedia>P. tannerae>P. nigrescens*).

5.7 Fungi

Eukaryotic microorganisms, opportunistic, can adhere to a variety of surfaces and evade host defense systems

- *Candida albicans* most common fungus recovered from root canal infections
- Observed under SEM in 4/10 infected teeth (Sen et al. 1995)
- Cultured from 47/692 (7%) root canal samples (Waltimo et al. 1997)
- Using PCR detected in 5/24 root canal samples, 0/19 aspirates (Baumgartner et al. 2000)
- Cultured yeasts from 10% of 60 root canal samples. More likely that yeasts would be recovered from root canals when also present in saliva (Egan et al. 2002)
- 28% necrotic canals were positive for filamentous fungi in Brazil (Gomes et al. 2010)
- *Candida albicans* can survive in anaerobic and nutrient-limited conditions (Richards et al. 2010, Ning et al. 2013)

1995	Sen BH, Piskin B, Demirci T	Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM	Endod Dent Traumatol 1995;11:6-9	In extracted infected teeth, yeasts were present in 4/10 specimens. Bacteria penetrated up to 150 um into dentinal tubules; tubule penetration and yeasts may play a role in persistent endodontic infections.
1997	Waltimo TMT, Siren EK, Torkko HLK, Olsen I, Haapasalo MPP	Fungi in therapy-resistant apical periodontitis	Int J Endod 1997;30: 96-101	Microorganisms were found in 692/967 root canal samples. 7% (47/692) showed growth of fungi. All fungi isolates except for one belonged to the genus *Candida*; *Candida albicans* (80%) was the most commonly isolated yeast species
2000	Baumgartner JC, Watts CM, Xia T	Occurrence of *Candida albicans* in infections of endodontic origin	J Endod 2000;26:695-8	Microbial samples collected from 24 root canals and 19 aspirates of cellulitis/abscesses. Used PCR to detect *C. albicans* in 5/24 root canal samples. All cellulitis/ abscess aspiration tested negative for *C. albicans*
2002	Egan MW, Spratt DA, Ng YL, Lam JM, Moles DR, Gulabivala K	Prevalence of yeasts in saliva and root canals of teeth associated with apical periodontitis	Int Endod J 2002;35:321-9	Yeasts occurred in 10% of 60 root canals. It was significantly (13.8 times) more probable that yeasts would be recovered from root canals when they were also present in the saliva
2010	Gomes C, Fidel S, Fidel R, de Moura Sarquis MI	Isolation and taxonomy of filamentous fungi in endodontic infections	J Endod 2010 Apr;36:626-9	In Brazil, samples from 60 clinical cases with pulp necrosis and PARL, 28% were positive for filamentous fungi.
2010	Richards D, Davies JK, Figdor D	Starvation survival and recovery in serum of *Candida albicans* compared with *Enterococcus faecalis*	OOOOE 2010;110:125-30	*In vitro*. Evaluated survival in limited nutrient conditions over 6 months. Similarly to *E. faecalis*, the yeast *Candida albicans* can survive in nutrient-limited conditions and can use serum as a source of nutrition and for recovery from starvation
2013	Ning Y, Hu X, Ling J, Du Y,	Candida albicans survival and biofilm	Int Endod J 2013;46:62-70.	*C. albicans* growth and survival were monitored in vitro for up to 8 months. CLSM used for semi-quantitative

	Liu J, Liu H, et al.	formation under starvation conditions		comparisons of the ultrastructure of biofilms formed on human dentin. *C*. albicans cells can survive and form biofilms in anaerobic and nutrient-limited conditions.

5.8 Spirochetes

- Difficult to culture. Found in primary infections using molecular methods (Siqueira and Rôças 2003, Baumgartner et al. 2003, Rôças and Siqueira 2005, Foschi et al. 2005, Montagnier et al. 2010)

2003	Baumgartner JC, Khemalee-lakul S, Xia T	Identification of spirochetes (treponemes) in endodontic infections	J Endod 2003;29:794-7	Used PCR. Found treponemes in 51/84 (60.7%) of samples from abscesses/cellulitis and 20/54 (37%) samples from infected root canals. Detected 1-5 species per sample.
2003	Siqueira JF Jr, Rôças IN.	*Treponema socranskii* in primary endodontic infections as detected by nested PCR	J Endod 2003;29:244-7	Used nested PCR. *T. socranskii* detected in 11/28 (39%) asymptomatic teeth, 5/12 acute apical periodontitis cases (39%) and 5/20 acute periradicular abscesses (25%)
2005	Rôças IN, Siqueira JF Jr,	Occurrence of two newly named oral treponemes - *Treponema parvum* and *Treponema putidum* - in primary endodontic infections	Oral Microbiol Immunol 2005;20:372-5	Re-analyzed samples from previous studies. *Treponema parvum* was detected in 52% of 21 root canals associated with chronic apical periodontitis, in 20% of 10 cases diagnosed as acute apical periodontitis, and in no abscessed case (n=19 samples). *Treponema putidum* was found in one case of acute apical periodontitis.
2005	Foschi F, Cavrini F, Montebugnoli L, Stashenko P, Sambri V, Prati C	Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients	Oral Microbiol Immunol 2005;20:289-95	Used PCR. *Treponema denticola* was detected in 56% of teeth with symptomatic chronic apical periodontitis
2010	Montagner F, Jacinto RC, Signoretti FG, Gomes BP	Treponema species detected in infected root canals and acute apical abscess exudates	J Endod. 2010 Nov;36(11):1796-9	Paired samples of infected RCs and AAAs from 20 subjects were analyzed. The most frequently detected species were *T. socranskii* (RC, 17/20; AAA, 15/20) and *T. denticola* (RC, 8/20; AAA, 11/20).

5.9 Viruses

- Found in pulp fibroblasts (Glick et al. 1991)
- Epstein-Barr virus may be associated with irreversible pulpitis and apical periodontitis (Li et al. 2009)
- Herpesviruses are present but not required for the development of acute apical abscesses and cellulitis of endodontic origin (Chen et al. 2009), HHV found in acute apical abscess aspirants (Ferreira et al. 2010).

1991	Glick M, Trope M, Bagasra O, Pliskin ME	Human immunodeficiency virus infection of fibroblasts of dental pulp in seropositive patients	OOO 1991;71:733-6	HIV was located in pulpal fibroblasts in 11 of 12 pulps from HIV seropositive patients.
2009	Li H, Chen V, Chen Y, Baumgartner JC, Machida CA	Herpesviruses in endodontic pathoses: association of Epstein-Barr virus with irreversible pulpitis and apical periodontitis	J Endod 2009;35:23-9	Epstein-Barr virus (EBV) DNA and RNA in endodontic pathoses cases in significantly higher percentages (43.9% and 25.6%) compared with healthy pulp controls (0%). Human cytomegalovirus (HCMV) DNA and RNA found in both endo patients (15.9% and 29.3%) and healthy pulp controls (42.1% and 10.5%). Herpes simplex virus (HSV-1) DNA found in low %s in endo patients (13.4%). One patient showed *Varicella zoster* virus (VZV).
2009	Chen V, Chen Y, Li H, Kent K, Baum-gartner JC, Machida CA	Herpesviruses in abscesses and cellulitis of endodontic origin	J Endod 2009;35:182-8	From patients exhibiting concurrent spontaneous pain (n = 28), nine abscesses contained HCMV, two abscesses contained EBV, one abscess contained HSV-1, and no abscesses contained VZV. Concluded that herpesviruses are present but not required for the development of acute apical abscesses and cellulitis of endodontic origin
2010	Ferreira DC, Paiva SS, Carmo FL, Rôças IN, Rosado AS, Santos KR, Siqueira JF Jr	Identification of herpesviruses types 1 to 8 and human papillomavirus in acute apical abscesses	J Endod. 2011 Jan;37(1):10-6	Analyzed human acute apical abscess aspirants for herpesviruses types 1 to 8 and human papillomavirus. 23/24 samples positive for target viruses with 14 samples (47%) positive for HHV-8 and 3 samples (11%) positive for HPV. EBV and HCMV were not present in any of the examined samples.

5.10 CJD

Transmissible spongiform encephalopathies (TSEs)

In cows: "Bovine spongiform encephalopathy", "Mad cow disease"

In humans: "Human prion disease", "Creutzfeldt-Jakob disease" (CJD)

- Accumulation of an abnormal form of prion protein in the brain
- This abnormal prion protein can be detected in lymphoid and neural tissue in CJD patients (Gill et al. 2001)
- Not detected in pulp of CJD patients (Blanquet-Grossard et al. 2000)
- No correlation between dental treatment and TSEs (Azarpazhooh and Fillery 2008)

Prion protein and sterilization:

- Prion protein highly resistant to routine sterilization procedures (Smith et al. 2002)
- Recommended prion protein decontamination procedures may corrode Ni-Ti instruments (Sonntag and Peters 2007)
- With risk of transmission, should endodontic instruments be single use only?

Prion protein may occur naturally in human teeth (Schneider et al. 2007)

2000	Blanquet-Grossard F, Sazdovitch V, Jean A, et al.	Prion protein is not detectable in dental pulp from patients with Creutzfeldt-Jakob disease	J Dent Res 2000 ;79:700	Prion protein was not detected in dental pulps from CJD patients
2001	Gill DS, Tredwin CJ, Gill SK, Ironside JW	The transmissible spongiform encephalopathies (prion diseases): a review for dental surgeons	Int Dent J 2001;51:439-46	Prion protein is detectable in lymphoid and neural tissue in CJD. Prion protein is resistant to routine sterilization procedures.
2002	Smith A, Dickson M, Aitken J, Bagg J	Contaminated dental instruments	J Hosp Infect 2002;51:233-5	Endodontic files may have close contact with terminal branches of the trigeminal nerve and thus there is potential for transmission of prions via contaminated surgical instruments. Based on LM and SEM examination of used endodontic files from private and public healthcare providers, suggested a need for improved cleaning methods for endodontic files / single use endodontic instruments
2007	Schneider K, Korkmaz Y, Addicks K, Lang H, Raab WH	Prion protein (PrP) in human teeth: an unprecedented pointer to PrP's function	J Endod 2007;33:110-3	In human teeth, cementoblasts and odontoblasts showed prominent staining for PrP at levels comparable to those of nerve fibers. Epithelial rests of Malassez, which are remnants of a cell type formerly forming enamel, were also positive. Thus, all PrP-positive cells in human dentition are in some way involved in calcified tissue formation. Suggests a previously undetected function of prion protein in healthy vertebrates
2007	Sonntag D, Peters OA	Effect of prion decontamination protocols on nickel-titanium rotary surfaces	J Endod 2007;33:442-6	Tested 7 brands. After 24 hr immersion in 3%NaOCl, 27.8% of instruments showed corrosion
2008	Azarpazhooh A, Fillery ED	Prion disease: the implications for dentistry	J Endod 2008;34:1158-66	Review. No reported definite or suspected cases of disease transmission arising from dental procedures, and there seems to be no correlation between dental treatment and TSEs.

5.11 "Unculturable" species reported in root canal infections

- *Dialister pneumosintes, Pseudoramibacter alactolyticus, Filifactor aloci, Catonella morbi*. *Granulicatella adiacens*, *Vagococcus fluvialis, P. baroniae*, and *P. multisaccharivorax* and others (Rôças and Siqueira 2009, Siqueira and Rôças 2002, 2003a, 2003b, 2006, Schirrmeister et al. 2009, Ribeiro et al. 2009 & others)
- Members of the domain *Archaea* (these are one of the three "domains" of life, are a highly diverse group of prokaryotes, distinct from bacteria and eukaryotes. Not generally thought to be "pathogens") identified in infected root canals e.g. *Methanobrevivacter oralis*-like phylotype (Vianna et al. 2006, Vickerman et al. 2007, Jiang et al. 2009)
- Pyrosequencing shows presence of unidentified species (Li et al. 2010, Siqueira et al. 2011)

2002	Siqueira JF Jr, Rôças IN.	*Dialister pneumosintes* can be a suspected endodontic pathogen	OOOOE 2002;94:494-8	Used nested PCR. *D. pneumosintes* detected in 17 of 22 asymptomatic cases (77.3%) and 4/10 root canals associated with acute apical periodontitis (40%). No relationship with the occurrence of symptoms. Overall, *D. pneumosintes* detected in 21/32 samples (65.6%)
2003a	Siqueira JF Jr, Rôças IN.	*Pseudoramibacter alactolyticus* in primary endodontic infections	J Endod 2003;29:735-8	*P. alactolyticus* detected in 76% of root-canal samples from teeth with asymptomatic periradicular lesions, in 60% of canals associated with acute apical periodontitis, and in 32% of pus aspirates from abscesses. No significant association with clinical symptoms observed. Overall, *P. alactolyticus* occurred in 56% of samples
2003b	Siqueira JF Jr, Rôças IN	Detection of *Filifactor alocis* in endodontic infections associated with different forms of periradicular diseases	Oral Microbiol Immunol 2003;18:263-5	*F. alocis* detected in 12/21 (57.1%) samples from teeth showing asymptomatic periradicular lesions and in 3/10 (30%) samples from root canals associated with acute apical periodontitis. Occurred in 8/19 (42.1%) pus aspirates from abscessed teeth. Overall, *F. alocis* detected in 23/50 (46%) samples.
2006	Siqueira JF Jr, Rôças IN	*Catonella morbi* and *Granulicatella adiacens*: new species in endodontic infections	OOOOE 2006;102:259-64	*C. morbi* occurred in 26% and *G. adiacens* in 14% of the samples taken from primary endodontic infections
2006	Vianna ME, Conrads G, Gomes BP, Horz HP	Identification and quantification of archaea involved in primary endodontic infections	J Clin Microbiol 2006;44:1274-82	*Methanobrevivacter* oralis-like 16S rDNA was amplified from samples from 5/20 necrotic single-rooted teeth with radiographic evidence of apical periodontitis and with no previous endodontic treatment.
2007	Vickerman MM, Brossard KA, Funk DB, Jesionowski AM, Gill SR	Phylogenetic analysis of bacterial and archaeal species in symptomatic and asymptomatic endodontic infections	J Med Microbiol 2007;56:110-8	*Methanobrevivacter* oralis-like 16S rDNA was amplified from the root canals of one symptomatic and one asymptomatic patient
2009	Schirrmeister JF, Liebenow	New bacterial compositions in root-	J Endod 2009;35:169-74	Detected *Vagococcus fluvialis* in initial samples from root canals undergoing retreatment

	AL, Pelz K, Wittmer A, Serr A, Hellwig E, Al-Ahmad A	filled teeth with periradicular lesions		
2009	Rôças IN, Siqueira JF Jr	Prevalence of new candidate pathogens *Prevotella baroniae, Prevotella multisaccharivorax* and as-yet-uncultivated *Bacteroidetes* clone X083 in primary endodontic infections	J Endod 2009;35: 1359-62	Case series. Samples from infected canals studied using 16S rRNA gene-based nested PCR. Found *Bacteroidetes* clone X083, *P. baroniae*, and *P. multisaccharivorax* in 50%, 35%, and 25% of the samples, respectively.
2009	Jiang YT, Xia WW, Li CL, Jiang W, Liang JP	Preliminary study of the presence and association of bacteria and archaea in teeth with apical periodontitis	Int Endod J 2009;42:1096-103	In Shanghai, China. Using RT-PCR, archae were detected in 16/42 (38%) samples from necrotic pulps and 6/17 (17%) samples from retreatment cases.
2010	Li L, Hsiao WW, Nandakumar R, Barbuto SM, Mongodin EF, Paster BJ, Fraser-Liggett CM, Fouad AF	Analyzing endodontic infections by deep coverage pyrosequencing	J Dent Res. 2010;89:980-4	Pyrosequencing technology should allow for more comprehensive analysis than traditional Sanger sequencing. Analysis of 7 root canal samples (1 retreatment) showed 47 vs. 28,590 sequences obtained per sample for Sanger sequencing vs. pyrosequencing, i.e. representing a 600-fold difference in "depth-of-coverage". Bottom line: bacterial communities in endodontic infections are more diverse than previously demonstrated.
2011	Ribeiro AC, Matarazzo F, Faveri M, Zezell DM, Mayer MP	Exploring bacterial diversity of endodontic microbiota by cloning and sequencing 16S rRNA	J Endod 2011;37:922-6	In samples from 12 infected canals there was wide bacterial diversity. The mean number of taxa per canal was 10.0, ranging from 3 to 21 per sample; 65.7% of the cloned sequences represented phylotypes for which no cultivated isolates have been reported.
2011	Siqueira JF Jr, Alves FR, Rôças IN	Pyrosequencing analysis of the apical rootcanal microbiota	J Endod. 2011; 37:1499-503	Used pyrosequencing to evaluate the microflora of 10 cryopulverized apical root segments of 10 extracted teeth. Generated 9,818 partial 16S rRNA gene sequences. Identified sequences belonging to 187 phylotypes, 84 genera and 10 phyla.

5.12 Bacteria aspirated from acute infections

- *Treponema denticola*
- *Porphyromonas endodontalis*
- *Dialister pneumosintes*
- *Tannerella forsythensis*
- *Porphyromonas gingivalis*
- *Filifactor alocis*
- *Fusobacterium* spp.
- *Propionibacterium propionicum*
- *Bacteroides melaninogenicus*
- *Streptococcus* spp.
- *Prevotella* spp.
- *Peptostreptococcus* spp.
- *Eubacterium* spp.

1982	Oguntebi B, Slee AM, Tanzer JM, Langeland K	Predominant microflora associated with human dental periapical abscesses	J Clin Microbiol 1982;15:964-66	Mixed but somewhat specific and relatively limited facultative and obligate anaerobic flora with *Fusobacterium nucleatum* and *Streptococcus mitis* as a frequent pair
1983	Williams BL, McCann GF, Schoenknecht FD	Bacteriology of dental abscesses of endodontic origin	J Clin Microbiol 1983;18:770-4	Syringe aspirates cultured from 10 dental abscesses of endodontic origin, all of which had penetrated beyond the bony alveolus to produce fluctuant swelling. 70% of the bacterial isolates were either strict anaerobes or microaerophilic. Mean 4.5 isolates per sample.
1986	Lewis MA, MacFarlane TW, McGowan DA	Quantitative bacteriology of acute dento-alveolar abscesses	J Med Microbiol 1986;21:101-4	Pus specimens obtained by needle aspiration of 50 acute dento-alveolar abscesses. Most samples contained a mixture of species (average 3.3); 20 (40%) of the abscesses contained anaerobes alone, 3 (6%) contained facultative anaerobes only and the remaining 27 (54%) contained mixtures of both types of bacteria, with anaerobes predominating.
2001	Siqueira JF, Rôças IN, Souto R, De Uzeda M, Colombo AP	Microbiological evaluation of acute periradicular abscesses by DNA-DNA hybridization	OOOOE 2001;92:451-7	Assessed prevalence of 49 bacterial species in acute periradicular abscess of endodontic origin using Checkerboard DNA-DNA hybridization. *B. forsythus* and *Porphyromonas gingivalis*- detected in 29.6% cases
2002	Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG	Molecular and cultural analysis of the microflora associated with endodontic infections	J Dent Res 2002 ;81:761-6	Performed cultural and molecular analyses (16S rDNA) of aspirate samples from 5 infected root canals. 26/65 taxa were found by the molecular method alone. A mean of 20.2 taxa was found in each sample.

5.13 Is there an association between specific microorganisms and symptoms?

Yes

Haapasalo 1986, Yoshida et al. 1987, Hashioka et al. 1992, Hahn et al. 1993, Gomes et al. 1996, Chavez 2002, Jacinto et al. 2003, Sakamoto et al. 2006, Gomes et al. 2006 and others

No

Baumgartner et al. 1999, Jung et al. 2000, Rôças et al. 2001, Fouad et al. 2002, Siqueira et al. 2003, Chu et al. 2005, Siqueira and Rôças 2009 and others

1986	Haapasalo M, Ranta H, Ranta K, Shah H	Black-pigmented *Bacteroides* spp. in human apical periodontitis	Infect Immun 1986;53:149-53	*B. gingivalis* and *B. endodontalis* were present only in acute infections, *B. intermedius* was found both in symptomatic and asymptomatic infections, and *B. denticola* occurred mostly in asymptomatic infections.
1987	Yoshida M, Fukushima H, Yamamoto K, Ogawa K, Toda T	Correlation between clinical symptoms and microorganisms isolated from root canals of teeth with periapical pathosis	J Endod 1987;13:24-8	Asymptomatic cases associated with no bacteria or predominantly facultative bacteria. Bacteria cultured in all cases with clinical symptoms. Obligate anaerobes related to presence of clinical symptoms
1992	Hashioka K, Yamasaki M, Nakane A, Horiba N, Nakamura H	The relationship between clinical symptoms and anaerobic bacteria from infected root canals	J Endod 1992;18:558-61	28 teeth from 25 patients with apical periodontitis. Cultured *Peptococcus, Peptostreptococcus, Eubacterium, Porphyromonas*, and *Bacteroides* related to percussion pain; *Porphyromonas* and *Bacteroides* related to odor.
1993	Hahn CL, Falkler WA Jr, Minah GE	Correlation between thermal sensitivity and microorganisms isolated from deep carious dentin	J Endod 1993;19:26-30	In 29 teeth with deep caries, Gram-positive cocci and Gram-negative rods were positively related to cold sensitivity.
1996	Gomes BPFA, Lilley JD, Drucker DB	Association of endodontic symptoms and signs with particular combinations of specific bacteria	Int J Endod 1996;29:69-75	Anaerobes cultured from 70% of painful teeth – *Prevotella* and *Peptostreptococcus* most common isolates. Swelling of the periodontium associated with *Eubacterium*, *Peptostreptococcus, Prevotella*; wet canal with *Prevotella*, *Eubacterium*, P*ropionibacterium*
1999	Baumgartner JC, Watkins BJ, Bae KS, Xia T	Association of black-pigmented bacteria with endodontic infections	J Endod 1999;25:413-5	Culture. 22/40 samples (55%) positive for BPB. 11/22 identified as *P. nigrescens*, 8/22 *P. inter-media*, 2/22 *P. gingivalis* and 1/22 *P. melaninogenica*. 16/22 teeth positive for BPB associated with purulent drainage. No relationship b/n BPBs and other clinical signs
2000	Jung IY, Choi BK, Kum KY, Roh BD, Lee SJ, Lee CY, Park DS	Molecular epidemiology and association of putative pathogens in root canal infection	J Endod 2000;26:599-604	18 symptomatic and 20 asymptomatic teeth from 36 subjects studied using PCR. *Fusobacterium* sp. (68.4%), *Peptostreptococcus micros* (44.7%) and *P. gingivalis* (26.3%). No significant association between any bacteria and symptoms.

2001	Rôças IN, Siqueira JF, Santos KRN, Coelho AMA	"Red complex" (*Bacteroides forsythus, Porphyromonas gingivalis* and *Trepo-nema denticola*) in endodontic infections: a molecular approach	OOOOE 2001:91: 468-71	Samples from 50 single rooted teeth with carious lesions, necrotic pulps, and radiographic periradicular bone loss collected using a K-file. At least one member of the Red Complex was found in 66% of cases (*T. denticola* 44%, *P. gingivalis* 30%, *B. forsythus* 26%). No significant correlation between the bacteria, either singularly or collectively, to a particular symptom.
2002	Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R, Hazlett K, Radolf JD	PCR-based identification of bacteria associated with endodontic infections	J Clin Microbiol 2002;40:32 23-31	Used PCR primers that targeted the bacterial 16S rRNA genes of ten pathogens. 24 teeth with necrotic pulps. Found that preoperative symptoms were significantly associated with the presence of *Streptococcus* spp. Black pigmented Gram negative anaerobes were not associated with symptoms.
2002	Chavez de Paz Villanueva LE	*Fusobacterium nucleatum* in endodontic flare-ups	OOOOE 2002;93:17 9-83	In 28 patients wanting emergency treatment after initiation of RCT, *F. nucleatum* was associated with most severe forms of flareups.
2003	Siqueira JF Jr	Microbial causes of endodontic flare-ups	Int Endod J 2003;36:45 3-63	Microorganisms are arguably the major causative agents of flare-ups.
2003	Siqueira JF, Rôças IN, Andrade AFB, Uzeda M	*Peptostreptococcus micros* in primary endodontic infections as detected by 16S rDNA-based PCR	J Endod 2003;29:11 1-3	*P. micros* was detected in 14/50 endodontic infection samples. No correlation between presence of *P. micros* and symptoms
2003	Jacinto RC, Gomes BP, Ferraz CC, Zaia AA, Filho FJ	Microbiological analysis of infected root canals from symptomatic and asymptomatic teeth with periapical periodontitis	Oral Microbiol Immunol 2003;18:28 5-92	Cultured microbial samples from 29 symptomatic and 19 asymptomatic canals. Root canals of symptomatic teeth harbored more obligate anaerobes more species than asymptomatic teeth. Relationships reported between Gram-negative anaerobes, and the presence of spontaneous or previous pain, tenderness to percussion, pain on palpation and swelling.
2005	Chu FC, Tsang CS, Chow TW, Samaranayake LP	Identification of cultivable microorganisms from primary endodontic infections with exposed and unexposed pulp space	J Endod 2005;31:4 24-9	Clinical study. Compared cultivable flora in canals with PA radiolucencies with exposed (n=45) and unexposed (n=43) pulp space. *Prevotella* sig more common in exposed group. *F. nucleatum* and *P. acne* significantly more common in unexposed canals. No differences in prevalence of *Actinomyces*, *Peptostreptococcus*, and *Campylobacter* between two groups of canals.
2006	Sakamoto M, Rôças IN, Siqueira JF Jr, Benno Y	Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections	Oral Microbiol Immunol 2006;21:1 12-22	Bacterial profiles of symptomatic endodontic infections generated by terminal restriction fragment length polymorphism analysis were clearly different from those of asymptomatic infections.
2006	Gomes BP, Jacinto RC, Pinheiro ET,	Molecular analysis of *Filifactor alocis*, *Tannerella forsythia*, and	J Endod 2006;32:9 37-40	Associations reported between (1) primary infections and *F. alocis* and *T. forsythia*, and (2) *F. alocis*, *T. forsythia* and *T. denticola* and signs and symptoms.

	Sousa EL, Zaia AA, Ferraz CC, Souza-Filho FJ	*Treponema denticola* associated with primary endodontic infections and failed endodontic treatment		
2009	Siqueira JF Jr, Rôças IN	Community as the unit of pathogenicity: an emerging concept as to the microbial pathogenesis of apical periodontitis	OOOOE 2009;107:870-8	Review. From the perspective of the single-pathogen concept, apical periodontitis can be considered as of no specific microbial etiology. Despite the high interindividual variability in endodontic microbial community composition, there are apparently some disease-related patterns.

5.14 Associations between specific species

Many specific associations described in human, animal and *in vitro* studies

1979	Sundqvist GK, Eckerbom MI, Larsson AP, Sjögren UT	Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections	Infect Immun 1979;25:685-93	Inoculated human endodontic bacteria subcutaneously into guinea pigs. Increased virulence associated with combination of *B. melaninogenicus* or *B. asaccharolyticus* and *Peptostreptococcus micros*
1982	Oguntebi B, Slee AM, Tanzer JM, Langeland K	Predominant microflora associated with human dental periapical abscesses	J Clin Microbiol 1982;15:964-66	*Fusobacterium nucleatum* and *Streptococcus mitis* as a frequent pair
1992	Sundqvist G	Associations between microbial species in dental root canal infections	Oral Microbiol Immunol 1992;7:257-62	Samples from 65 infected human root canals analyzed according to species, frequency of occurrence and proportion of total isolated flora. Strong positive associations found between *F. nucleatum* & *P. micros*, *P. endodontalis*; *Selenomonas sputigena* and *W. recta*; *P. intermedia* & *P. micros*; *P. anaerobius* & eubacteria
1992	Baumgartner JC, Falkler WA Jr, Beckerman T	Experimentally induced infection by oral anaerobic microorganisms in a mouse model	Oral Microbiol Immunol 1992;7:253-6	A mixed culture of *F. nucleatum* with either *Porphyromonas gingivalis* or *Prevotella intermedia* was significantly more pathogenic than *F. nucleatum* in pure culture in mice.
1998	Siqueira Junior J, Magalhaes FA, Lima KC, de Uzeda M	Pathogenicity of facultative and obligate anaerobic bacteria in monoculture and combined with either *Prevotella intermedia* or *Prevotella nigrescens*	Oral Microbiol Immunol 1998;13:368-72	Induced abscesses using mouse model. Synergism between bacterial strains was only apparent when associating *Porphyromonas endodontalis* with *P. intermedia* or *P. nigrescens*.
2001	Jung I, Choi B, Kum K, Yoo Y, Yoon T, Lee S, Lee	Identification of oral spirochetes at the species level and their association with other	OOOOE 2001;92:329-34	79 patients with single root necrotic teeth with radiographic evidence of bone resorption. *T. maltophilum* was found most frequently (compared to the other oral spirochetes investigated) and significantly

	C	bacteria in endodontic infections		associated with *P. gingivalis* and *B. forsythus*.
2001	Siqueira JF Jr, Rôças IN, Oliveira JC, Santos KR	Molecular detection of black-pigmented bacteria in infections of endodontic origin	J Endod 2001;27:563-6	Used 16S rDNA--directed PCR to assess prevalence of BPB in samples from 54 infected teeth (10 with acute periradicular abscesses). *P. gingivalis* was always found associated with *P. endodontalis* in abscessed teeth.
2002	Peters LB, Wesselink PR, van Winkelhoff AJ	Combinations of bacterial species in endodontic infections	Int J Endod 2002;35:698-702	In samples from canals of 58 asymptomatic endodontic infections, significant relationships were found between *P. intermedia* and *P. micros*, *P. intermedia* and *P. oralis*, *A. odontolyticus* and *P. micros*, *Bifidobacterium* spp. and *Veillonella* spp.
2003	Baumgartner JC, Khemaleelakul S, Xia T	Identification of spirochetes (treponemes) in endodontic infections	J Endod 2003;29:794-7	PCR. Found treponemes in 51/84 (60.7%) of samples from abscesses/cellulitis and 20/54 (37%) samples from infected root canals. 1-5 species per sample. Significant association between *Treponema maltophilum* and *T. socranskii, T. denticola*
2004	Chavez de Paz LE, Molander A, Dahlén G	Gram-positive rods prevailing in teeth with apical periodontitis undergoing root canal treatment	Int J Endod 2004;37:579-87	139 teeth undergoing RCT analyzed. Used culture and SDS-page methods. Concluded that a possible association existing between *Lactobacillus* spp. and Gram-positive cocci in root canals of teeth with apical periodontitis receiving treatment
2006	Johnson EM, Flannagan SE, Sedgley CM	Coaggregation interactions between oral and endodontic *E. faecalis* and bacterial species isolated from persistent apical periodontitis	J Endod 2006;32:946-50	*In vitro*: Coaggregation interactions were evaluated between *E. faecalis* clinical isolates (n=53) and species previously shown to survive and induce apical periodontitis in monkeys: *P. anaerobius*, *P. oralis*, *F. nucleatum*, and *S. anginosus*. All *E. faecalis* strains (n = 53) coaggregated with *F. nucleatum*.
2006	Khemaleelakul S, Baumgartner JC, Pruksakom S	Autoaggregation and coaggregation of bacteria associated with acute endodontic infections	J Endod 2006;32:312-8	*In vitro:* Assessed 62 bacterial strains from 10 acute endodontic infections. Autoaggregation detected in 35/62 (56.45%) of the bacteria. Coaggregation of bacteria occurred 148/183 pairs (80.87%) using dye-staining assay with confocal microscopy. Coaggregation was observed for each of the 15 genera assayed, especially *Prevotella, Streptococcus* and *Fusobacterium*
2009	Metzger Z, Lin YY, Dimeo F, Ambrose WW, Trope M, Arnold RR	Synergistic pathogenicity of Porphyromonas gingivalis and Fusobacterium nucleatum in the mouse subcutaneous chamber model	J Endod 2009;35:86-94	*In vivo*. Mouse model. Determined viable counts of mono- and mixed- infections in subcutaneous chambers for up to 40 days. The species tested benefited from the presence of the other depending on the specific strain used.

5.15 Is there an association between microorganisms in root canals and geographic location?

Yes

- Flora may vary according to geographic location (Siqueira et al. 2005, Rôças et al. 2006)

2005	Siqueira JF, Jung IY, Rôças IN, Lee CY	Differences in prevalence of selected bacterial species in primary endodontic infections from two distinct geographic locations	OOOOE 2005;99:641-7	Most prevalent species found in South Korean samples were *F. nucleatum* (38% of the cases), *T. forsythia* (26%) and *T. maltophilum* (24%). Overall, *P endodontalis, D. pneumosintes, Filifactor alocis, T denticola* and *T. forsythia* were detected in more Brazilian samples than in South Korean samples.
2006	Rôças IN, Baumgartner JC, Xia T, Siqueira JF Jr	Prevalence of selected bacterial named species and uncultivated phylotypes in endodontic abscesses from two geographic locations	J Endod 2006;32:1135-8	Some bacterial taxa can differ in the frequencies they occur in root canal samples from different locations. *T. denticola* and *T. forsythia* were detected in more Brazilian samples than Portland, OR samples. *Dialister invisus* (70% of the cases), *P. endodontalis* (63%) and *Dialister pneumosintes* (55%) were most frequent taxa in Oregon samples.

5.16 Summary – primary endodontic infections

- Biofilms, Multiple species ("polymicrobial")
- Predominantly anaerobic Gram negative rods

Culture - based identification

Facultative anaerobes	Anaerobes
Gram positive cocci	
Streptococcus	*Micromonas*
Enterococcus	*Peptostreptococcus*
	Peptococcus
Gram negative cocci	
Neisseria	*Veillonella*
Gram positive rods	
Lactobacillus	*Actinomyces*
	Eubacterium
	Propionibacterium
Gram negative rods	
Enterobacter	*Porphyromonas*
Pseudomonas	*Prevotella*
Eikonella	*Fusobacterium*
Capnocytophaga	*Bacteroides* family
	Dialister
	Filifactor
Spirochetes	
	Treponema
Fungi	*Candida*
Viruses	HIV, Epstein-Barr virus, human cytomegalovirus, Herpes simplex virus-1

DNA-based identification

- Multiple species ("polymicrobial")
- Significantly more species than in culture-based studies
- Many species as yet unidentified and unculturable

Proteomic analyses

- Multiple proteins involved in metabolism, housekeeping, adhesion, biofilm formation, antibiotic resistance, pathogenesis
- Many "hypothetical" proteins with as yet no known function

Part 6

Endodontic microbiology and unsuccessful endodontic treatment

6.1 Microorganisms are found in failed endodontically treated teeth

Reviews - Nair 2006, Siqueira and Rôças 2008
Histological - Nair et al. 1999, Ricucci et al. 2009
Culture - Sundqvist et al. 1998, Molander et al. 1998, Hancock et al. 2001, Peciuliene et al. 2001, Cheung and Ho 2001, Pinheiro et al. 2003
PCR-based methods Siqueira and Rôças 2004, Chugal et al. 2011
PCR-based reverse checkerboard hybridization Rôças and Siqueira 2013
Combined culture and molecular methods Sedgley et al. 2006, Williams et al. 2006, Anderson et al. 2012

1998	Molander A, Reit C, Dahlén G, Kvist T	Microbiological status of root-filled teeth with apical periodontitis	Int Endod J 1998;31:1-7	Examined the microbiological status of 100 root-filled teeth with radiographically verified apical periodontitis. Microbes found in 68 of 100 teeth. Predominance of Gram positive facultative anaerobes (69%). Most frequently isolated strains were *Enterococcus* (32 teeth)
1998	Sundqvist G, Figdor D, Persson S, Sjögren U	Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment	OOOOE 1998;85:86-93	54 root canal treated teeth after 4-5 years (asymptomatic, presence of PA radiolucency) were selected for retreatment. Microbial flora of retreatment cases: single species predominantly Gram positive. Most common isolates *E. faecalis*. Overall success rate of retreatment was 74%; lower success rate with teeth with positive cultures at time of root filling. Different flora to previously untreated teeth.
1999	Nair PN, Sjögren U, Figdor D, Sundqvist G	Persistent periapical radiolucencies of root-filled human teeth, failed endodontic treatments, and periapical scars	OOOOE 1999 ;87:617-27	Histological descriptions of 6 cases that demonstrated persistent periapical radiolucent lesions after conventional root canal treatment. Persistent PA radiolucencies after endodontic treatment may be due to persistent infection in root canal, formation of cyst, or healing by scar formation
2001	Hancock III HH, Sigurd-sson A, Trope M, Moisei-witsch J	Bacteria isolated after unsuccessful endodontic treatment in a North American population	OOOOE 2001;91;57 9-86	Bacteria were cultivated in 34 of the 54 retreatment cases. The microbial flora was mainly of 1 to 2 strains of predominantly Gram-positive organisms. *E. faecalis* was identified in 30% of teeth with a positive culture
2001	Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M	Isolation of yeasts and enteric bacteria in root filled teeth with chronic apical periodontitis	Int J Endod 2001;34:42 9-34	Sampled 40 root filled teeth with chronic apical periodontitis. In culture-positive teeth, prevalence of yeasts was 18% and enteric bacteria 64%. IKI improved antimicrobial effect of treatment.

2001	Cheung GS, Ho MW	Microbial flora of root canal-treated teeth associated with asymptomatic periapical radiolucent lesions	Oral Microbiol Immunol 2001;16:332-7	Studied samples from 24 teeth which had received nonsurgical RCT >4 years previously, presenting with an acceptable coronal restoration and a periapical radiolucent area. Samples obtained after removal of the old root canal filling. Number of bacterial genera recovered ranged from 0 to 6, with facultative Gram-positive cocci being the most prevalent
2003	Pinheiro ET, Gomes BPFA, Feraz CCR, Sousa ELR, Teixeira FB, Souza-Filho FJ	Microorganisms from canals of root-filled teeth with periapical lesions.	Int Endod J 2003;36:1-11	Microflora in 51/60 canals after failure of RCT were limited to a small number of predominantly Gram-positive microbial species. Facultative anaerobes, especially *E. faecalis*, were the most commonly isolated microorganisms. Polymicrobial infections and obligate anaerobes were frequently found in canals of symptomatic root-filled teeth.
2004	Siqueira JF Jr, Rôças IN	Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment	OOOOE 2004;97:85-94	Samples from 22 root-filled teeth with persistent PA lesions analyzed for 19 taxa using PCR. All samples positive for at least 1 target species. *E. faecalis* was the most prevalent species-detected in 77% of the cases. Mean number of species in samples filled up to 2 mm short of the radiographic apex was 3 (range, 1-5), and greater than 2 mm from the apex was 5 (range, 2-11).
2006	Nair PN	On the causes of persistent apical periodontitis: a review	Int Endod J 2006;39:249-81	Review
2006	Sedgley CM, Nagel AC, Dahlén G, Reit C, Molander A	Real-time quantitative PCR and culture analysis of *Enterococcus faecalis* in root canals	J Endod 2006;32:173-7	Consecutive root canal samples obtained from 40 primary infection and 48 retreatment cases divided into 2 equal aliquots that were independently analyzed by culture and qPCR by investigators blinded to the other results. *E. faecalis* detected in more retreatment than primary infection samples (89.6% versus 67.5%). Real-time qPCR more sensitive than cultivation ($p < 0.0001$).
2006	Williams JM, Trope M, Caplan DJ, Shugars DC	Detection and quantitation of *E. faecalis* by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment	J Endod 2006;32:32:715-21	Clinical study. 29 cases. *E. faecalis* up to 3 times more prevalent in refractory than primary infections in samples taken at access, post-instrumentation/irrigation and postcalcium hydroxide treatment. Real-time qPCR more sensitive than cultivation ($p < 0.001$). Viable but not-culturable *E. faecalis* detected by RT-PCR in four samples that were negative by cultivation.
2008	Siqueira JF Jr, Rôças IN	Clinical implications and microbiology of bacterial persistence after treatment procedures	J Endod 2008;34:1291-1301	Review
2009	Ricucci D, Siqueira JF Jr,	Histologic investigation of root canal-treated teeth	J Endod. 2009;35(4	All specimens exhibited periradicular inflammation. In general, intraradicular bacterial colonization was

	Bate AL, Pitt Ford TR	with apical periodontitis: a retrospective study from twenty-four patients	):493-502	heavier in symptomatic failed teeth.
2011	Chugal N, Wang JK, Wang R, He X, Kang M, Li J, Zhou X, Shi W, Lux R	Molecular characterization of the microbial flora residing at the apical portion of infected root canals of human teeth	J Endod. 2011 Oct;37(10) :1359-64	Clinical. 18 teeth with necrotic pulps and 8 retreatment cases. Apical bacterial communities in primary infections were more diverse than in secondary infections. Identified Fusobacteria, Actinomyces species, and Anaeroglobus in both types of infection.
2012	Anderson AC, Hellwig E, Vespermann R, Wittmer A, Schmid M, Karygianni L, Al-Ahmad A	Comprehensive analysis of secondary dental root canal infections: a combinat-ion of culture and culture-independent approaches reveals new insights	PLoS One 2012;7:e49 576.	21 samples from previously root-filled teeth with PA lesions. Microorganisms were found in 12 samples with culture-dependent and -independent methods combined. The number of bacterial species ranged from 1 to 12 in one sample. The majority of the 26 taxa belonged to the phylum Firmicutes (14 taxa), followed by Actinobacteria, Proteobacteria and Bacteroidetes. Found 13 taxa not previously identified in root filled teeth.
2013	Rôças IN, Siqueira JF, Jr	Characterization of microbiota of root canal-treated teeth with posttreatment disease	J Clin Microbiol 2012;50:17 21-4	42 root filled teeth. All asymptomatic with radiographic evidence of apical periodontitis. Most prevalent taxa were *Propionibacterium* species, *Fusobacterium nucleatum*, streptococci, and *Pseudoramibacter alactolyticus*

6.2 Does chemomechanical instrumentation completely disinfect root canals?

Inaccessible biofilms in complex root canal systems of mandibular first molar roots cannot be removed by contemporary instruments and irrigation alone in one-visit treatment (Nair et al. 2005)

1963	Zeldow BJ, Ingle JI	Correlation of the positive culture to the prognosis of endodontically treated teeth: a clinical study	JADA 1963; 66:9-13	Early study. Greater success rate was achieved after obtaining a negative culture. However, some cases healed despite microorganisms present at time of obturation. Used aerobic culturing.
1987	Bystrom A, Happonen R-P, Sjögren U, Sundqvist G	Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis	Endod Dent Traumatol 1987;3:58-63	In 79 teeth with PA lesions, obturation done only after negative culture. After 2 yr followup, 85% healed completely, 10% reduced in size. Of 10%, 3 taken to surgery showed little inflammation, mostly fibrous tissue.
1987	Allard U, Stromberg U, Stromberg T	Endodontic treatment of experimentally induced apical periodontitis in dogs	Endod Dent Traumatol 1987;3:240 -4	In microbially-induced PA lesions in dogs, all canals were contaminated at obturation. All went on to heal at 4 mo. NSRCT was successful even if there was an established infection at time of obturation.

1995	Peters LB, Wesselink PR, Moorer WR	The fate and role of bacteria left in dentinal tubules	Int J Endod 1995;28:95-9	Bacteria left in dentinal tubules following root canal cleaning, shaping and obturation do not appear to jeopardize the success of treatment.
1997	Sjögren U, Figdor D, Persson S, Sundqvist G	Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis	Int Endod J 1997;30:297-306	Investigated role of infection on prognosis of endodontic therapy by following-up teeth that had single visit endo. Complete healing occurred in 94% of cases that yielded a negative culture; success rate 68% in cases with positive culture; absence of bacteria at time of obturation increased probability of a successful outcome. Important to completely eliminate bacteria from the root canal system before obturation
2002	Peters LB, Wesselink PR	Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms	Int J Endod 2002;35:660-7	Reported that the presence of a positive bacterial culture ($CFU<10^2$) at the time of filling did not influence the outcome of treatment. Total 39 patients. Healing monitored radiographically for up to 4.5 years.
2005	Nair PN, Henry S, Cano V, Vera J	Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment	OOOOE 2005;99:231-52	Used light and transmission electron microscopy to study mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Concluded that inaccessible biofilms in complex root canal systems of mandibular first molar roots cannot be removed by contemporary instruments and irrigation alone in one-visit treatment
2007	Sathorn C, Parashos P, Messer HH	How useful is root canal culturing in predicting treatment outcome	J Endod 2007;33:220-5	Review of studies using culture methods. Concludes that "currently practiced intracanal sampling techniques suffer from deficiencies that limit their predictive value"

6.3 Microflora in untreated canals versus previously root-filled canals

Primary endodontic infections

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

Previously treated canals

- Biofilms
- Polymicrobial, reduced microbial load
- Frequently recover Gram positive facultatively anaerobic cocci: *Enterococcus* spp.
- Starvation-survival capacity is likely a selection factor for microbial participation in post-treatment disease (Brundin et al. 2009)

- Enterococcus spp. predominate in culture-positive root-filled teeth with chronic apical periodontitis (Molander et al. 1998, Sundqvist et al. 1998, Hancock et al. 2001, Peciuliene et al. 2001, Pinheiro et al. 2003 and many others)
- Enterococci less dominant, or not detected in some DNA-based identification studies (Fouad et al. 2005)

6.3.1 Enterococci

- Genus *Enterococcus* (Division: Firmicutes; Class: Bacilli; Order: Lactobacillales; Family: Enterococcaceae)
- There are currently 54 *Enterococcus* species. Main species found in humans:
  - *E. faecalis*
  - *E. faecium*
- *E. faecalis* have been detected in the oral cavity (Sedgley et al. 2004), as a small proportion of the overall bacterial load (Sedgley et al. 2005). Presence may be associated with periodontal status (Sedgley et al. 2006).
- Source of oral enterococci is likely to be food. Review (Zehnder and Guggenheim 2009)
- Enterococci in food could enter a pulpless root canal via coronal leakage (Kampfer et al. 2007)
- Food-borne enterococci can incorporate into oral biofilms (Al-Ahmad et al. 2010)
- Enterococci associated with persistence of infection during endodontic treatment (Siren et al. 1997)
- *E. faecalis* most frequent *Enterococcus* species in root canals of failed endodontically treated teeth (Molander et al. 1998, Sundqvist et al. 1998, Hancock et al. 2001, Peciuliene et al. 2001, Pinheiro et al. 2003, Sedgley et al. 2006)
- *E. faecalis* was detected in more retreatment than primary infection samples (89.6% versus 67.5%) using real-time qPCR (Sedgley et al. 2006)
- *E. faecalis* up to 3 times more prevalent in refractory than primary infections (Williams et al. 2006)
- *E. faecalis* was detected in 22% of samples from 40 retreatment cases using PCR and molecular sequencing techniques (Fouad et al. 2005)
- *E. faecalis* more frequently recovered from asymptomatic than symptomatic cases (Rôças et al. 2004)
- Enterococci from teeth undergoing retreatment were associated with normal periapex (Kaufman et al. 2005)
- No significant difference was observed when comparing the occurrence of *E. faecalis* in root-filled teeth with and without periradicular lesions (Zoletti et al. 2006)
- Early *E. faecalis* biofilms - bacterial adherence is significantly influenced by last irrigant used: least adherence when NaOCl was used (Kishen et al. 2008)
- Antibiotic resistance genes can transfer between *E. faecalis* and *S. gordonii* in root canals *ex vivo* (Sedgley et al. 2008)
- *E. faecalis* strains from saliva and infected root canals have the potential to recruit PMNs in the infectious sites leading to inflammation via up-regulation of PMN IL-1α, TNF-α, MMP-8, and COX-2 (Ma et al. 2011)

1997	Siren EK, Haapasalo MPP, Ranta	Microbiological findings and clinical treatment procedures in endodontic cases selected for	Int J Endod 1997;30:91-5	Of cases (*n*=40) with enteric bacteria 55% had been open during the treatment, while in the group (*n*=40) where only non-enteric bacteria were found 30% had

	K, Salmi P, Kerusuo EN	microbiological investigation		been open. Enteric bacteria were also more frequently isolated in cases with a high number of appointments before sampling.
2004	Rôças IN, Siqueira JF Jr, Santos KR	Association of *Enterococcus faecalis* with different forms of periradicular diseases	J Endod 2004;30:315-20	Samples from cases of untreated teeth with different forms of periradicular diseases. Used 16S rDNA-based nested PCR. Reported that *E. faecalis* was more associated with asymptomatic cases of primary endo infections than with symptomatic ones. *E. faecalis* more likely to be found in cases of failed endodontic therapy than in primary infections.
2004	Sedgley C, Lennan SL, Clewell DB	Prevalence, phenotype and genotype of oral enterococci.	Oral Microbiol Immunol 2004;19:95-101	Enterococci were detected in oral rinse samples from 11% of 100 patients receiving endodontic treatment and 1% of 100 dental students with no history of endodontic treatment (P=0.0027).
2005	Fouad AF, Zerella J, Barry J, Spangberg LS	Molecular detection of *Enterococcus* species in root canals of therapy-resistant endodontic infections	OOOOE 2005;99:112-8	*E. faecalis* was detected in 22% of samples from 40 retreatment cases using PCR and molecular sequencing techniques
2005	Kaufman B, Spangberg L, Barry J, Fouad AF	*Enterococcus* spp. in endodontically treated teeth with and without periradicular lesions	J Endod 2005;31:851-856	58 teeth that had received RCT >1 yr previously and required retreatment were sampled. DNA extraction and PCR amplification were performed using 16S rDNA bacterial primers. Enterococci from teeth undergoing retreatment were associated with normal periapex.
2005	Sedgley CM, Nagel AC, Shelburne CE, Clewell DB, Appelbe O, Molander A	Quantitative real-time PCR detection of oral *Enterococcus faecalis* in humans	Arch Oral Biol 2005;50:575-83	Real-time qPCR assays *E. faecalis* comprised 0.0006-0.0047% of a total bacterial load that ranged from 5.92 x 10(5) to 5.69 x 10(7) cells/ml of oral rinse. *E. faecalis* was detected in five (17%) of 30 oral rinse samples from endodontic patients in concentrations from 114 to 490 cells/ml.
2006	Sedgley C, Buck G, Appelbe O	Prevalence of *Enterococcus faecalis* at multiple oral sites in endodontic patients using culture and PCR	J Endod 2006;32:104-9	21 endodontic patients. *E. faecalis* detected in more tongue than gingival sulcus, oral rinse, and root canal samples (43, 14, 10, and 10%, respectively), and in more patients with gingivitis/periodontitis compared to healthy periodontium (73% versus 20%; p = 0.03)
2006	Sedgley CM, Nagel AC, Dahlén G, Reit C, Molander A	Real-time quantitative PCR and culture analysis of *Enterococcus faecalis* in root canals	J Endod 2006;32:173-7	Consecutive root canal samples obtained from 40 primary infection and 48 retreatment cases divided into two equal aliquots that were independently analyzed by culture and qPCR by investigators blinded to the other results. *E. faecalis* detected in more retreatment than primary infection samples (89.6% versus 67.5%). Real-time qPCR more sensitive than cultivation ($p < 0.0001$).
2006	Zoletti GO, Siqueira JF Jr, Santos KR	Identification of *Enterococcus faecalis* in root-filled teeth with or without periradicular	J Endod 2006;32:722-6	No significant difference was observed when comparing the occurrence of *E. faecalis* in root-filled teeth with and without periradicular lesions ($p > 0.05$)

		lesions by culture-dependent and-indep-endent approaches		
2006	Williams JM, Trope M, Caplan DJ, Shugars DC	Detection and quantitation of *E. faecalis* by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment	J Endod 2006;32: 32:715-21	Clinical study. 29 cases. *E. faecalis* up to 3 times more prevalent in refractory than primary infections in samples taken at access, post-instrumentation/irrigation and postcalcium hydroxide treatment. Real-time qPCR more sensitive than cultivation ($p < 0.001$). Viable but not-culturable *E. faecalis* detected by RT-PCR in four samples that were negative by cultivation.
2007	Kampfer J, Gohring TN, Attin T, Zehnder M	Leakage of food-borne *Enterococcus faecalis* through temporary fillings in a simulated oral environment	Int Endod J 2007;40:47 1-7	*In vitro*. Using *E. faecalis,* showed that food-derived microbiota could enter the necrotic root canal system via microleakage alongside Cavit. First to show a potential link between enterococci in food and their presence in pulpless root canals.
2008	Kishen A, Sum CP, Mathew S, Lim CT	Influence of irrigation regimens on the adherence of *Enterococcus faecalis* to root canal dentin	J Endod 2008;34:85 0-4	*In vitro*. Pre-treated dentin blocks with different irrigants, then exposed samples to *E. faecalis* for 1hr. Adhesion (pull-off) force measured using atomic force microscopy. Bacterial adherence sig influenced by last irrigant used: least adherence when NaOCl was used
2008	Sedgley CM, Lee EH, Martin MJ, Flannagan SE	Antibiotic resistance gene transfer between *Streptococcus gordonii* and *Enterococcus faecalis* in root canals of teeth *ex vivo*	J Endod 2008;34:57 0-4	*Ex vivo*. Antibiotic resistance gene transferred between different species in root canals. Findings demonstrate that horizontal genetic exchange in endodontic infections might facilitate adoption of an optimal genetic profile for survival.
2009	Zehnder M, Guggenheim B	The mysterious appear-ance of enterococci in filled root canals	Int Endod J 2009;42:27 7-87	Review.
2009	Brundin M, Figdor D, Sundqvist G, Sjogren U	Starvation response and growth in serum of *Fusobacterium nucleatum*, *Peptostreptococcus anaerobius*, *Prevotella intermedia*, and *Pseudoramibacter alactolyticus*	OOOOE 2009;108: 129-34	*In vitro*. Analyzed starvation-survival behavior over 60 days of 4 species representative of the untreated root canal infection. Results: species prevalent in post-treatment infection are well equipped to endure starvation and survive in low numbers on minimal serum. Conclusions: starvation-survival capacity is likely a selection factor for microbial participation in post-treatment disease.
2010	Al-Ahmad A, Maier J, Follo M, Spitzmüll-er B, Wittmer A, Hellwig E, Hübner J, Jonas D	Food-borne enterococci integrate into oral biofilm: an in vivo study	J Endod. 2010 Nov;36(11):1812-9	6 healthy volunteers wore dental splints loaded with enamel slabs. After 3 days, volunteers ate cheese containing enterococci. Results: *E. faecalis*, *E. faecium*, and *E. avium* isolated from the initial biofilm, 5-day-old biofilm, and cheese, showed genetic homogeneity. Concluded that food-borne enterococci, particularly *E. faecalis*, might survive in oral biofilm and become a source for endo infections.

2011	Ma Z, Wang Y, Zhu X, Zhang C, Li S, Jin L, Shen Y, Haapasalo M	Role of polymorphonuclear neutrophils in the clearance of *Enterococcus faecalis* derived from saliva and infected root canals	J Endod. 2011 Mar;37(3): 346-52	Evaluated 15 endodontic and 9 saliva strains of *E. faecalis*. Murine PMNs killed ~ 80% of bacterial cells in 1hr. qPCR showed that IL-1α, TNF-α, MMP-8, and COX-2 mRNA were up-regulated in *E. faecalis*-stimulated PMNs or in *E. faecalis*-invaded muscular tissues. MMP-8 mRNA level was positively related to COX-2 mRNA level. Histological evaluation showed that all strains could recruit PMNs to the local infectious sites and cause abscess formation.

Potential reasons for survival of *E. faecalis* in the root canal

- Ability of components of dentin as well as serum to inhibit medicaments such as calcium hydroxide, chlorhexidine and iodine potassium iodide (Portenier et al. 2001)
- Ability to invade dentinal tubules and adhere to collagen in presence of human serum (Love 2001)
- *E. faecalis* readily invades dentinal tubules, but does not adhere preferentially to tubule walls (Chivatxaranukul et al. 2008)
- A proton pump which allows *E. faecalis* to survive $Ca(OH)_2$ treatment (Evans et al. 2002)
- Bacterial species recovered from infected root canal dentin that were alkali-resistant at pH 9.0 and/or pH 10.0 belonged mainly to the genus *Enterococcus* (Nakajo et al. 2004)
- Immunosuppression mediated by *E. faecalis* could contribute to the pathogenesis of endodontic failures (Son et al. 2004)
- Age of dentin? Viable counts in *E. faecalis* biofilms were higher when grown on dentin from older (>60yr) vs younger (<30yr) patients (Ozdemir et al. 2010)
- Expression of virulence factors, e.g. gelatinase and response to pheromones (Sedgley et al. 2005)
    - Ace, the collagen binding protein produced by *E. faecalis*, mediates adhesion of *E. faecalis* to particulate dentin (Kowalski et al. 2006)
- Influence of phenotype:
    - Ageing *E. faecalis* are more resistant to medicaments (Portenier et al. 2005)
    - Variations in efficacy of different medicaments depending on biofilm or planktonic phenotype (Abdullah et al. 2005)
    - A minor increase in pH up to 8.5 increases the collagen-binding ability of *E. faecalis*, *in vitro* (Kayaoglu et al. 2005)
    - Viable *E. faecalis* entombed at the time of root filling could provide a long-term nidus for subsequent infection (Sedgley et al. 2005)
    - Found that 5/6 clinical *E. faecalis* strains induced little or no release of hydrolytic enzymes from the PMN cells (Reynaud af Geijersstam et al. 2005)
    - In general, multiple antibiotic resistance not found (Sedgley et al. 2005, Reynaud af Geijersstam et al. 2006)
    - The same root canal can harbor two different strains of *E. faecalis* (Reynaud af Geijersstam et al. 2006, Pinheiro et al. 2006)
    - *ftsZ*, a gene involved in cell division, may play a role in survival of *E. faecalis* following prolonged exposure to alkaline pH levels (Appelbe and Sedgley 2007)

- Different clinical strains vary in their biofilm formation capabilities (Duggan and Sedgley 2007). *E. faecalis* viable cells were found in biofilms in the presence of *S. mutans* in a strain-dependent manner (Deng et al. 2009)
- Clinical isolates of oral and endodontic *E. faecalis* were not strong biofilm-formers (Duggan and Sedgley 2007)
- Surface adherence and intracellular survival of *E. faecalis* within macrophages was sig higher in biofilm bacteria compared to planktonic (Mathew et al. 2010)
- Starved cells of *E. faecalis* ATCC 29212 formed biofilm on dentin with reduced efficiency compared with cells in exponential phase and stationary phase (Liu et al. 2010)
- The expression of gelE by clinical *E. faecalis* isolates from retreatment cases was higher in cases with apical radiolucency than in those without (Wang et al. 2011)
- Efflux pump inhibitors such as Verapimil may render *E. faecalis* biofilms more susceptible to antimicrobials (Upadya et al. 2011)

2001	Portenier I, Haapasalo H, Rye A, Waltimo T, Orstavik D, Haapasalo M	Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin	Int Endod J 2001;34:184-8	*In vitro*. Antibacterial activity against *E. faecalis* by $Ca(OH)_2$ was totally inactivated by dentin powder, hydroxylapatite (HA) or bovine serum albumin (BSA). Activity against *E. faecalis* by CHX (0.05%) was strongly inhibited by BSA and slowed down by dentin. HA had little or no inhibitory effect on CHX. Activity against *E. faecalis* by 0.2%/0.4% IKI was totally inhibited by dentin, but unaffected by HA or BSA.
2001	Love RM	*Enterococcus faecalis*--a mechanism for its role in endodontic failure	Int Endod J 2001;34:399-405	Postulated that a virulence factor of *E. faecalis* in failed endodontically treated teeth may be related to ability of *E. faecalis* to invade dentinal tubules and adhere to collagen in the presence of human serum
2002	Evans M, Davies JK, Sundqvist G, Figdor D	Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide	Int Endod J 2002;35:221-8	*In vitro*. Survival of *E. faecalis* in $Ca(OH)_2$ appears to be unrelated to stress induced protein synthesis. A functioning proton pump is critical for survival of *E. faecalis* at high pH
2004	Son HH, Lim S, Shon W, Kim HS, Lee W	Effects of sonicated *Enterococcus faecalis* extracts on interleukin-2 and interleukin-4 production by human T cells	J Endod 2004;30:701-3	*In vitro*. Expressions of IL-2 and IL-4 by lymphocytes were significantly inhibited when T cells were preexposed to sonicated *E. faecalis* extracts. Suggests that immunosuppression mediated by *E. faecalis* could contribute to the pathogenesis of endodontic failures
2004	Nakajo K, Nakazawa F, Iwaku M, Hoshino E	Alkali-resistant bacteria in root canal systems	Oral Microbiol Immunol 2004;19:390-4	*In vitro*. Bacteria recovered from homogenized dentin powder from human infected root canal walls were assessed for alkali resistance at pH 9.0 and/or pH 10.0. Resistant bacteria belonged mainly to the genus *Enterococcus*, also some *Streptococcus* strains
2005	Portenier I, Waltimo T, Orstavik D, Haapasalo M	The susceptibility of starved, stationary phase, and growing cells of *Enterococcus faecalis* to endodontic medicaments	J Endod 2005;31:380-6	*In vitro*. Ageing *E. faecalis* cells were more resistant to endodontic antimicrobials (saturated $Ca(OH)_2$ solution, 0.05% chlorhexidine digluconate and 0.0001% NaOCl)

2005	Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, Dahlén G	Virulence, phenotype and genotype characteristics of endodontic *Enterococcus* Spp.	Oral Microbiol Immunol 2005;20:10-9	*In vitro*. Investigated virulence, phenotype and genotype of 33 endodontic enterococcal isolates. Phenotypic and genotypic evidence of potential virulence factors were identified in endodontic *Enterococcus* spp., specifically production of gelatinase and response to pheromones. Most strains susceptible to the 12 antibiotics tested except 4 strains resistant to tetracycline
2005	Abdullah M, Ng YL, Gulabivala K, Moles DR, Spratt DA	Susceptibilities of two *Enterococcus faecalis* phenotypes to root canal medications	J Endod 2005;31:30-6	*In vitro*. Compared efficacy of antimicrobials using clinical isolate of *E. faecalis* grown as biofilm or planktonic suspension. Difference in gradients of bacterial killing among biofilm, planktonic suspension or pellet presentation was dependent upon the test agent except with NaOCl and $Ca(OH)_2$ (no difference detected). NaOCl most effective - 100% kills for all presentations of *E. faecalis* after 2 min contact time
2005	Reynaud af Geijersstam A, Sorsa T, Stackelberg S, Tervahartiala T, Haapasalo M	Effect of *E. faecalis* on the release of serine proteases elastase and cathepsin G, and collagenase-2 (MMP-8) by human polymorphonuclear leukocytes (PMNs)	Int Endod J 2005;38:667-77	*In vitro*. Studied 6 *E. faecalis* strains isolated from treatment resistant cases of apical periodontitis. Five strains induced little or no release of hydrolytic enzymes from the PMN cells. These findings may partly explain the clinical observation that root canal infections dominated by *E. faecalis* are usually symptom free.
2005	Sedgley CM, Lennan SL, Appelbe OK	Survival of *Enterococcus faecalis* in root canals *ex vivo*	Int Endod J 2005;38:735-42	*Ex vivo*. *E. faecalis* inoculated into root canals maintained viability for 12-months *ex vivo*. The clinical implications are that viable *E. faecalis* entombed at the time of root filling could provide a long-term nidus for subsequent infection
2005	Kayaoglu G, Erten H, Orstavik D	Growth at high pH increases *Enterococcus faecalis* adhesion to collagen	Int Endod J 2005;38:389-96	*In vitro*. A "minor" increase in pH up to 8.5 increases the collagen-binding ability of *E. faecalis*
2006	Reynaud Af Geijersstam AH, Ellington MJ, Warner M, Woodford N, Haapasalo M	Antimicrobial susceptibility and molecular analysis of *Enterococcus faecalis* originating from endodontic infections in Finland and Lithuania	Oral Microbiol Immunol 2006;21:164-8	Evaluated 59 *E. faecalis* strains isolated from treatment resistant cases of apical periodontitis. Reported high level resistance to rifampicin, susceptibility or intermediate susceptibility to ampicillin and penicillin. Also showed that the same root canal can harbor two different strains of *E. faecalis*
2006	Pinheiro ET, Anderson MJ, Gomes BP, Drucker DB	Phenotypic and genotypic identification of enterococci isolated from canals of root-filled teeth with periapical lesions	Oral Microbiol Immunol 2006;21:137-44	Evaluated 22 *E. faecalis* strains isolated from root-filled teeth with periapical lesions. Noted genetic heterogeneity between strains. Showed that the same root canal can harbor two different strains of *E. faecalis*

2006	Kowalski WJ, Kasper EL, Hatton JF, Murray BE, Nallapareddy SR, Gillespie MJ	*Enterococcus faecalis* adhesin, Ace, mediates attachment to particulate dentin	J Endod 2006;32:634-7	*In vitro*. Showed that Ace, the collagen binding protein produced by *E. faecalis*, mediates adhesion of *E. faecalis* to particulate dentin
2007	Duggan J, Sedgley CM	Biofilm formation of oral and endodontic *Enterococcus faecalis*	J Endod 2007;33:815-8	*In vitro*. *E. faecalis* strains recovered from root canals (n=33), the oral cavity (n=21) and non-oral/non-endodontic sources (n=16) did not differ in capacity to form biofilms. In contrast to strains from other clinical sources in particular endocarditis strains, clinical isolates of oral and endodontic *E. faecalis* were not strong biofilm-formers
2007	Appelbe OK, Sedgley CM	Effects of prolonged exposure to alkaline pH on *Enterococcus faecalis* survival and specific gene transcripts	Oral Microbiol Immunol 2007;22:169-74	*In vitro*. Investigated survival and gene expression of *E. faecalis* maintained in alkaline media. *E. faecalis* maintained for one week showed survival levels of 100% (pH 7), 1% (pH 10), 0.001% (pH 11), and 0.00001% (pH 12). Transcripts of *ftsZ*, a gene involved in cell division, increased by 37-fold after 120 hrs at pH 10 at 37°C suggesting that *ftsZ* may play a role in *E. faecalis* survival in prolonged alkaline pH levels
2008	Chivatxaranukul P, Dashper SG, Messer HH	Dentinal tubule invasion and adherence by *Enterococcus faecalis*	Int Endod J 2008; 41:873-82	*In vitro*. *E. faecalis* readily invades dentinal tubules, but does not adhere preferentially to tubule walls.
2009	Bryce G, O'Donnell D, Ready D, Ng YL, Pratten J, Gulabivala K	Contemporary root canal irrigants are able to disrupt and eradicate single- and dual-species biofilms	J Endod 2009;35:1243-8	*In vitro*. Biofilm disruption and cell viability were influenced by the species, their coassociation in dual-species biofilms, the test agent, and the duration of exposure. Gram-negative obligate anaerobe species were more susceptible to cell removal than gram-positive facultative anaerobes. The effectiveness of NaOCl as an endodontic irrigant was reinforced.
2009	Deng DM, Hoogenkamp MA, Exterkate RA, Jiang LM, van der Sluis LW, Ten Cate JM, Crielaard W	Influence of *Streptococcus mutans* on *Enterococcus faecalis* biofilm formation	J Endod 2009;35:1249-52	*In vitro*. Tested 8 clinical strains of *E. faecalis* for biofilm formation on hydroxyapatite disks in the presence and absence of a *S. mutans* biofilm. Found that sig more *E. faecalis* viable cells were found in biofilms in the presence of *S. mutans* in a strain-dependent manner.
2010	Mathew S, Yaw-Chyn L, Kishen A	Immunogenic potential of *Enterococcus faecalis* biofilm under simulated growth conditions	J Endod 2010 May;36:842-6	*In vitro*. Compared interactions of *E. faecalis* from biofilms or planktonic when incubated with macrophages. Surface adherence and intracellular survival of *E. faecalis* within macrophages was sig higher in biofilm bacteria.
2010	Liu H, Wei X, Ling J, Wang	Biofilm formation capability of *E. faecalis*	J Endod 2010;36:6	*In vitro*. SEM. Starved cells of *E. faecalis* ATCC 29212 formed biofilm on dentin with reduced efficiency

	W, Huang X	cells in starvation phase and its susceptibility to NaOCl	30-5	compared with cells in exponential phase and stationary phase. Biofilms of starved cells were more resistant to 5.25% NaOCl than those of stationary cells.
2010	Ozdemir HO, Buzoglu HD, Calt S, Stabholz A, Steinberg D	Effect of EDTA and NaOCl irrigation on *E. faecalis* biofilm colonization in young and old human root canal dentin: *in vitro* study	J Endod 2010;36:842-6	*In vitro*. Compared *E. faecalis* biofilm formation on dentin of extracted teeth from young (<30yr) and old (>60yr) pts. Measured viable counts and histology (SEM and CLSM). Conclusions: (1) Viable counts in "old" group were higher, (2) EDTA and NaOCl sig reduces the amount of intracanal biofilm.
2011	Wang L, Dong M, Zheng J, Song Q, Yin W, Li J, Niu W	Relationship of biofilm formation and *gelE* gene expression in Enterococcus faecalis recovered from root canals in patients requiring endodontic retreatment	J Endod. 2011;37:631-6	The expression of *gelE* and biofilm formation in *E. faecalis* strains isolated from root canal retreatment cases was assessed. The expression of *gelE* was stronger in the cases of apical radiolucency than in those without ($P < .05$). The expression of *gelE* was higher in the biofilm-positive than in biofilm-negative strains ($P < .05$).
2011	Upadya M, Shrestha A, Kishen A	Role of efflux pump inhibitors on the antibiofilm efficacy of calcium hydroxide, chitosan nanoparticles, and light-activated disinfection	J Endod. 2011;37:1422-6	*In vitro*. Resistance to antimicrobials by *E. faecalis* may be associated with biofilm extracellular polymeric substance. Efflux pump inhibitors such as Verapimil may render *E. faecalis* biofilms more susceptible to antimicrobials.

6.4 Summary

Genera cultured from canals undergoing endodontic retreatment

- Culture studies often show fewer species and reduced microbial load compared with primary infections
- Polymicrobial
- *E. faecalis* predominates

Facultative anaerobes	Anaerobes
Gram positive cocci	
Enterococcus	*Micromonas* (formerly *Peptostreptococcus*)
Streptococcus	*Staphylococcus*
Gram negative cocci	
Veillonella	*Anaeroglobus*
Gram positive rods	
Lactobacillus	*Actinomyces*
Bacillus	*Propionibacterium* (formerly *Arachnia*)
Pseudoramibacter	*Eubacterium*
Gram negative rods	
Escherichia	*Porphyromonas*
Pseudomonas	*Prevotella*
Proteus	*Fusobacterium*
Klebsiella	*Bacteroides* family
Enterobacter	*Wolinella*
Tannerella	
Fungi	
Candida	

DNA-based identification

- Multiple species ("polymicrobial")
- Less predominance of *E. faecalis* than in culture studies
- Significantly more species than in culture-based studies
- Many species as yet unidentified and unculturable

Part 7

Microorganisms in periapical lesions

7.1 Do microorganisms survive in asymptomatic periapical lesions?

No (Fish 1939, Walton and Ardjmand 1992, Ricucci et al. 2006)
Yes (Tronstad et al. 1987, Sunde et al. 2003)

Do microorganisms survive in symptomatic lesions?

Yes (Sjogren et al. 1988, Ricucci et al. 2015 and others)

- "Anaerobic bacteria are able to survive and maintain an infectious disease process in periapical tissues" (Tronstad et al. 1987)
- In periapical lesions of root-filled teeth with asymptomatic apical periodontitis observed microcolonies (CLSM), and detected *P. gingivalis, P. intermedia, T. forsythensis, Streptococcus* spp. and unidentified morphotypes (FISH)(Sunde et al. 2003)
- Not detected in periapical tissues (monkey study – light microscopy) (Walton and Ardjmand 1992)
- Bacteria detected in abscesses and cysts, but not in asymptomatic granulomas – light microscopy (Ricucci et al. 2006, Ricucci et al. 2015)

1939	Fish EW	Bone infection	JADA1939; 26: 691-712	Early attempt to disprove focal infection theory. Found that microorganisms were confined to "Zone of infection" • Zone of infection - innermost zone, necrotic, contains bacteria; center of abscess • Zone of contamination - cell destruction evident; abscess wall, exudative • Zone of irritation - contains osteoclasts and histiocytes; granulomatous zone • Zone of stimulation – encapsulation evident
1987	Tronstad L, Barnett F, Riso K, Slots J	Extraradicular endodontic infections	Endod Dent Traumatol 1987;3:86-90	Examined specimens from 8 asymptomatic patients with refractory periapical lesions, 5 with sinus tracts. 2/3 of the canals were negative for bacteria and all the periapical lesions were positive for Gram positive anaerobes. Concluded that anaerobic bacteria are able to survive and maintain an infectious disease process in periapical tissues.
1988	Sjögren U, Happonen RP, Kahnberg KE, Sundqvist G	Survival of *Arachnia propionica* in periapical tissue	Int J Endod 1988;21:277-82	Described a case of refractory infection in the periapical area. *Arachnia propionica* was isolated, showing that microaerophilic Gram positive pleomorphic organisms can infect periapical lesions.
1992	Walton RE, Ardjmand K	Histological evaluation of the presence of bacteria	J Endod 1992;18:216-27	Induced 7-month periapical lesions (n=18) in monkeys. Obtained block sections - avoided sampling contamination of lesion. Sections Brown and Brenn-

		in induced periapical lesions in monkeys		stained for bacteria. Results: Bacteria detected in necrotic tissue in canals. Bacteria were NOT detected in periapical tissues (except intracellularly or as bolus at foramen).
2003	Sunde PT, Olsen I, Gobel UB, Theegarten D, Winter S, Debelian GJ, Tronstad L, Moter A	Fluorescence in situ hybridization (FISH) for direct visualization of bacteria in periapical lesions of asymptomatic root-filled teeth	Microbiology 2003;149:1095-102.	39 PA lesions from asymptomatic root filled teeth with PARL and no sinus tracts or endo-perio lesions. Evaluated with CLSM and FISH. In 50% of lesions rods, spirochaetes and cocci were spread out in some areas while other parts seemed bacteria-free. Bacteria were also seen to form microcolonies. Probes showed *P. gingivalis, P. intermedia, T. forsythensis, Streptococcus* spp. and unidentified morphotypes detected in asymptomatic periapical lesions of root-filled teeth.
2006	Ricucci D, Pascon EA, Ford TR, Langeland K	Epithelium and bacteria in periapical lesions	OOOOE 2006;101:239-49	Histological evaluation of 50 untreated teeth that had lesions attached to their apices. Bacteria were found in all teeth. Granulomas were most common, and most epithelialized lesions were cysts. Bacteria were only detected periapically in abscesses or cysts.
2015	Ricucci D, Siqueira JF, Jr., Lopes WS, Vieira AR, Rocas IN J Endod 2015;41:265-73	Extraradicular infection as the cause of persistent symptoms: a case series	J Endod 2015;41:265-73	3 cases. Different forms of extraradicular infection were associated with three symptomatic cases. Observed (1) necrotic debris, heavily colonized by ramifying bacteria, in cyst lumen, (2) numerous bacterial aggregations through the inflammatory tissue in granuloma, (3) bacterial biofilms on external apical root surface, filling large lateral canal and other apical ramifications, and between layers of cementum detached from root surface

7.2 Sampling

Microbiological sampling of periapical tissue presents difficulties

- Early papers were questioned over sampling methods – contamination by oral flora possible
- Attempt to improve sampling - after reflecting flap, irrigated the cortical bone with saline in an effort to remove any contaminants introduced during flap resection (Iwu et al. 1990)
- Phenotypic typing showed that following marginal incision, bacteria from the periodontal pocket might reach the underlying tissues by surgeon-released bacteremia or direct translocation of microorganisms (Sunde et al. 2000)
- 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 always "destroy" the DNA

<u>There is no method available yet that can discriminate between DNA from microorganism at site of interest (lesion) and from contaminant</u>

1990	Iwu C, Macfarlane TW, Mac-Kenzie. D, Stenhouse D	The microbiology of periapical granulomas	OOO 1990;69:502-5	Evaluated bacterial content of periapical granulomas using a wash method sampling technique and anaerobic culturing. 85% had positive cultures with 55% facultative anaerobes, 45% strict anaerobes. Most frequent isolates *Veillonella*, *Streptococcus*, *Actinomyces*, *Propionibacterium* and *Bacteroides*.
2000	Sunde PT, Olsen I, Lind PO, Tronstad L	Extraradicular infection: a methodological study	Endod Dent Traumatol 2000;16:84-90	Compared marginal and submarginal incisions. Phenotypic profiling indicated that following marginal incision, bacteria from the periodontal pocket might have reached the underlying tissues by surgeon-released bacteremia or direct translocation.
2000	Sunde PT, Tronstad L, Eribe ER, Lind PO, Olsen I	Assessment of periradicular microbiota by DNA-DNA hybridization	Endod Dent Traumatol 2000;16:191-6	Bacterial DNA detected in 36 lesions from patients with apical periodontitis (previously root filled). Submarginal incision less species than intrasulcular. *A. actinomycetemcomitans* and *B. forsythus* in more than 60% of lesions. *P. endodontalis* "abundant" in periapical tissue.
2000	Gatti JJ, Dobeck JM, Smith C, Socransky SS, Skobe Z	Bacteria of asymptomatic periradicular endodontic lesions identified by DNA-DNA hybridization	Endod Dent Traumatol 2000;16:197-204	Used checkerboard DNA-DNA hybridization to detect bacterial DNA in 36 lesions from patients with asymptomatic, chronic suppurative periodontitis (all previously root filled). Submarginal incision better than intrasulcular in avoiding sample contamination. Bacterial DNA found in all lesions. *Bacteroides forsythus* and *Actinomyces naeslundii* predominant.

7.3 Microflora identified in sinus tracts

- In 9/12 cases, species present in the root canal were also in the sinus tracts (Weiger et al. 1995)

1995	Weiger R, Manncke B, Werner H, Lost C	Microbal flora of sinus tracts and root canals of non-vital teeth	Endod Dent Traumatol 1995;11:15-9	Seventy-one bacterial strains were isolated from 12 endodontic-related sinus tracts. In 9/12 cases, species present in the root canal were also in the sinus tracts

7.4 Microflora identified in periapical samples

- **Gram positive filamentous rods have been recovered from periapical lesions**
  - *Actinomyces israelii* (Sundqvist and Reutvering 1980, Happonen 1986)
  - *Arachnia propionica* (Sjögren et al. 1988) (now *Propionibacterium propionicus*)
  - *A. israelii, A. viscosus, A. naeslundii, and A. meyeri* (Sunde et al. 2002)
  - Actinomycotic aggregates observed (Ricucci and Siqueira 2008) – case report

- **Other microorganisms, including viruses**
  - HIV DNA in periradicular lesion from an HIV-positive patient (Elkins et al. 1994)
  - 31.8% *Actinomyces*, 22.7% *Propionibacterium* spp., 18.2% *Streptococcus* spp. (Abou-Rass and Bogen 1998)
  - Black-pigmented anaerobic rods were infrequent (Bogen and Slots 1999)
  - Predominantly anaerobes (Abou-Rass and Bogen 1998, Sunde et al. 2000a)
  - *A. actinomycetemcomitans* and *B. forsythus* in more than 60% of lesions. *P. endodontalis* was "abundant" in periapical tissue (Sunde et al. 2000)
  - Bacterial DNA found in 36 chronic suppurative periodontitis (all previously root filled) lesions. *Bacteroides forsythus* and *Actinomyces naeslundii* predominant (Gatti et al. 2000)
  - Facultative organisms, such as *Staphylococcus*, *Enterococcus*, *Enterobacter*, *Pseudomonas*, *Stenotrophomonas*, *Sphingomonas*, *Bacillus*, or *Candida* species were recovered from 27/36 lesions (Sunde et al. 2002)
  - Herpes virus not found (Slots et al. 2003). *Candida* spp. not found (Waltimo et al. 2003)
  - Cytomegalovirus, Epstein-Barr virus found in periapical lesions (Yildirim et al. 2006)
  - Fungi – *Aspergillus mycetoma* (Giardino et al. 2006) - case report
  - Cytomegalovirus found in monocytes/macrophages and T-lymphocytes, but not in B lymphocytes in periapical lesions (Sabeti et al. 2009)
  - Periradicular lesions exhibited a diverse microbial profile with many uncultivated phylotypes. *E. faecalis* and *Burkholderia cepacia*, *Atopobium rimae*, *Peptostreptococcus micros*, S*treptococcus* genomospecies C8, *Dialister* sp E2_20 E1, and *Eubacterium* strain A35MT were associated with periradicular lesions (Subramanian and Mickel 2009)
  - EBV DNA and EBNA-2 messenger RNA found in apical periodontitis lesions at higher frequencies (72.5% and 50%) compared with controls (both 2.5%) (Hernandi et al. 2010)

- **SEM identification of biofilms on external surface of apices**
  - Previously treated teeth (Noiri et al. 2002)
  - Primary teeth with periapical lesions (Rochas et al. 2008)

1980	Sundqvist G, Reuterving C-O	Isolation of *Actinomyces israelii* from periapical lesion	J Endod 1980;6: 602-6	Case report - describes repeatedly isolating *Actinomyces israelii* from the root canal of a tooth with a periapical lesion over 6 months
1986	Happonen R-P	Periapical actinomycosis: A follow-up study of 16 surgically treated cases	Endod Dent Traumatol 1986;2:205 -9	Suggested that periapical actinomycoses are more common than previously believed. All cases had long endodontic histories; there may be an association between actinomycotic infections and periapical lesions
1988	Sjögren U, Happonen RP, Kahnberg KE, Sundqvist G	Survival of *Arachnia propionica* in periapical tissue	Int J Endod 1988;21:27 7-82	Described a case of refractory infection in the periapical area. *Arachnia propionica* was isolated, showing that microaerophilic Gram positive pleomorphic organisms can colonize and survive in periapical lesions.
1994	Elkins DA, Torabinejad M, Schmidt RE, Rossi JJ, Kettering JD	Polymerase chain reaction detection of HIV DNA in human periradicular lesions	J Endod 1994;20:38 6-8	Used PCR to determine that periradicular lesion from an HIV positive patient contained HIV DNA

1998	Abou-Rass M, Bogen G	Microorganisms in closed periapical lesions	Int Endod J 1998;31:39-47	In 13 cases, apical surgical samples yielded 63.6% obligate anaerobes and 36.4% facultative anaerobes. Prevalence of isolated species was 31.8% *Actinomyces*, 22.7% *Propionibacterium*, 18.2% *Streptococcus* spp.
1999	Bogen G, Slots J	Black-pigmented anaerobic rods in closed periapical lesions	Int Endod J 1999;32:204-10	Black-pigmented anaerobic rods were infrequent inhabitants of closed periapical lesions (*n*=20)
2000	Sunde PT, Olsen I, Lind PO, Tronstad L	Extraradicular infection: a methodological study	Endod Dent Traumatol 2000;16:84-90	Sampled 30 patients with root-filled teeth and periapical radiolucencies. The predominant cultivable bacteria were anaerobic.
2000	Sunde PT, Tronstad L, Eribe ER, Lind PO, Olsen I	Assessment of periradicular microbiota by DNA-DNA hybridization	Endod Dent Traumatol 2000;16:191-6	Bacterial DNA detected in 36 lesions from patients with apical periodontitis (previously root filled). *A. actinomycetemcomitans* and *B. forsythus* in more than 60% of lesions. *P. endodontalis* was "abundant" in periapical tissue.
2000	Gatti JJ, Dobeck JM, Smith C, Socransky SS, Skobe Z	Bacteria of asymptomatic periradicular endodontic lesions identified by DNA-DNA hybridization	Endod Dent Traumatol 2000;16:197-204	Used Checkerboard DNA-DNA hybridization to detect bacterial DNA in 36 lesions from patients with asymptomatic, chronic suppurative periodontitis (all previously root filled). Bacterial DNA found in all lesions. *Bacteroides forsythus* and *Actinomyces naeslundii* predominant
2002	Noiri Y, Ehara A, Kawahara T, Takemura N, Ebisu S	Participation of bacterial biofilms in refractory and chronic periapical periodontitis	J Endod 2002;28:679-83	SEM. Viewed 6 teeth and 5 extruded gutta-percha points associated with refractory periapical. Bacterial biofilms were seen at the extraradicular area of 9 samples
2002	Sunde PT, Olsen I, Debelian GJ, Tronstad L	Microbiota of periapical lesions refractory to endodontic therapy	J Endod 2002 ;28:304-10	Studied 36 teeth with refractory apical periodontitis. 148 microbial strains detected among 67 microbial species. 51% of strains were anaerobic. Gram+ species constituted 79.5% of the flora. *Staphylococcus*, *Enterococcus*, *Enterobacter*, *Pseudomonas*, *Steno-trophomonas*, *Sphingomonas*, *Bacillus*, *Candida* species were recovered from 27 of the lesions (75%). *Actino-myces israelii*, *A. viscosus, A. naeslundii*, and *A. meyeri* found in periapical microbiota of 9/36 teeth.
2003	Waltimo T, Kuusinen M, Jarvensivu A, et al.	Examination on *Candida* spp. in refractory periapical granulomas	Int Endod J 2003;36;643-7	Using PCR analyses of 103 periapical granulomas concluded that *Candida* spp. do not seem to occur in granulomas
2003	Slots J, Sabeti M, Simon JH	Herpesviruses in periapical pathosis: an etiopathogenic relationship?	OOOOE 2003;96:327-31	Review. Cytomegalovirus or Epstein-Barr virus active infections detected in >90% of granulomas of symptomatic and large periapical lesions. Dual infection with CMV and Epstein-Barr virus is closely associated with symptomatic lesions. Herpes simplex virus active infection has no apparent relationship to PA disease.
2006	Yildirim S, Yapar M,	Human cytomegalo-virus, Epstein-Barr	Oral Microbiol Immunol	In periapical symptomatic pathosis of 12 deciduous teeth, seven (58%) of the periapical lesions yielded

	Kubar A, Slots J	virus and bone resorption-inducing cytokines in periapical lesions of deciduous teeth	2006;21:107-11	human CMV and eight (67%) Epstein-Barr virus. Only one (8%) periapical lesion showed neither virus. Also showed that increased receptor activator of nuclear kappa B ligand (RANKL) expression in periapical lesions may be of pathogenetic significance
2006	Giardino L, Pontieri F, Savoldi E, Tallarigo F	*Aspergillus mycetoma* of the maxillary sinus secondary to overfilling of a root canal	J Endod 2006;32:692-4	Most recent of periodic case reports of noninvasive aspergillosis (a fungal infection) of the maxillary sinus. [Thought to be associated with overextension of root canal filling materials into maxillary sinus, particularly those containing zinc, which is a growth factor for *Aspergillus* species. Disease can present as chronic sinusitis in non-immunocompromised patients]
2008	Ricucci D, Siqueira JF	Apical actinomycosis as a continuum of intraradicular and extraradicular infection: case report and critical review on its involvement with treatment failure	J Endod 2008;34:1124-9	Case report. Apical actinomycotic aggregates formed a continuum with aggregates remaining in apical ramifications. State that there is no clear evidence that apical actinomycosis is an independent entity leading to persistent apical periodontitis lesions.
2008	Rocha CT, Rossi MA, Leonardo MR, Rocha LB, Nelson-Filho P, Silva LA	Biofilm on the apical region of roots in primary teeth with vital and necrotic pulps with or without radiographically evident apical pathosis	Int Endod J 2008;41:664-9	SEM. Evaluated 18 extracted primary teeth. Observed microbial biofilms on external root surface of teeth with pulp necrosis and radiographically visible periapical pathosis, but not on teeth with normal pulps.
2009	Subramanian K, Mickel AK	Molecular analysis of persistent periradicular lesions and root ends reveals a diverse microbial profile	J Endod 2009;35:950-7.	Thirty-four samples of periradicular tissue and resected root ends were collected. Total bacterial levels were estimated using real-time PCR and 16S rRNA genes were sequenced for bacterial identification. Bacteria were detected more consistently and at higher levels in root ends. PA lesions exhibited diverse microbial profile with many uncultivated phylotypes. *E. faecalis* and *Burkholderia cepacia* predominated in both samples.
2009	Sabeti M, Daneshmand A, Simon JH, Slots J	Cytomegalovirus-infected inflammatory cells in dental periapical lesions	Oral Microbiol Immunol 2009;24:434-6	In periapical granulomatous tissue collected from 15 extracted teeth with symptomatic periapical lesions, cytomegalovirus was identified in 10 lesions and in monocytes/macrophages and T-lymphocytes, but not in B lymphocytes. Used flow cytometry to identify cytomegalovirus infected cells.
2010	Hernádi K, Szalmás A, Mogyorósi R, Czompa L, Veress G, Csoma E et al.	Prevalence and activity of Epstein-Barr virus and human cytomegalovirus in symptomatic and asymptomatic apical periodontitis lesions	J Endod 2010;36:1485-9	Compared EBV and HCMV prevalence in 40 periapical lesion samples and 40 healthy pulp controls. EBV DNA and EBNA-2 messenger RNA were found in apical periodontitis lesions at significantly ($p < 0.0001$) higher frequencies (72.5% and 50%, respectively) compared with controls (both 2.5%).

7.5 Summary

Genera cultured from periapical lesions

- Multiple species ("polymicrobial")
- Predominantly Gram positive and Gram negative anaerobes
- Covers symptomatic and asymptomatic cases

Facultative anaerobes	Anaerobes
Gram positive cocci	
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* family
Tannerella	
Fungi	
Aspergillus	
Candida?	

DNA-based identification

- Multiple species ("polymicrobial")
- Many species as yet unidentified and unculturable
- Viruses (HIV, Cytomegalovirus, Epstein-Barr virus)

Part 8

Effects of endodontic treatment on microflora

8.1 Sodium hypochlorite
Is NaOCl an effective antimicrobial intracanal irrigant?
YES

Clinical studies:

- Classic studies: Irrigation with saline inadequate for disinfection of canal (Bystrom and Sundqvist 1981, Cvek et al. 1976), 0.5 percent NaOCl better than saline (Bystrom and Sundqvist 1983), no significant difference between 0.5% and 5% NaOC1 (Bystrom and Sundqvist 1985, Cvek et al. 1976)
- NaOCl irrigation with ProFile 29 Series rotary instrumentation reduced canal bacteria during endodontic treatment. However this method could not consistently render canals bacteria-free. Intracanal calcium hydroxide for at least 1 wk rendered 92.5% of the canals bacteria free (Shuping et al. 2000)
- Cuspid (n=2) and bicuspid canals (n=11) were bacteria-free after using 1% NaOCl and 0.04 taper ProFile rotary files to a #8 size (Card et al. 2002)
- Cross-sectional study. 20 retreatment cases – all samples culture negative after biomechanical preparation using NaOCl and EDTA (Schirrmeister et al. 2007)
- 2.5% NaOCl did not eliminate endotoxin (Martinho and Gomes 2008)

- **With combined rotary and hand instrumentation:**
  - *In vivo*, compared to 2% chlorhexidine gel, 2.5% NaOCl had a higher kill capacity and removed more cells from the root canal (Vianna et al. 2006)

***In vivo* dog study:**

- Biomechanical preparation with 1% and 5% NaOCl did not inactivate the effects of the endotoxin (Tanomaru et al. 2003)

***In vitro* studies:**

- NaOCl did not detoxify endotoxin (de Oliveira et al. 2007)
- Water, EDTA, ethanol, 0.12% chlorhexidine, chlorhexidine + sodium hypochlorite, and sodium hypochlorite alone showed little breakdown of LPS (Buck et al. 2001)
- Frequency of irrigation, and mechanical agitation of the irrigant during preparation, more important than concentration of NaOCl (Moorer and Wesselink 1982, Siqueira et al. 2000)
- 100-fold increase in killing efficacy against *E. faecalis* by NaOCl at 45°C compared to 20°C (Sirtes et al. 2005). Increased temperature also enhanced tissue dissolution, but agitation had a greater effect (Stojicic et al. 2010).
- Organic solvent action of NaOCl decreases at lower pH (Christensen et al. 2008)
- Addition of surface modifiers did not improve bactericidal activity of NaOCl against *E. faecalis* (Williamson et al. 2009)
- 5 min immersion in NaOCl does not achieve file sterility (Gnau et al. 2009)
- *E. faecalis* biofilms more resistant to 5.25% NaOCl than stationary cells (Liu et al. 2010)

- *E. faecalis* biofilms removed by 1% NaOCl (Bhuva et al. 2010) and 1% and 6% NaOCl (Dunavant et al. 2006)
- In polymicrobial biofilms, 6% NaOCl rendered bacteria nonviable and physically removed the biofilm (Clegg et al. 2006)
- Mixed-species biofilms of *F. nucleatum* and *P. micros* showed a time-dependent synergy in growth and resistance to NaOCl (Ozok et al. 2007)
- 1% NaOCl penetrated dentin depth only 50%-80% compared to 6% NaOCl (Zhou et al. 2010)
- 5.25% NaOCl causes tunnelling erosion of mineralized dentin (Zhang et al. 2010)
- Level of maturation of biofilm affects their susceptibility to antimicrobial agents. Older biofilms are less susceptible than younger biofilms (Stojicic et al. 2013)

1976	Cvek M, Nord CE, Hollender L	Antimicrobial effect of root canal debridement in teeth with immature root. A clinical and microbiological study	Odontol Revy 1976;27:1-10	Clinical study. Examined the antibacterial effect on a mixed flora following use *in vivo* of 0.9% saline, 0.5% and 5% NaOCl as irrigants in mature and immature root canals. While saline had a minimal antibacterial effect, both concentrations of NaOCl were equally effective
1981	Bystrom A, Sundqvist G	Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy	Scand J Dent Res 1981;89:321-8	Clinical study. Presence of bacteria in 17 single-rooted teeth with PA lesions studied throughout treatment. Root canals irrigated with NaCl. Proportion of bacteria changed during treatment; bacteria surviving between appointments increased significantly. Irrigation with NaCl inadequate for disinfection of canal.
1983	Bystrom A, Sundqvist G	Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy	OOO 1983;55:307-12	Clinical study. Bacteriologic evaluation of the effect of 0.5% NaOCl in endodontic therapy. Better than saline.
1985	Bystrom A, Sundqvist G	The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy	Int J Endod 1985;18:35-40	Clinical study. Evaluated the antibacterial effectiveness of irrigants (5% NaOC1, 0.5% NaOC1, and EDTA + 5% NaOC1. Teeth were sampled at start of subsequent visit. No significant difference between 0.5% and 5% NaOC1
1982	Moorer WR, Wesselink PR	Factors promoting the tissue dissolving capacity of sodium hypochlorite	Int Endod J 1982;15:187-96	*In vitro*. Detailed investigations into interaction of three concentrations (0.6%, 1.2% and 3%) of NaOCl with various concentrations of protein hydrolysate. Recommended concentration between 0.5% and 2%.
2000	Shuping GB, Orstavik D, Sigurdsson A, Trope M	Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications	J Endod 2000;26:751-5	Clinical study. Evaluated bacterial reduction with NiTi ProFile 29 series and 1.25% NaOCl in 42 subjects. Canals sampled before, during and after instrumentation, and after treatment with $Ca(OH)_2$. Greater reduction of bacteria with NaOCl irrigation, compared with sterile saline [saline data from a previous study Dalton et al. 1998]. After instrumentation with NaOCl, 61.9% canals bacteria-free. After $Ca(OH)_2$ for at least 1 wk 92.5% canals bacteria-free.
2000	Siqueira Junior JF,	Chemomechanical reduction of the bacterial population in the root	J Endod 2000 ;26:331-4	*In vitro*. 1%, 2.5%, and 5.25% NaOCl all more effective than saline solution. No difference between

	Rôças IN, Favieri A, Lima KC	canal after instrument-ation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite		concentrations of NaOCl. Root canals inoculated with *E. faecalis*. Suggested that regular exchange and the use of large amounts of irrigant should maintain the antibacterial effectiveness of the NaOCl solution, compensating for the effects of concentration
2001	Buck RA, Cai J, Eleazer PD, Staat RH, Hurst HE	Detoxification of endotoxin by endodontic irrigants and calcium hydroxide	J Endod 2001;27:325-7	*In vitro*. Studied effects of endo irrigants and $Ca(OH)_2$ on LPS using mass spectrometry/gas chromatography. Water, EDTA, ethanol, 0.12% CHX, CHX+NaOCl, and NaOCl alone showed little breakdown of LPS. $Ca(OH)_2$ detoxified LPS (5 days better than 10 min or 30 min).
2002	Card SJ, Sigurdsson A, Orstavik D, Trope M	The effectiveness of increased apical enlargement in reducing intracanal bacteria	J Endod 2002 ;28:779-83	Clinical study. Instrumented canals (n=13) were bacteria-free after using 1% NaOCl and 0.04 taper ProFile rotary files to a #8 size. For molars, 22/27 mesial roots of molars were also bacteria-free. Further preparation using Lightspeed to apical #60 24/27 molars were bacteria-free.
2003	Tanomaru JM, Leonardo MR, Tanomaru Filho M, Bonetti Filho I, Silva LA	Effect of different irrigation solutions and calcium hydroxide on bacterial LPS	Int Endod J 2003;36;733-9	*In vivo* dog study. Compared 1% and 5% sodium hypochlorite, 2% CHX and saline solution/ $Ca(OH)_2$dressing. Biomechanical preparation with the irrigating solutions did not inactivate the effects of the endotoxin but the $Ca(OH)_2$intracanal dressing did appear to inactivate the effects induced by endotoxin.
2005	Sirtes G, Waltimo T, Schaetzle M, Zehnder M	The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy	J Endod 2005;31:669-71	*In vitro*. Killing efficacy of diluted NaOCl solutions against 48-h incubations of *E. faecalis* was compared at 45°C and 20°C. The 1% NaOCl solution at 45°C dissolved pulp tissues as effectively as the 5.25% solution at 20°C, while the 60°C/1% solution was significantly more effective. A 100-fold increase in killing efficacy was observed between NaOCl solutions at 20°C and 45°C.
2006	Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR	The effect of exposure to irrigant solutions on apical dentin biofilms in vitro	J Endod 2006;32:434-7	*In vitro*. Apical root sections. SEM and viable counts of artificial polymicrobial biofilms immersed in various irrigants (NaOCl, EDTA, CHX, MTAD). 6% NaOCl was the only irrigant capable of both rendering bacteria nonviable and physically removing the biofilm
2006	Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL	Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms	J Endod 2006;32:527-31	*In vitro*. Flow cell system. *E. faecalis* biofilms immersed in various irrigants (NaOCl, SmearClear, REDTA, CHX, MTAD). 1% and 6% NaOCl most efficient in removing the biofilm
2006	Vianna ME, Horz HP, Gomes BP, Conrads G	*In vivo* evaluation of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue	Int Endod J 2006;39;484-92	Clinical study. 32 single teeth with necrotic pulps. Used RT-qPCR and culture to analyze load and microbial reduction and compare two different irrigants, 2% CHX and 2.5% NaOCl). NaOCl had a higher capacity to kill microorganisms and was able to remove more cells from the root canal.

2007	de Oliveira LD, Jorge AO, Carvalho CA, Koga-Ito CY, Valera MC	*In vitro* effects of endodontic irrigants on endotoxins in root canals	OOOOE 2007;104:135-42	*In vitro*. *Escherichia coli* endotoxin was inoculated into 84 root canals. $Ca(OH)_2$and polymyxin B detoxified endotoxin in root canals and altered properties of LPS to stimulate the antibody production by B-lymphocytes. NaOCl and CHX did not detoxify endotoxin
2007	Schirrmeister JF, Liebenow AL, Braun G, Wittmer A, Hellwig E, Al-Ahmad A	Detection and eradication of microorganisms in root-filled teeth associated with periradicular lesions: an *in vivo* study	J Endod 2007;33:536-40	Clinical study. Cross-sectional study. 20 teeth undergoing retreatment Used PCR and culture to analyze presence of bacteria. After root canal preparation and irrigation using 2.5% NaOCl and EDTA, no microorganisms could be detected in teeth.
2007	Özok AR, Wu MK, Luppens SBI, Wesselink PR	Comparison of growth and susceptibility to sodium hypochlorite of mono- and dual-species biofilms of *Fusobacterium nucleatum* and *Peptostreptococcus (micromonas) micros*	J Endod 2007;33:819-22	*In vitro*. Compared growth and susceptibility to different concentrations of sodium hypochlorite (NaOCl) of mono- and dual-species biofilms of *F. nucleatum* or *P. micros* grown in polystyrene wells at 24 or 96 hr. As age of biofilms increased, so did their resistance to NaOCl. Mixed-species biofilms showed a time-dependent synergy in growth and resistance to NaOCl
2008	Christensen CE, McNeal SF, Eleazer P	Effect of lowering the pH of sodium hypochlorite on dissolving tissue *in vitro*	J Endod 2008;34:449-52	*In vitro*. No sig diff between the pH 12 and 9 groups. Sig difference between pH 12 and 9 versus pH 6. Higher concentrations and greater time periods all lead to greater amounts of tissue dissolution
2008	Martinho FC, Gomes BP	Quantification of endotoxins and cultivable bacteria in root canal infection before and after chemomechanical preparation with 2.5% sodium hypochlorite	J Endod 2008;34:268-72	Clinical study. Chemomechanical preparation with 2.5% NaOCl was moderately effective against bacteria but less effective against endotoxins in root canal infection. 24 root canals.
2009	Williamson AE, Cardon JW, Drake DR	Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*	J Endod 2009;35:95-7	*In vitro*. 1- and 3-min exposures to 6% NaOCl and Chlor-EXTRA (<6% NaOCl with surface modifier) were sig superior to 2% CHX and CHX-Plus. Addition of surface modifiers did not improve bactericidal activity compared to the original formulations
2009	Gnau HL, Goodell GG, Imamura GM	Rapid chairside sterilization of endodontic files using 6% NaOCl	J Endod 2009;35:1253-4	*In vitro*. Endodontic files from four manufacturers demonstrated a 6% contamination rate and up to 5 min immersion in NaOCl did not achieve file sterility.
2010	Bhuva B, Patel S, Wilson R, Niazi S,	The effectiveness of passive ultrasonic irrigation on intraradicular *E. faecalis* biofilms in	Int Endod J 2010 Mar;43:241-50	*In vitro*. SEM. In extracted teeth both conventional syringe irrigation and passive ultrasonic irrigation with 1% NaOCl were effective at completely removing intraradicular *E. faecalis* biofilms.

	Beighton D, Mannocci F	extracted single-rooted human teeth		
2010	Zou L, Shen Y, Li W, Haapasalo M	Penetration of sodium hypochlorite into dentin	J Endod 2010 May;36:793-6	*In vitro*. Evaluated ability of NaOCl to bleach human dentin blocks stained with crystal violet for up to 20min. Depth of penetration with 1% NaOCl was 50%-80% of the depth using 6% NaOCl.
2010	Liu H, Wei X, Ling J, Wang W, Huang X	Biofilm formation capability of *E. faecalis* cells in starvation phase and its susceptibility to NaOCl	J Endod 2010 Apr;36:630-5	*In vitro*. SEM. Starved cells of *E. faecalis* ATCC 29212 formed biofilm on dentin with reduced efficiency compared with cells in exponential phase and stationary phase. Biofilms were more resistant to 5.25% NaOCl than those of stationary cells.
2010	Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M	Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant	J Endod. 2010 Sep;36(9):1558-62	*In vitro*. Compared NaOCl under various conditions to dissolve bovine tissue. Dissolution of tissue increased almost linearly with the concentration of sodium hypochlorite. Increased temperature also enhanced tissue dissolution, but agitation had a greater effect.
2010	Zhang K, Tay FR, Kim YK, Mitchell JK, Kim JR, Carrilho M, Pashley DH, Ling JQ	The effect of initial irrigation with two different sodium hypochlorite concentrations on the erosion of instrumented radicular dentin	Dent Mater. 2010 Jun;26(6):514-23	*In vitro*. SEM and TEM were used to examine the erosion of instrumented canal walls irrigated with 5.25% NaOCl/EDTA or 1.3% NaOCl/EDTA. The superficial destructive effect of NaOCl on mineralized dentin is irreversible and is present irrespective of whether EDTA is subsequently employed as the final active irrigant.
2013	Stojicic S, Shen Y, Haapasalo M	Effect of the source of biofilm bacteria, level of biofilm maturation, and type of disinfecting agent on the susceptibility of biofilm bacteria to antibacterial agents	J Endod 2013:39:473–7	Showed that the source of biofilm bacteria, level of biofilm maturation, and type of disinfecting agent affect the susceptibility of multispecies biofilm bacteria to antibacterial agents. The change of biofilm bacteria from sensitive to resistant against disinfecting agents occurred between 2 and 3 weeks of biofilm maturation

8.2 Chlorhexidine

Is chlorhexidine an effective intracanal irrigant/medicament?

- Chlorhexidine is not necessarily active against all bacterial endospores, fungal spores or viruses
- Bacteriostatic or bactericidal (depending on concentration used) for a wide range of Gram positive and Gram negative bacteria
- Does not dissolve tissue

***Clinical studies*:**

- CHX as an irrigant was no more effective than NaOCl and did not have a prolonged bactericidal effect (Ringel et al. 1982)
- Use of CHX as an additional rinse after irrigation with 1% NaOCl reduced the number of cases with cultivable bacteria at the conclusion of the first visit (Zamany et al. 2003)
- No difference when comparing the antibacterial effects of 2.5% NaOCl and 0.12% CHX used as irrigants during the treatment of infected canals (Siqueira et al. 2007)
- 7-15 day intracanal medication with 2% CHX liquid did not reduce (1) the proportion of teeth with negative cultures, or (2) the bacterial concentration (Paquette et al. 2007)
- 2% CHX gel is effective root canal disinfectant and additional intracanal dressing did not significantly improve the bacteria reduction (Wang et al. 2007)
- Chemomechanical preparation using 2% CHX gel reduced endotoxin levels by 44.4% (Vianna et al. 2007)
- Neither 2.5% NaOCl or 2% CHX gel were effective in eliminating endotoxin from infected root canals (Gomes et al. 2009)
- Case series: comparable 2-4 yr outcome after medication with 2% CHX liquid or $Ca(OH)_2$ (Tervit et al. 2009)
- No difference between 0.12% CHX and 2.5% NaOCl in microbial reduction (Rôças and Siqueira 2011)

***In vitro*:**

- Might allow substantive antimicrobial activity in instrumented root canals (White et al. 1997, Rosenthal et al. 2004)
- Canal dressing for 1 week with 2% CHX may provide residual antimicrobial activity against *E. faecalis* (Basrani et al. 2002)
- Antibacterial activity against *E. faecalis* by chlorhexidine (0.05%) was strongly inhibited by bovine serum albumin and slowed down by dentin (Portenier et al. 2001)
- CHX-killed *E. faecalis* were less potent than heat-killed *E. faecalis* in the production of tumor necrosis factor alpha (TNF-alpha) by a murine macrophage cell line (Lee et al. 2009)
- CHX irrigant less effective than NaOCl (SEM) (Yamashita et al. 2003)
- CHX more effective against *E. faecalis* than was $Ca(OH)_2$ paste or a mixture of CHX with $Ca(OH)_2$ paste *in vitro* (Schafer and Bossman 2005)
- 2% CHX gel more effective than $Ca(OH)_2$ at 400 micron depth in human dentin blocks (Krithikadatta et al. 2007)
- Addition of surface modifiers did not improve bactericidal activity compared to the original formulations (Williamson et al. 2009)
- CHX-Plus showed higher levels of bactericidal activity than 2% CHX against 3wk biofilm at 1, 3 and 10 min exposure times (Shen et al. 2009). Activity of CHX and CHX-Plus enhanced by low intensity ultrasonic or sonic agitation (Shen et al. 2010)
- Bacteria in mature biofilms and nutrient-limited biofilms on hydroxyapatite discs were more resistant to CHX killing than in young biofilms (Shen et al. 2011)
- 2% CHX does not dissolve biofilms (del Carpio-Perochena et al. 2011)

CHX precipitation reactions:

- CHX and NaOCl form a precipitate when combined (Basrani et al. 2007)
- CHX and NaOCl precipitate blocks dentinal tubules (Bui et al. 2008)
- CHX plus EDTA produces a white precipitate by forming a salt with EDTA rather than undergoing a chemical reaction (Rasimick et al. 2008)
- Adding (CaOH)2 to CHX leads to immediate and total degradation of CHX (Barbin et al. 2008)
- PCA (4-chloroaniline) is in NaOCl/CHX precipitate and 2.0% CHX at 45°C (but not 37°C) (Basrani et al. 2009)
- CHX and NaOCl precipitate contains parachloroaniline (Basrani et al. 2010)
- CHX and NaOCl precipitate does NOT contains parachloroaniline (Thompson and Sem 2010)
- CHX and NaOCl precipitate contains two chemical fragments derived from CHX (PCU and PCGH), neither of which are PCA (Nowicki and Sem 2011)

1982	Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM	*In vivo* evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants	J Endod 1982;8:200-4	Clinical study. Comparison between 0.2% CHX and 2.5% NaOCl. Anaerobic culturing of 60 teeth with necrotic pulps and positive cultures at start of treatment, showed that 2.5% NaOCl was more effective than 0.2% CHX. The prevalence of positive cultures and culture reversals over 3 appointments indicated that CHX did not have a prolonged bactericidal effect.
1997	White RR, Hays GL, Janer LR.	Residual antimicrobial activity after canal irrigation with chlorhexidine	J Endod 1997;23:229-31	*In vitro*. Indicated that CHX irrigants (2% and 0.12%) could instill substantive antimicrobial activity in instrumented root canals.
2001	Portenier I, Haapasalo H, Rye A, Waltimo T, Orstavik D, Haapasalo M	Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin	Int Endod J 2001;34:184-8	*In vitro*. Antibacterial activity against *E. faecalis* by CHX (0.05%) was strongly inhibited by bovine serum albumin and slowed down by dentin. Hydroxylapatite had little or no inhibitory effect on CHX.
2002	Basrani B, Santos JM, Tjaderhane L, Grad H, Gorduysus O, Huang J, Lawrence HP, Friedman S	Substantive antimicrobial activity in chlorhexidine-treated human root dentin	OOOOE 2002;94:240-5	*In vitro*. Assessed the substantive antimicrobial activity of different medicaments in human root dentin in 98 instrumented root canals medicated for 7 days with different CHX solutions. After medication, canals were inoculated with *E. faecalis* for 21 days. Canal dressing for 1 week with 2% CHX may provide residual antimicrobial activity against *E. faecalis*.
2003	Yamashita JC, Tanomaru Filho M, Leonardo MR, Rossi MA, Silva LA	Scanning electron microscopic study of the cleaning ability of chlorhexidine as a root-canal irrigant	Int Endod J 2003;36:391-4	*In vitro*. The apical third of the root canals was not cleaned as well as the middle and coronal thirds. Cleaning by CHX and saline was inferior compared to the cleaning by NaOCl with and without EDTA.
2003	Zamany A, Safavi K, Spangberg LS	The effect of chlorhexidine as an endodontic disinfectant	OOOOE 2003;96:578-81	Clinical study. Assessed whether addition of a 2% CHX rinse to a 1% NaOCl irrigation protocol enhanced disinfection of the root canal system *in vivo*. Cultivable

				bacteria were retrieved at the conclusion of the first visit in 1/12 CHX cases whereas in the control group 7/12 cases showed growth.
2004	Rosenthal S, Spangberg L, Safavi K	Chlorhexidine substantivity in root canal dentin	OOOOE 2004;37:105-13	*In vitro*. Reported that CHX is retained in bovine root canal dentin in antimicrobially effective amounts for up to 12 weeks.
2005	Schafer E, Bossmann K	Antimicrobial efficacy of chlorhexidine and two calcium hydroxide formulations against *E. faecalis*	J Endod 2005;31:53-6	*In vitro*. CHX more effective than against three day inoculum of *E. faecalis* than was $Ca(OH)_2$ paste or a mixture of CHX with $Ca(OH)_2$ paste *in vitro* in extracted teeth prepared to size 40.
2007	Siqueira JF, Rôças IN, Paiva SS, Guimarães-Pinto T, Magalhães KM, Lima KC	Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis	OOOOE 2007;104:122-30	Clinical study. 32 teeth. After chemomechanical preparation no significant difference in number of cases with negative cultures using either with 0.12% CHX (8/16) or NaOCl (6/16) as an irrigant
2007	Paquette L, Legner M, Fillery ED, Friedman S	Antibacterial efficacy of chlorhexidine gluconate intracanal medication *in vivo*	J Endod 2007;33:788-95	Clinical study. 22 teeth with apical periodontitis. Used culture and microscopy with Live/Dead staining to assess viability. Instrumented teeth medicated with 2% CHX liquid for 7-15 days. Intracanal CHX did not reduce (1) the proportion of teeth with negative cultures, or (2) the bacterial concentration.
2007	Basrani BR, Manek S, Sodhi RNS, Fillery E, Manzur A	Interaction between sodium hypochlorite and chlorhexidine gluconate	J Endod 2007;33:966-9	*In vitro.* Combined different concentrations of CHX and NaOCl and observed formation of precipitate. Advise removing NaOCl before placing CHX into the canal.
2007	Krithikadatta J, Indira R, Dorothykalyani AL	Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments	J Endod 2007;33:1473-6	*In vitro*. Human dentin blocks contaminated with *E. faecalis* for 21 days. Exposed to disinfectants for up to 5 days. Evaluated viable bacteria at 200 μm and 400 μm depths. Results: % reduction in bacteria 100%, 86.5%, 62.5% and 58.5% with 2% CHX gel, 2% metronidazole, bioactive glass and $Ca(OH)_2$, respectively.
2007	Wang CS, Arnold RR, Trope M, Teixeira FB	Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria	J Endod 2007;33:1283-9	Clinical study. Culture methods. 43 patients with apical periodontitis. Evaluated efficacy of 2% CHX gel on intracanal bacteria reduction during rotary instrumentation and intracanal Ca(OH)2 /2% CHX gel dressing. Concluded that 2% CHX gel is effective disinfectant and additional dressing did not sig improve the bacteria reduction.
2007	Vianna ME, Horz HP, Conrads G,	Effect of root canal procedures on endotoxins and	Oral Microbiol Immunol 2007;22:41	Clinical study. 24 teeth. Chemo-mechanical preparation performed using 2% CHX gel. Used Limulus amoebocyte lysate assay to measure amount of

	Zaia AA, Souza-Filho FJ, Gomes BP	endodontic pathogens	1-8	endotoxin. Endotoxin present in 100% of the initial samples. After chemo-mechanical preparation mean endotoxin reduction of 44.4% was found. After 7 days of intracanal dressing [using eith Ca(OH)2 paste; 2% CHX gel; and Ca(OH)2 + 2% CHX gel], endotoxin concentration decreased by only 1.4%. No difference was found among different intracanal medicaments.
2008	Bui TB, Baumgartner JC, Mitchell JC	Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin	J Endod 2008;34:18 1-5	*In vitro*. Forty-four extracted single-rooted human teeth were instrumented and irrigated with both NaOCl and CHX to produce a precipitate. Root canal surfaces were analyzed with ESEM. Results: NaOCl/CHX precipitate tends to occlude the dentinal tubules.
2008	Rasimick BJ, Nekich M, Hladek MM, Musikant BL, Deutsch AS	Interaction between chlorhexidine digluconate and EDTA	J Endod 2008;34:15 21-3	*In vitro*. CHX plus EDTA produces a white precipitate. Showed that CHX forms a salt with EDTA rather than undergoing a chemical reaction. Parachloroaniline, a potentially carcinogenic decomposition product of chlorhexidine, was not detected in precipitate.
2008	Barbin LE, Saquy PC, Guedes DF, Sousa-Neto MD, Estrela C, Pécora JD	Determination of para-chloroaniline and reactive oxygen species in chlorhexidine and chlorhexidine associated with calcium hydroxide	J Endod 2008;34:15 08-14	*In vitro*. Mass spectrometry. Looked for parachloroaniline (PCA) and reactive oxygen species (ROS) in samples of 0.2% CHX and Ca(OH)2 mixed with 0.2% CHX. Addition of (CaOH)2 to CHX leads to an immediate and total degradation of CHX.
2009	Williamson AE, Cardon JW, Drake DR	Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*	J Endod 2009;35:95 -7	*In vitro*. 1- and 3-min exposures to 6% NaOCl and Chlor-EXTRA (<6% NaOCl with surface modifier) were sig superior to 2% CHX and CHX-Plus. Addition of surface modifiers did not improve bactericidal activity compared to the original formulations
2009	Lee JK, Baik JE, Yun CH, Lee K, Han SH, Lee W, Bae KS, Baek SH, Lee Y, Son WJ, Kum KY	Chlorhexidine gluconate attenuates the ability of lipoteich-oic acid from *Entero-coccus faecalis* to stimulate toll-like receptor 2	J Endod 2009;35:21 2-5	*In vitro.* ELISA. CHX-killed *E. faecalis* was less potent than heat-killed *E. faecalis* in the production of tumor necrosis factor alpha (TNF-alpha) by a murine macrophage cell line (Attenuate – weaken)
2009	Basrani BR, Manek S, Fillery E	Using diazotization to characterize the effect of heat or sodium hypochlorite on 2.0% chlorhexidine	J Endod 2009;35: 1296-9	*In vitro*. Confirmed the presence of an aromatic amine [like PCA, or Para Chloro Aniline (4-chloroaniline)] in NaOCl/CHX precipitate and also in 2.0% CHX at 45°C (but not 37°C).
2009	Tervit C, Paquette L, Torneck CD,	Proportion of healed teeth with apical periodontitis medicated	J Endod 2009;35: 1182-5	Case series. 2-4 yr follow-up on previous study of 22 teeth with apical periodontitis medicated with CHX. Reported comparable outcome after medication with

	Basrani B, Friedman S	with two percent chlorhexidine gluconate liquid: a case-series study		2% CHX liquid and calcium hydroxide.
2009	Gomes BP, Martinho FC, Vianna ME	Comparison of 2.5% sodium hypochlorite and 2% chlorhexidine gel on oral bacterial lipopolysaccharide reduction from primarily infected root canals	J Endod 2009;35: 1350-3	Clinical study. Used Limulus amebocyte lysate assay. Neither 2.5% NaOCl or 2% CHX gel were effective in eliminating endotoxin from infected root canals.
2009	Shen Y, Qian W, Chung C, Olsen I, Haapasalo M	Evaluation of the effect of two chlorhexidine preparations on biofilm bacteria in vitro: a three-dimensional quantitative analysis	J Endod. 2009 Jul;35(7):981-5	*In vitro*. CHX-Plus showed higher levels of bactericidal activity than 2% CHX against 3wk biofilm at 1, 3 and 10 min exposure times ($p < 0.001$)
2010	Basrani BR, Manek S, Mathers D, Fillery E, Sodhi RN	Determination of 4-chloroaniline and its derivatives formed in the interaction of sodium hypochlorite and chlorhexidine by using gas chromatography	J Endod 2010;36: 312-4	*In vitro.* Aim: to identify the NaOCl/CHX precipitate using gas chromatography-mass spectrometry. The results showed an absence of other aniline derivatives in the precipitate. Only PCA was found. Recommended: "it would appear prudent to minimize its formation by avoiding the use of CHX together with NaOCl."
2010	Thomas JE, Sem DS	An *in vitro* spectroscopic analysis to determine whether para-chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine	J Endod 2010;36: 315-7	*In vitro.* Aim: determine whether para-chloroaniline (PCA) is formed through the reaction of mixing NaOCl and CHX. Used nuclear magnetic resonance spectroscopy. No PCA was found.
2010	Shen Y, Stojicic S, Qian W, Olsen I, Haapasalo M	The synergistic antimicrobial effect by mechanical agitation and two chlorhexidine preparations on biofilm bacteria	J Endod. 2010 Jan;36(1):100-4	*In vitro.* Low-intensity ultrasonic or sonic agitation improves the action of CHX and CHX-Plus against biofilm bacteria (even in the absence of disruption of the biofilm).
2011	Rôças IN, Siqueira JF Jr	Comparison of the *in vivo* antimicrobial effectiveness of NaOCl and CHX used as root canal irrigants: a molecular microbiology study	J Endod. 2011 Feb;37(2): 143-50	Clinical study. 47 teeth with necrotic canals and asymptomatic apical periodontitis. Used PCR/reverse-capture checkerboard assay to analyze samples. Compared 0.12% CHX and 2.5% NaOCl as irrigants. Both reduced taxa and levels of microorganisms. No difference between CHX and NaOCl.
2011	Shen Y, Stojicic S,	Antimicrobial efficacy of chlorhexidine	J Endod. 2011	*In vitro*. Bacteria in mature biofilms and nutrient-limited biofilms on hydroxyapatite discs were more

	Haapasalo M	against bacteria in biofilms at different stages of development	May;37(5): 657-61	resistant to CHX killing than in young biofilms.
2011	Nowicki JB, Sem DS	An *in vitro* spectroscopic analysis to determine the chemical composition of the precipitate formed by mixing sodium hypochlorite and chlorhexidine	J Endod. 2011 Jul;37(7):983-8	*In vitro.* One-dimensional and 2-dimensional nuclear magnetic resonance spectroscopy: comparison with a standard sample. Breakdown products appear to be parachlorophenylurea (PCU) and parachloro-phenyl-guanidyl-1,6-diguanidyl-hexane (PCGH). Conclusions: the precipitate contains two chemical fragments derived from CHX (PCU and PCGH), neither of which are PCA.
2011	del Carpio-Perochena AE, Bramante CM, Duarte MA et al.	Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin	J Endod. 2011 Aug;37(8): 1134-8	*In vivo/in vitro*. 120 bovine dentin specimens were infected intraorally using a removable orthodontic device. Biofilms were exposed to 2% CHX, or 1%, 2.5%, and 5.25% NaOCl. 2% CHX does not dissolve biofilms.

8.3 EDTA

***In vitro*:**

- Alternate irrigation with NaOCl and EDTA rather than using EDTA all at once as a final rinse after NaOCl enhanced biofilm removal (Soares et al. 2010)
- EDTA in combination with NaOCl sig reduces the amount of intracanal biofilm (Ozdemir et al. 2010)
- EDTA affected the membrane integrity in all organisms but failed to remove more than a few cells in biofilms of *E. faecalis, L. paracasei,* and *S. anginosus* (Chavez de Paz et al. 2010)

Non-endo literature:

- EDTA can act as an antibiofilm acid for limiting *S. aureus* biofilm attachment by decreasing iron availability (Al-Azemi et al. 2011)
- "The ability of EDTA to chelate and potentiate the cell walls of bacteria and destabilize biofilms by sequestering calcium, magnesium, zinc, and iron makes it a suitable agent for use in the management of biofilms" (Finnegan and Percival 2015, review of wound care)

2010	Soares JA, Roque de Carvalho MA, Cunha Santos SM et al.	Effectiveness of chemomechanical preparation with alternating use of sodium hypochlorite and EDTA in eliminating intracanal *Enterococcus faecalis* biofilm	J Endod 2010;36:894-8.	*In vitro*. Alternate irrigation with NaOCl and EDTA rather than using EDTA all at once as a final rinse after NaOCl enhanced *E. faecalis* biofilm removal.
2010	Ozdemir HO, Buzoglu HD, Calt S,	Effect of EDTA and NaOCl irrigation on *E. faecalis* biofilm colon-	J Endod 2010 May;36:842-6	*In vitro*. Compared *E. faecalis* biofilm formation on dentin of extracted teeth from young (<30yr) and old (>60yr) pts. Measured viable counts and histology

	Stabholz A, Steinberg D	ization in young and old human root canal dentin: *in vitro* study		(SEM and CLSEM). Conclusions: (1) Viable counts in "old" group were higher, (2) EDTA and NaOCl sig reduces the amount of intracanal biofilm.
2010	Chavez de Paz LE, Bergenholtz G, Svensater G	The effects of antimicrobials on endodontic biofilm bacteria	J Endod 2010;36:70-7.	*In vitro*. 24hr biofilms exposed for 5 min to 1% NaOCl, 2.5% CHX, and EDTA (50 mmol/L). NaOCl affected membrane integrity (MI) of all organisms and removed most biofilm cells. EDTA affected MI in all organisms but failed to remove more than a few cells in biofilms of *E. faecalis, L. paracasei,* and *S. anginosus*. CHX had mild effect on MI of *E. faecalis*.
2011	Al-Azemi A, Fielder MD, Abuknesha RA, Price RG	Effects of chelating agent and environmental stresses on microbial biofilms: relevance to clinical microbiology	J Appl Microbiol 2011;110:1307-13	*In vitro*. Significant inhibition of *Staphylococcus aureus* biofilm formation was observed in the presence of EDTA.
2015	Finnegan S, Percival S	EDTA: An Antimicrobial and Antibiofilm Agent for Use in Wound Care	Adv Wound Care 2015;4:415-21.	Review: "The ability of EDTA to chelate and potentiate the cell walls of bacteria and destabilize biofilms by sequestering calcium, magnesium, zinc, and iron makes it a suitable agent for use in the management of biofilms"

8.4 Smear layer

- The smear layer is a layer of organic and inorganic material produced by instrumentation (Violich and Chandler 2010). Chelators and acids can remove the inorganic component of the smear layer.
- Presence of smear layer delayed antimicrobial effects of medications (Orstavik and Haapasalo 1990)
- Removal of the smear layer enhanced sealability (Behrend et al. 1996)

1990	Orstavik D, Haapasalo M	Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules	Endod Dent Traumatol 1990;6:142-9	*In vitro*. *E. faecalis* and *S. sanguis* grew 300-400um into slabs of bovine dentinal tubules after 14-21 days. Presence of a smear layer delayed, but did not prevent, antimicrobial effects of medications
1996	Behrend GD, Cutler CW, Gutmann JL	An *in vitro* study of smear layer removal and microbial leakage along root-canal fillings	Int Endod J 1996;29:99-107	*In vitro*. Removal of the smear layer resulted in increased resistance to bacterial penetration
2010	Violich DR, Chandler NP	The smear layer in endodontics - a review	Int Endod J 2010 Jan;43:2-15	Review. The smear layer is a layer of organic and inorganic material produced by instrumentation. Can prevent penetration of intracanal medicaments into dentinal tubules and influence adaptation of filling materials to canal walls. Data needed to determine role of smear layer in outcomes of root canal treatment.

MTAD

- "Biopure MTAD" (Dentsply) developed for use as antimicrobial agent and to remove smear layer. A mixture of a tetracycline isomer, citric acid, and a detergent (Torebinejad et al. 2003)
- Developers report MTAD has more favorable properties than conventional irrigants NaOCl and EDTA (Torebinejad et al. 2003, Shabahang and Torebinejad 2003, and other studies)
- The presence of dentine or bovine serum albumin caused a marked delay in killing two *E. faecalis* strains by MTAD and 0.2% chlorhexidine (Portenier et al. 2006)
- 6% NaOCl and 2% CHX were equally effective and statistically significantly superior to BioPure MTAD and 17% EDTA ($p < 0.05$) in antifungal activity (Ruff et al. 2006)
- Potential for iatrogenic tetracycline staining with MTAD? (Tay *et al*. 2006)
- 1.3% NaOCl/BioPure MTAD left nearly 50% of canals contaminated with *E. faecalis*. 5.25% NaOCl/15% EDTA consistently disinfected all canals (Baumgartner et al. 2007)
- In bovine teeth, NaOCl and doxycycline were more effective than control in killing *E. faecalis* at the shallow bur depth, but at the deeper bur depth only NaOCl was superior (Krause et al. 2007)
- Tetracycline-resistant strains in infected root canals may survive irrigation with solutions containing tetracycline (Rossi-Fedele and Roberts 2007)
- Antimicrobial efficacy reported using 8 strains of *E. faecalis* (Newberry et al. 2007)
- Clinical study - final rinse with MTAD and medication with CHX did not reduce bacterial counts beyond levels achieved by canal preparation with NaOCl (Malkhassian et al. 2009)
- Review of MTAD in endodontics (Singla et al. 2011)
- MTADN: The addition of nisin (an antibacterial peptide effective against Gram-positive bacteria) to MTAD improved antibacterial efficacy against some gram-positive bacteria associated with persistent intracanal infection (Tong et al. 2012).

2003	Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S	A new solution for the removal of the smear layer	J Endod 2003;29:170-5	*In vitro*. Reported that a tetracycline isomer, an acid, and a detergent (MTAD) used as a final rinse on the surface of instrumented root canals was effective in removing the smear layer
2003	Shabahang S, Torabinejad M	Effect of MTAD on *Enterococcus faecalis*-contaminated root canals of extracted human teeth	J Endod 2003;29:576-9	*In vitro*. Combination of 1.3% NaOCl as irrigant and MTAD as final rinse was sig more effective against *E. faecalis* than 5-min application of MTAD, 1.3% NaOCl, 5.25% NaOCl or 1-min application of EDTA then irrigation with 5 ml of 1.3% NaOCl or 5.25% NaOCl
2006	Portenier I, Waltimo T, Orstavik D, Haapasalo M	Killing of *Enterococc-us faecalis* by MTAD and chlorhexidine di-gluconate with or with-out cetrimide in the presence or absence of dentine powder or BSA	J Endod 2006;32:138-41	*In vitro*. The presence of dentine or bovine serum albumin caused a marked delay in killing two *E. faecalis* strains by MTAD and 0.2% chlorhexidine
2006	Ruff ML, McClanahan SB, Babel BS	*In vitro* antifungal efficacy of four irrigants as a final rinse	J Endod 2006;32:331-3	*In vitro*. Extracted teeth. 6% NaOCl and 2% CHX equally effective and significantly superior to BioPure MTAD and 17% EDTA ($p < 0.05$) in antifungal activity

2006	Tay FR, Mazzoni A, Pashley DH, Day TE, Ngoh EC, Breschi L	Potential iatrogenic tetracycline staining of endodontically treated teeth via NaOCl/ MTAD irrigation: a preliminary report	J Endod 2006;32:354-8	*In vitro*. Suggested rinsing NaOCl-treated dentin with ascorbic acid, a reducing agent, before the application of MTAD to avoid photo-oxidative degradation of tetracycline in MTAD that can result in staining of dentin
2007	Baumgartner JC, Johal S, Marshall JG	Comparison of the antimicrobial efficacy of 1.3% NaOCl/Bio-Pure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation	J Endod 2007;33:48-51	*In vitro*. Showed consistent disinfection of infected human root canals with 5.25% NaOCl/15% EDTA. In contrast, the combination of 1.3% NaOCl/BioPure MTAD left nearly 50% of the canals contaminated with *E. faecalis*
2007	Krause TA, Liewehr FR, Hahn CL	The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on *Enterococcus faecalis*	J Endod 2007;33:28-30	*In vitro*. Used two experimental models – bovine teeth and agar diffusion. In tooth model, NaOCl and doxy-cycline were more effective than control in killing *E. faecalis* at shallow bur depth, but at deeper bur depth NaOCl was superior. In agar diffusion model, NaOCl produced less inhibition than MTAD or doxycycline
2007	Rossi-Fedele G, Roberts AP	A preliminary study investigating the survival of tetracycline resistant *Enterococcus faecalis* after root canal irrigation with high concentrations of tetracycline	Int Endod J 2007;40;772-7	*In vitro*. Compared two *E. faecalis* strains [one tetracycline (tet) sensitive and one tet-resistant] survival to exposure to a tet-containing irrigation solution in bovine root canals. The tet-containing solution prevented growth of sensitive *E. faecalis*, but resistant strain was able to survive a 5 min exposure at 30 mg mL(-1). Implications: Tetracycline-resistant strains in infected root canals may survive irrigation with solutions containing tetracycline.
2007	Newberry BM, Shabahang S, Johnson N, Ap RM, Torabinej	The antimicrobial effect of biopure MTAD on eight strains of *Enterococcus faecalis*: an in vitro investigation	J Endod 2007;33:1352-4	*In vitro*. Root canals contaminated with *E. faecalis* (8 strains tested) were irrigated with 1.3% NaOCl for 15 minutes, and rinsed with 4-5 mL MTAD. Roots were immersed in MTAD for 5 min then vortexed in BHI to remove excess MTAD. After 1 week incubation in BHI no broth turbidity was observed. Growth in dentin shavings from one sample was observed.
2009	Malkhassian G, Manzur AJ, Legner M, Fillery ED, Manek S, Basrani BR, Friedman S	Antibacterial efficacy of MTAD final rinse and two percent chlorhexidine gel medication in teeth with apical periodontitis: a randomized double-blinded clinical trial	J Endod 2009;35:1483-90	Clinical study. Randomized double-blinded trial. Obtained samples before and after canal preparation with NaOCl, after final rinse, after CHX was flushed, and after final irrigation. Enumerated bacteria. Canal preparation alone reduced bacterial counts by 95%. Concluded that final rinse with MTAD and medication with CHX did not reduce bacterial counts beyond levels achieved by canal preparation with NaOCl.
2011	Singla MG, Garg A, Gupta S	MTAD in endodontics: an update review	OOOOE 2011;112:e70-6	Review.
2012	Tong Z, Zhou L, Kuang R,	In vitro evaluation of MTAD and nisin in	J Endod 2012;38:490	*In vitro*. Nisin is an antibacterial peptide effective against Gram-positive bacteria. Investigated MTAD in

	Lv H, Qu T, Ni L	combination against common pathogens associated with root canal infection	-4	combination with nisin. Concluded that addition of nisin improved the antibacterial efficacy of MTAD against pathogens, especially for some gram-positive bacteria associated with persistent intracanal infection.

QMix

- QMix contains EDTA, CHX and a surfactant. Developed for final rinse after NaOCl to remove smear layer and bacteria. "QMix and NaOCl were superior to CHX and MTAD under laboratory conditions in killing *E. faecalis* and plaque bacteria in planktonic and biofilm culture."Ability to remove smear layer by QMix was comparable to EDTA." (Stojicic et al. 2012)
- QMix was equally effective as 6% NaOCl in killing *E. faecalis* in dentin (Ma et al. 2011)

2011	Ma J, Wang Z, Shen Y, Haapasalo M	A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy	J Endod. 2011 Oct;37(10): 1380-5	*In vitro*. New infection model: dentinal tubules are infected with *E. faecalis* by centrifugation of the suspension into tubules. Samples are then exposed to test antimicrobials and evaluated using LIVE/DEAD stains and CLSM. Demonstrated that QMix (EDTA, CHX and a surfactant) was equally effective in killing bacteria in dentin as 6% NaOCl.
2012	Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M	Antibacterial and smear layer removal ability of a novel irrigant, QMix	Int Endod J 2012;45:363 -71	*In vitro*. Developed QMix which contains EDTA, CHX and a surfactant. For final rinse after NaOCl to remove smear layer and bacteria. Reported that "QMix and NaOCl were superior to CHX and MTAD under laboratory conditions in killing *E. faecalis* and plaque bacteria in planktonic and biofilm culture. Ability to remove smear layer by QMix comparable to EDTA."

8.5 Calcium hydroxide

Does calcium hydroxide affect endodontic microflora?

- **Systematic reviews**
  "Calcium hydroxide remains the best medicament available to reduce residual microbial flora further" (Law and Messer 2004)
  "Calcium hydroxide has limited effectiveness in eliminating bacteria from human root canal when assessed by culture techniques" (Sathorn et al. 2007)
- **Clinical studies**
  - 7 day intracanal application $Ca(OH)_2$ was effective in eliminating bacteria. 10 minute application ineffective (Sjögren et al. 1991)
  - After 1 week medication with $Ca(OH)_2$ 14/22 canals showed no growth (Orstavik et al. 1991)
  - Root canals dressed with $Ca(OH)_2$ for 1 wk showed significant reduction of bacterial growth (Yared and Bou Dagher 1994)

- After instrumentation with NaOCl irrigation, 61.9% of canals were rendered bacteria-free. Intracanal $Ca(OH)_2$ for at least 1 wk rendered 92.5% of the canals bacteria free (Shuping et al. 2000)
- $Ca(OH)_2$ and sterile saline slurry limits but does not totally prevent regrowth of endodontic bacteria following 4 weeks medication (Peters et al. 2002)
- No difference in efficacy between $Ca(OH)_2$ mixed in aqueous 2% CHX versus aqueous $Ca(OH)_2$ slurry alone in the disinfection of the pulp space (Zerella et al. 2005)
- After one week, the antibacterial efficacy of either $Ca(OH)_2$, 2% CHX, or a combination of both $Ca(OH)_2$ /CHX was comparable (Manzur et al. 2007)
- One-week intracanal dressing with $Ca(OH)_2$/CPMC paste reduced culture-positive cases (Siqueira et al. 2007)
- One-week intracanal dressing with $Ca(OH)_2$/glycerin and $Ca(OH)_2$/ camphorated paramonochlorophenol/glycerin reduced culture-positive cases with no difference between groups (Rôças and Siqueira 2011)
- Intracanal dressings were not efficient at reducing bacterial load, but the 14-day intracanal dressing with $Ca(OH)_2$ paste was significantly more effective than 2% CHX gel, particularly in cases with apical periodontitis (Teles et al. 2014)

- ***In vitro:***
  - $Ca(OH)_2$ hydrolyzes the lipid moiety of bacterial LPS (Safavi and Nichols 1993) and may alter the biological properties of LPS (Safavi and Nichols 1994).
  - $Ca(OH)_2$ effective in killing *Actinomyces israelii* (Barnard et al. 1996)
  - Longer term (5 days versus 1 day) $Ca(OH)_2$ detoxified LPS molecules by hydrolysis of ester bonds in the fatty acid chains of the lipid A moiety (Buck et al. 2001)
  - Calcium hydroxide inactivates lipoteichoic acid from *E. faecalis* through deacylation of the lipid moiety (Baik et al. 2011)
  - $Ca(OH)_2$ and polymyxin B detoxified endotoxin in root canals and altered properties of LPS to stimulate the antibody production by B-lymphocytes. Sodium hypochlorite and chlorhexidine did not detoxify endotoxin (de Oliveira et al. 2007)
  - 10 day application of $Ca(OH)_2$ needed to disinfect dentinal tubules (Orstavik and Haapasalo 1990)
  - Anaerobic Gram-negative bacteria are more susceptible to $Ca(OH)_2$ pastes than facultative Gram-positive microorganisms (Gomes et al. 2002)
  - $Ca(OH)_2$ inactivated by dentin, hydroxyapatite and serum (Portenier et al. 2001, Haapasalo et al. 2007 review)
  - $Ca(OH)_2$ treated roots contained significantly fewer viable bacteria than did untreated roots (Parmar et al. 2011)

1990	Orstavik D, Haapasalo M	Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules	Endod Dent Traumatol 1990;6:142-9	Used *in vitro* dentin model to test speed and depth of infection by different bacteria, and their susceptibility to Calasept, CMCP, IKI, chlorhexidine, 5.25%NaOC1, and 17% EDTA. Calasept required up to 10d to disinfect the canals. IKI more effective than NaOCl
1991	Sjögren U, Figdor D, Spangberg L,	The antimicrobial effect of calcium hydroxide as a short-	Int J Endod 1991;24:119-25	Clinical study. Showed that $Ca(OH)_2$ was effective in eliminating bacteria with a 7 day application but not effective with a 10 minute application in the canal

	Sundqvist G	term intracanal dressing		system.
1991	Orstavik D, Kerekes K, Molven O	Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study	Int J Endod 1991;24:1-7	Clinical study. Showed that $Ca(OH)_2$ was effective in eliminating bacteria with a 7 day application in 14/22 cases.
1993	Safavi KE, Nichols FC	Effect of calcium hydroxide on bacterial lipopolysaccharide	J Endod. 1993 Feb;19(2): 76-8	*In vitro*. $Ca(OH)_2$ hydrolyzes the lipid moiety of bacterial LPS, resulting in the release of free hydroxy fatty acids
1994	Safavi KE, Nichols FC	Alteration of biological properties of bacterial lipopolysaccharide by calcium hydroxide treatment	J Endod. 1994 Mar;20(3): 127-9	*In vitro*. Prostaglandin E2 was identified in supernatants of LPS-stimulated monocytes but not in those stimulated with $Ca(OH)_2$-treated LPS. Treatment with $Ca(OH)_2$ may alter the biological properties of LPS.
1994	Yared GM, Bou Dagher FE	Influence of apical enlargement on bacterial infection during treatment of apical periodontitis	J Endod 1994;20;535-7	Clinical study showed that 41 of 60 root canals dressed with $Ca(OH)_2$ for 1 wk showed no bacterial growth
1996	Barnard D, Davies J, Figdor D	Susceptibility of *Actinomyces israelii* to antibiotics, sodium hypochlorite and calcium hydroxide	Int Endod J 1996;29:320-6	*In vitro*. Both sodium hypochlorite solution and calcium hydroxide were found to be highly effective in killing *A. israelii*
2000	Shuping GB, Orstavik D, Sigurdsson A, Trope M	Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications	J Endod 2000;26:751-5	Clinical study. Evaluated bacterial reduction with NiTi rotary instrumentation and 1.25% NaOCl irrigation using 42 subjects with chronic apical periodontitis. After instrumentation with NaOCl irrigation, 61.9% of canals were rendered bacteria-free. The placement of $Ca(OH)_2$ for at least 1 wk rendered 92.5% of the canals bacteria free.
2001	Buck RA, Cai J, Eleazer PD, Staat RH, Hurst HE	Detoxification of endotoxin by endodontic irrigants and calcium hydroxide	J Endod 2001;27:325-7	*In vitro* study. Examined the effects of endodontic irrigants and $Ca(OH)_2$ on LPS using mass spectrometry/gas chromatography. Aqueous solutions of LPS mixed with an endo irrigant for 30 min and with $Ca(OH)_2$ for 1, 2, or 5 days. Water, EDTA, ethanol, 0.12% CHX, CHX + NaOCl, and NaOCl alone showed little breakdown of LPS. Longer term (5 vs 1day) $Ca(OH)_2$ detoxified LPS molecules by hydrolysis of ester bonds in the fatty acid chains of the lipid A moiety.
2001	Portenier I, Haapasalo H, Rye A, Waltimo T,	Inactivation of root canal medicaments by dentine,	Int Endod J 2001;34:184-8	*In vitro*. Antibacterial activity against *E. faecalis* by $Ca(OH)_2$ was totally inactivated by the presence of 28 mg of dentin powder, hydroxylapatite or bovine serum

	Orstavik D, Haapasalo M	hydroxylapatite and bovine serum albumin		albumin
2002	Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR	Effects of instrument-ation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions	Int Endod J 2002;35:13-21	Clinical study. Evaluated the fate of microorganisms in root canals of teeth with infected pulps and periapical bone lesions with and without the use of $Ca(OH)_2$ medication for 4 weeks. Concluded that a $Ca(OH)_2$ and sterile saline slurry limits but does not totally prevent regrowth of endodontic bacteria.
2002	Gomes BP, Ferraz CC, Garrido FD, Rosalen PL, Zaia AA, Tei-xeira FB, de Souza-Filho F	Microbial susceptibility to calcium hydroxide pastes and their vehicles	J Endod 2002;28:758-61	Anaerobic Gram-negative bacteria are more susceptible to $Ca(OH)_2$ pastes than facultative Gram-positive microorganisms.
2004	Law A, Messer H	An evidence-based analysis of the antibacterial effectiveness of intracanal medicaments	J Endod 2004;30:689-94	Evidence-based review evaluating the antibacterial effectiveness of intracanal medicaments used in the management of apical periodontitis. Concluded that the main component of antibacterial action appears to be associated with instrumentation and irrigation, although canals cannot be reliably rendered bacteria free. $Ca(OH)_2$ remains the best medicament available to reduce residual microbial flora further.
2005	Zerella JA, Fouad AF, Spangberg LS	Effectiveness of a calcium hydroxide and chlorhexidine digluconate mixture as disinfectant during retreatment of failed endodontic cases	OOOORE 2005;100:756-61	Clinical study. No difference in efficacy between a slurry of $Ca(OH)_2$ mixed in aqueous 2% chlorhexidine (CHX) versus aqueous $Ca(OH)_2$ slurry alone in the disinfection of the pulp space of 40 failed root-filled teeth during endodontic retreatment.
2007	Haapasalo M, Qian W, Portenier I, Waltimo T	Effects of dentin on the antimicrobial properties of endodontic medicaments	J Endod 2007;33:917-25	Review
2007	Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S	Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a random-ized clinical trial	J Endod 2007;33:114-8	Clinical study. Sampled 33 teeth with chronic apical periodontitis before and after instrumentation and after 1 week dressing with either (1) $Ca(OH)_2$, (2) 2% CHX, or (3) a combination of both $Ca(OH)_2$/CHX. Reported that the antibacterial efficacy of the three regimens was comparable.
2007	Siqueira JF, Magalhães KM, Rôças IN	Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/ camphorated	J Endod 2007;33:667-72	Clinical study. 11 teeth. Chemomechanical preparation with 2.5% NaOCl as an irrigant reduced the number of bacteria in the canal but failed to render the canal free of cultivable bacteria in more than one-half of the cases.

		para-monochlorophenol paste as an intracanal dressing		A 7-day intracanal dressing with $Ca(OH)_2$/CPMC paste increased the number of culture-negative cases
2007	Sathorn C, Parashos P, Messer H	Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis	Int Endod J 2007;40:2-10	Systematic review. Eight studies qualified for analyses. Six studies reported a statistically significant reduction in bacteria after medication and two studies did not. Meta analysis on the combined data did not show a difference. Authors concluded that "Calcium hydroxide has limited effectiveness in eliminating bacteria from human root canal when assessed by culture techniques".
2007	de Oliveira LD, Jorge AO, Carvalho CA, Koga-Ito CY, Valera MC	*In vitro* effects of endodontic irrigants on endotoxins in root canals	OOOOE 2007;104:135-42	*In vitro*. *Escherichia coli* endotoxin was inoculated into 84 root canals. $Ca(OH)_2$ and polymyxin B detoxified endotoxin in root canals and altered properties of LPS to stimulate the antibody production by B-lymphocytes. NaOCl and CHX did not detoxify endotoxin
2011	Parmar D, Hauman CH, Leichter JW, McNaughton A, Tompkins GR	Bacterial localization and viability assessment in human ex vivo dentinal tubules by fluorescence confocal laser scanning microscopy	Int Endod J. 2011 Jul;44(7):644-51	*Ex vivo*. Obtained root slices from 12 teeth infected with *E. faecalis*, and bacterial evaluated viability using live/dead stains and CLSM. Percentage survival of bacteria was sig less in teeth (n=6) treated with $Ca(OH)_2$ (29%-50%) compared to untreated control teeth (n=6) (83%-96%).
2011	Rôças IN, Siqueira JF Jr	*In vivo* antimicrobial effects of endodontic treatment procedures as assessed by molecular microbiologic techniques	J Endod. 2011 Mar;37(3):304-10	Clinical study using 24 teeth with necrotic canals and apical periodontitis. Used PCR/reverse-capture checkerboard assay to analyze samples. Compared $Ca(OH)_2$ in either glycerin or in camphorated paramonochlorophenol/glycerin as one week interappointment medications. Both reduced taxa and levels of microorganisms. No difference between groups.
2014	Teles AM, Manso MC, Loureiro S, Silva R, Madeira IG, Pina C et al.	Effectiveness of two intracanal dressings in adult Portuguese patients: a qPCR and anaerobic culture assessment	Int Endod J 2014;47:32-40	Clinical study. Used RT-qPCR and viable counts to compare effectiveness of CHX and $Ca(OH)_2$. Concluded that intracanal dressings were not efficient at reducing bacterial load, but the 14-day intracanal dressing with $Ca(OH)_2$ performed significantly better than CHX, particularly in cases with apical periodontitis

8.6 Antibiotics

- See AAE Colleagues for Excellence Newsletters:
  - Summer 2006 "Antibiotics and the treatment of endodontic infections"
  - Winter 2012 "Use and Abuse of Antibiotics"
- Use of antibiotics in endodontics should be highly limited and restricted to a few selected cases

- Historical – intracanal penicillin and polyantibiotic paste used until allergy/resistance issues raised (Grossman 1945, 1946, 1952)
- Prophylactic antibiotics do not prevent flare-ups (Walton and Chiappinelli 1992, 1993, Nagle et al. 2000, Henry et al. 2001, Lindeboom et al. 2005, Keenan et al. 2006)
- Triple antibiotic paste (metronidazole, ciprofloxacin, and minocycline) was effective as intracanal medicament in immature dog teeth (Windley et al. 2005) and has been used for regenerative endodontic procedures - see Regendo Literature.
- For review of local application of antibiotics, see Mohammadi and Abbott 2009

1945	Grossman LI	Treatment of pulpless teeth with a concentrated sulfonamide solution	JADA 1945;32:1432-6	Historical – intracanal sulfonamide
1946	Grossman LI	Penicillin treatment of pulpless teeth	J Endod 1946;1:30-2	Historical – intracanal penicillin
1952	Grossman LI	Polyantibiotic treatment of pulpless teeth	JADA 1951;43:265-78	Historical – intracanal polyantibiotic paste
1992	Walton RE, Chiappinelli J	Prophylactic penicillin: effect on posttreatment symptoms following root canal treatment of asymptomatic periapical pathosis	J Endod 1993;19:466-70	Clinical study. The use of prophylactic penicillin did not affect the incidence of post-treatment flare-ups in cases of pulpal necrosis and asymptomatic periapical lesions
1993	Walton RE, Chiappinelli J	Prophylactic penicillin: effect on posttreatment symptoms following root canal treatment of asymptomatic periapical pathosis	J Endod 1993;19:466-70	Clinical study. The use of prophylactic penicillin did not affect the incidence of post-treatment flare-ups in cases of pulpal necrosis and asymptomatic periapical lesions
2000	Nagle D, Reader A, Beck M, Weaver J	Effect of systemic penicillin on pain in untreated irreversible pulpitis	OOOOE 2000;90:636-40	Clinical study. Prospective, randomized, double-blind study. Penicillin 500mg qid for 7 days did not relieve painless pulpitis. Penicillin should not be prescribed for untreated irreversible pulpitis. [*This study was identified in a Cochrane Systemic Review by Keenan* et al. *(2006) as providing a high level of evidence*]
2001	Henry M, Reader A, Beck M	Effect of penicillin on postoperative endodontic pain and swelling in symptomatic necrotic teeth	J Endod 2001;27:117-23	Clinical study. Penicillin given postoperatively did not reduce pain, percussion pain, swelling, or the number of analgesic medications taken for symptomatic necrotic teeth with periapical radiolucencies
2005	Lindeboom JA, Frenken JW, Valkenburg P,	The role of preoperative prophylactic antibiotic administration in periapical endodontic surgery: a randomized,	Int Endod J 2005;38:877-81	Clinical study. No statistically significant difference was found between clindamycin prophylaxis and placebo with regard to the prevention of postoperative infection in endodontic surgical procedures.

	van den Akker HP	prospective double-blind placebo-controlled study		
2005	Windley W 3rd, Teixeira F, Levin L, Sigurdsson A, Trope M	Disinfection of immature teeth with a triple antibiotic paste	J Endod 2005;31:439-43	*In vivo*. Dog study. Triple antibiotic paste (metronidazole, ciprofloxacin, and minocycline) was effective in the disinfection of immature teeth with apical periodontitis.
2006	Keenan JV, Farman AG, Fedorowicz Z, Newton JT	A Cochrane systematic review finds no evidence to support the use of antibiotics for pain relief in irreversible pulpitis	J Endod 2006;32:87-92	Cochrane Systematic Review promotes evidence-based outcomes studies using several databases to identify randomized controlled trials for inclusion. Here, clinical outcome, expressed in terms of pain relief, was examined. Cited the paper by Nagle et al. (2000) as providing a high level of evidence that there is no significant difference in pain relief for patients with untreated irreversible pulpitis who received antibiotics versus those who did not.
2009	Mohammadi Z, Abbott PV	On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology	Int Endod J 2009;42:555-67	Review of literature 1981-2008.

Are multiply resistant endodontic microorganisms a clinical problem? Not so far……

(Dahlén et al. 2000, Khemaleelakul et al. 2002, Baumgartner and Xia 2003, Sedgley et al. 2005, Jacinto et al. 2006, LeCorn et al. 2007, Skucaite et al. 2010)

- Penicillin resistant genes more prevalent in primary than in persistent infections (Jungermann et al. 2011)
- Treatment using 2.5% NaOCl irrigation reduced all except tetracycline and erythromycin resistance genes (Rôças and Siqueira 2013)
- In 47 strains from infected root canals, found some resistance to tetracycline, fosfomycin, doxycycline, rifampicin and vancomycin (lactobacilli only), but not to pencillin, clindamycin, amoxycillin, gentamicin, moxifloxacin, metronidazole (Al-Ahmad et al. 2014)

2000	Dahlén G, Samuelsson W, Molander A, Reit C	Identification and antimicrobial susceptibility of enterococci isolated from the root canal	Oral Microbiol Immunol 2000;15:309-12	Clinical study. 29 isolates. Some resistant to benzylpenicillin, ampicillin, clindamycin, metronidazole, and tetracycline, but all sensitive to erythromycin and vancomycin.
2002	Khemaleelakul S, Baumgartner	Identification of bacteria in acute endodontic infections	OOOOE 2002;94:746-55	Clinical study. Studied needle aspirates from 17 patients with acute endodontic abscesses/cellulites in Thailand. 127 strains isolated by culture – with most frequent

	JC, Pruksakorn S	and their antimicrobial susceptibility		genera *Prevotella* and *Streptococcus*. Penicillin V was effective against majority of isolates.
2003	Baumgartner JC, Xia T	Antibiotic susceptibility of bacteria associated with endodontic abscesses	J Endod 2003;29:44-7	Clinical study. 98 isolates from needle aspirates. Did not identify species, but 85% of strains susceptible to Pen V, 91% to amoxicillin, 100% to amoxicillin and clavulanic acid, 96% to clindamycin, 45% to metronidazole alone (but 93% if combined with Pen V and 99% with amoxicillin)
2005	Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, Dahlén G	Virulence, phenotype and genotype characteristics of endodontic *Enterococcus* spp.	Oral Microbiol Immunol 2005;20:10-9	Clinical study. Tested the susceptibility to different antibiotics of 33 *Enterococcus* isolates from root canals. All were susceptible to ampicillin, benzylpenicillin, chloramphenicol, erythromycin, fusidic acid, kanamycin, rifampin, streptomycin and vancomycin. One strain (*E. faecium*) was resistant to gentamicin. Five strains were resistant to tetracycline. NB. Enterococci are intrinsically resistant to clindamycin
2006	Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ	Incidence and antimicrobial susceptibility of *Porphyromonas gingivalis* isolated from mixed endodontic infections	Int Endod J 2006;39:62-70	Clinical study. Tested the susceptibility to different antibiotics of 20 *P. gingivalis* isolates from root canals. All were susceptible to amoxicillin, amoxicillin + clavulanate, cephaclor, clindamycin, benzylpenicillin, metronidazole and tetracycline. One strain was resistant to erythromycin and eight strains were resistant to azythromycin
2007	LeCorn DW, Vertucci FJ, Rojas MF, Progulske-Fox A, Bélanger M	*In vitro* activity of amoxicillin, clinda-mycin, doxycycline, metronidazole, and moxifloxacin against oral *Actinomyces*	J Endod 2007;33:557-60	All antibiotics tested except metronidazole inhibited the 15 oral *Actinomyces* species tested. Metronidazole was not active against any of the species.
2010	Skucaite N, Peciuliene V, Vitkauskiene A, Machiulskiene V	Susceptibility of endo-dontic pathogens to antibiotics in patients with symptomatic apical periodontitis	J Endod. 2010 Oct;36(10):1611-6	Clinical study. Studied species from root canals (35 cases) and apical abscess aspirants (23 cases). All microorganisms were highly sensitive to penicillin G, amoxicillin, and ampicillin. Susceptibilities to clindamycin and erythromycin were 73.8% and 54.7%, respectively. About 40% of the isolates were resistant to tetracycline.
2013	Rôças IN, Siqueira JF, Jr	Detection of antibiotic resistance genes in samples from acute and chronic endodontic infections and after treatment	Arch Oral Biol 2013;58:1123-8.	Clinical study. DNA extracts from abscess aspirates (n=25) and root canals of teeth with asymptomatic apical periodontitis (n=24) were sampled before and after treatment. Antibiotic resistance genes were detected by using RT-PCR. The most prevalent genes were tetracycline and erythromycin resistance genes. Treatment eliminated resistance genes from most cases.
2014	Al-Ahmad A, Ameen H, Pelz K,	Antibiotic resistance and capacity for biofilm formation of	J Endod 2014;40:223-30.	Clinical study. 47 isolates belonging to 32 species from infected filled root canals were studied for resistance to antibiotics using the Etest. Found resistance to

	Karygianni L, Wittmer A, Anderson AC, et al	different bacteria isolated from endodontic infections associated with root-filled teeth		tetracycline, fosfomycin, doxycycline, rifampicin and vancomycin (lactobacilli only), but not to pencillin, clindamycin, amoxycillin, gentamicin, moxifloxacin, metronidazole

8.7 Other antimicrobial approaches

- Iodine potassium iodide (IKI) has been used in retreatment procedures with aim of eliminating enterococci and yeasts:
  Clinical studies:
  - Pretreatment with 5% IKI prior to calcium hydroxide placement might reduce the frequency of persisting strains of *E. faecalis* (Molander et al. 1999)
  - Irrigation with IKI for 5 minutes prior to obturation reduced bacterial load (Peciuliene et al. 2001)

  ***In vitro*:**
  - 2% IKI effective in killing *Candida albicans* (Waltimo et al. 1999)
  - IKI eliminated *E. faecalis* from infected dentinal tubules in 15-mins (Baker et al. 2004)

In vitro:

- Ozone is known to act as a strong antimicrobial agent against bacteria, fungi, and viruses. Ozonated water had nearly the same antimicrobial activity as 2.5% sodium hypochlorite and was less cytotoxic (Nagayoshi et al. 2004). Ozone had an antibacterial effect on planktonic *E. faecalis* cells and those suspended in fluid, but little effect when embedded in biofilms (Hems et al. 2005). Aqueous and gaseous ozone were dose- and strain-dependently effective against different mono-species biofilms in root canals but one min exposure tio NaOCl was as effective (Huth et al. 2009)
- Ultraviolet light enhanced antimicrobial efficacy of NaOCl irrigation (Metzger et al. 2007)
- Bioglass eliminated *E. faecalis* infection in bovine dentin blocks (Zehnder et al. 2004). Using nano-sized bioglass afforded a ten-fold increase in silica release and solution pH elevation by more than three units, and killing efficacy was substantially higher (Waltimo et al. 2008)
- Incorporation of cationic nanoparticulates to sealers improved the direct antibacterial property and the ability to leach out antibacterial components (Kishen et al. 2009)
- MTA and bioaggregate (BA) both had antimicrobial effect that was enhanced with the addition of dentin powder. (Zhang et al. 2009)
- Stabilized chlorine dioxide less effective than 3% NaOCl against polymicrobial biofilms grown in bovine incisor root canals (Lundstrom et al. 2010)
- Liquorice extract had bactericidal activity against *E. faecalis* (Badr *et al*. 2011)
- 0.2% cetrimide had bactericidal activity against *E. faecalis* biofilms (Baca *et al*. 2011)
- EndoSequence Root Repair premixed putty or syringeable paste were similarly antibacterial against *E. faecalis* from root canal infections (Lovato and Sedgley 2011)
- Vanadium chloroperoxidase (an enzyme derived from a fungus) had an antimicrobial effect on 24 hr *E. faecalis* biofilms (Persoon et al. 2012)
- N-acetylcysteine was bactericidal against planktonic and biofilm forms of *E. faecalis* ATCC 29212 (Quah et al. 2012)

- Benzalkonium chloride coating on dentin may be effective as an antibiofilm agent at the initial biofilm formation stage (Jaramillo et al. 2012)
- Phage therapy: phage application reduced biomass of 24hr and 96hr *P. aeruginosa* A14 biofilms grown on microplates but not when grown in the extracted tooth model (Phee et al. 2013).
- Cold plasma treatment of 8 or 10 minutes had antimicrobial activity against *E. faecalis*, and destroyed biofilm structure (Pan et al. 2013).
- Medicated gutta percha:
- The addition of antimicrobials to gutta percha and sealers has variable antimicrobial efficacy *in vitro* (Shur et al. 2003, Lin *et al*. 2004, Hoelscher et al. 2006, Melker et al. 2006)
- Chlorhexidine-impregnated gutta percha points did not possess an *in vitro* inhibitory activity strong enough to eliminate *E. faecalis* completely from infected dentinal tubules (Lui et al. 2004)
- Sealers:
- Root fillings with gutta-percha and AH Plus or Grossman's sealer were effective in killing *E. faecalis* in dentinal tubules. Other endodontic sealers, as well as $Ca(OH)_2$, were less effective (Saleh et al. 2004)
- After 8-months incubation at 37ºC, viable *E. faecalis* was recovered from more teeth sealed with RoekoSeal (95%) compared to AH-Plus (40%) and Roth's sealer (45%) (Sedgley 2007)
- Fewer bacteria adhered to Apexit Plus. Real Seal SE and gutta-percha showed the highest number of adherent bacteria (Senges et al. 2011)

1999	Molander A, Reit C, Dahlén G	The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide	Endod Dent Traumatol 1999;15: 205-9	Clinical study. Fifty human teeth, with radiographically verified apical periodontitis, were microbiologically sampled. 3-7 days pretreatment with IPI prior to calcium hydroxide placement might reduce the frequency of persisting strains of *E. faecalis*.
1999	Waltimo TM, Orstavik D, Siren EK, Haapasalo MP	*In vitro* susceptibility of *Candida albicans* to four disinfectants and their combinations	Int Endod J 1999;32: 421-9	*In vitro*. *C. albicans* cells were highly resistant to calcium hydroxide. Sodium hypochlorite (5% and 0.5%) and iodine (2%) potassium iodide (4%) killed all yeast cells within 30sec, while chlorhexidine acetate (0.5%) showed complete killing after 5 min.
2001	Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M	Isolation of yeasts and enteric bacteria in root filled teeth with chronic apical periodontitis	Int J Endod 2001;34: 429-34	Clinical study. Sampled 40 root filled teeth with chronic apical periodontitis. In culture-positive teeth, prevalence of yeasts was 18% and enteric bacteria 64%. IKI improved antimicrobial effect of treatment.
2003	Shur AL, Sedgley CM, Fenno JC	The antimicrobial efficacy of 'MGP' gutta-percha *in vitro*	Int Endod J 2003;36: 616-21	*In vitro*. Compared to iodoform-free gutta-percha, iodoform-containing 'MGP' gutta-percha had an inhibitory effect *in vitro* on *S. aureus and F. nucleatum*, but not on *E. faecalis, E. coli* or *P. aeruginosa*. Iodoform-free gutta-percha inhibited *S. sanguis* and *A. odontolyticus*
2004	Lui JN, Sae-Lim V, Song KP, Chen NN	*In vitro* antimicrobial effect of chlorhexidine-impregnated gutta percha points on *Enterococcus*	Int Endod J 2004;37: 105-13	*In vitro*. Assessed antimicrobial activity of CHX-impregnated gutta percha points on *E. faecalis* using human roots. Chlorhexidine-impregnated points did not possess an *in vitro* inhibitory activity

		faecalis		strong enough to eliminate *E. faecalis* completely from infected dentinal tubules.
2004	Saleh IM, Ruyter IE, Haapasalo M, Orstavik D	Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers *in vitro*	Int Endod J 2004;37: 193-8	*In vitro*. Investigated the ability of different endodontic sealers and calcium hydroxide to kill *E. faecalis* in experimentally infected dentinal tubules. Root fillings *in vitro* with gutta-percha and AH Plus or Grossman's sealer were effective in killing *E. faecalis* in dentinal tubules. Other endodontic sealers (Ketac-Endo, Apexit, RoekoSeal) and calcium hydroxide were less effective
2004	Baker NE, Liewehr FR, Buxton TB, Joyce AP	Antibacterial efficacy of calcium hydroxide, iodine potassium iodide, betadine, and betadine scrub with and without surfactant against *E. faecalis in vitro*	OOOOE 2004;98: 359-64	*In vitro*. IKI was the only agent able to eliminate *E. faecalis* from bovine root dentin when used with a 15-minute contact time. The addition of surfactant did not enhance the antibacterial action of any medicament.
2004	Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M	Antimicrobial effect of ozonated water on bacteria invading dentinal tubules	J Endod 2004;30: 778-81	*In vitro*. Examined the effect of ozonated water against *E. faecalis* and *S. mutans* infected bovine dentin. Ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl. Ozonated water less cytotoxic against L-929 mouse fibroblasts than NaOCl.
2004	Zehnder M, Söderling E, Salonen J, Waltimo T	Preliminary evaluation of bioactive glass S53P4 as an endodontic medication in vitro	J Endod 2004;30: 220-4	*In vitro*. BAG eliminated *E. faecalis* infection in bovine dentin blocks. Preincubation with human dentin boosted BAG-killing efficacy against strains of *E. faecalis, C. albicans, P. aeruginosa, S. sanguis*, and *S. mutans*
2005	Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA	An *in vitro* evaluation of the ability of ozone to kill a strain of *Enterococcus faecalis*	Int Endod J 2005;38: 22-9	*In vitro*. Evaluated ozone as an antibacterial agent against *E. faecalis* in broth and biofilm cultures. Ozone had an antibacterial effect on *E. faecalis* suspended in fluid, but little effect when embedded in biofilms. It was less effective than NaOCl.
2006	Hoelscher AA, Bahcall JK, Maki JS	*In vitro* evaluation of the antimicrobial effects of a root canal sealer-antibiotic combination against *E. faecalis*	J Endod 2006;32: 145-7	*In vitro*. Sealer-antibiotic combinations containing amoxicillin, penicillin, clindamycin, and doxycycline, but not metronidazole, enhanced the antimicrobial effect of Kerr EWT sealer alone.
2006	Melker KB, Vertucci FJ, Rojas MF, Progulske-Fox A, Belanger M	Antimicrobial efficacy of medicated root canal filling materials	J Endod 2006;32: 148-51	*In vitro*. Tetracycline containing gutta-percha inhibited *Actinomyces israelii, A. naeslundii, Enterococcus faecalis,* and *Fusobacterium nucleatum*. Resilon points did not have antimicrobial activity. Standard gutta-percha and MGP inhibited *F. nucleatum* and *A. naeslundii*, with MGP also inhibiting *A. israelii*
2007	Sedgley CM	The influence of root canal sealer and gelatinase activity on extended survival of *Enterococcus faecalis* in obturated root	J Endod 2007;33: 561-6	*In vitro*. After 8-months incubation at 37ºC, viable *E. faecalis* was recovered from more teeth sealed with RoekoSeal (95%) compared to AH-Plus (40%)(p=0.0004) and Roth's sealer (45%)(p=0.0012).

		canals *in vitro*		
2007	Metzger Z, Better H, Abramovitz I	Immediate root canal disinfection with ultraviolet light: an *ex vivo* feasibility study	OOOOE 2007;104:425-33	*Ex vivo*. Used root canals infected with *E. faecalis*. Compared 5% NaOCl and UV light exposure: NaOCl alone achieved negative cultures in 47% of cases, compared to 96% using NaOCl followed by UV ($P < .001$). Illumination of root canals with ultraviolet light may be an effective supplementary means to achieve immediate disinfection of infected root canals.
2008	Kishen A, Shi Z, Shrestha A, Neoh KG	An investigation on the antibacterial and antibiofilm efficacy of cationic nanopart-iculates for root canal disinfection	J Endod 2008;34:1515-20	*In vitro*. Incorporation of cationic nanoparticulates to sealers improved the direct antibacterial property and the ability to leach out antibacterial components. There was a significant reduction in the adherence of *E. faecalis* to nanoparticulates-treated dentin.
2008	Waltimo T, Brunner TJ, Vollenweider M, Stark WJ, Zehnder M	Antimicrobial effect of nanometric bioactive glass 45S5	J Dent Res 2008;86:754-7	*In vitro*. The shift from micron- to nano-sized bioglass afforded a ten-fold increase in silica release and solution pH elevation by more than three units. Killing efficacy was substantially higher with the new material against all tested strains.
2009	Huth KC, Quirling M, Maier S, Kamereck K, Alk-hayer M, Paschos E, Welsch U, Miethke T, Brand K, Hickel R	Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model	Int Endod J 2009;42:3-13	*In vitro* study. Aqueous and gaseous ozone were dose- and strain-dependently effective against different mono-species biofilms in root canals. One min exposure tio NaOCl was as effective.
2009	Zhang H, Pappen FG, Haapasalo M	Dentin enhances the antibacterial effect of mineral trioxide aggreg-ate and bioaggregate	J Endod 2009;35:221-4	*In vitro*. Direct exposure test. Used a clinical *E. faecalis* strain. MTA and BA both had antimicrobial effect that was enhanced with the addition of dentin powder. May be attributable to high pH (>12)
2010	Lundstrom JR, Williamson AE, Villhauer AL, Dawson DV, Drake DR	Bactericidal activity of stabilized chlorine dioxide as an endodontic irrigant in a polymicrobial biofilm tooth model system	J Endod 2010;36:1874-8	*In vitro*. Compared to 3% NaOCl, stabilized chlorine dioxide had significantly less bactericidal activity against polymicrobial biofilms grown in bovine incisor root canals (*S. sanguinis*, *A. viscosus*, and *P. nigrescens*).
2011	Badr AE, Omar N, Badria FA	A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament	Int Endod J 2011;44:51-8	*In vitro*. Liquorice extract either separately or as liquorice /Ca(OH)(2) mixture had a bactericidal effect against *E. faecalis* and retained compatibility with fibroblasts in tissue culture compared to Ca(OH)(2)
2011	Baca P, Junco P, Arias-Moliz MT, González-Rodríguez	Residual and antimicrobial activity of final irrigation protocols on Enterococcus faecalis biofilm in dentin	J Endod 2011;37:363-6	*In vitro*. Dentin blocks with 3-wk old E. faecalis biofilms were exposed for 24hr to 2.5% NaOCl, 2% CHX, 0.2% cetrimide (CTR), 17% EDTA, 7% maleic acid (MA), and regimens of 2.5% NaOCl followed by 17% EDTA or 7% MA and 0.2% CTR or 2% CHX.

	MP, Ferrer-Luque CM			0.2% CTR alone or in combinations with 2% CHX achieved the best antimicrobial activity.
2011	Senges C, Wrbas KT, Altenburger M, Follo M, Spitzmüller B, Wittmer A, Hellwig E, Al-Ahmad A	Bacterial and *Candida albicans* adhesion on different root canal filling materials and sealers	J Endod 2011;37: 1247-52	*In vitro*. Tested: AH-Plus, Tubli Seal, gutta-percha, Real Seal SE, EndoREZ, Apexit Plus, GuttaFlow, and dentin using (a) salivary bacteria (b) selected individual species. Visualization using SEM and CLSM. Fewer bacteria adhered to Apexit Plus. Real Seal SE and gutta-percha showed the highest number of adherent bacteria.
2011	Lovato KF, Sedgley CM	Antibacterial activity of endosequence root repair material and proroot MTA against clinical isolates of *Enterococcus faecalis*	J Endod 2011;37: 1542-6	*In vitro*. Aim: to determine whether EndoSequence Root Repair premixed putty (ESP) or syringeable paste (ESS) were antibacterial against *E. faecalis* from root canal infections. ESP, ESS, and MTA had similar antibacterial efficacy against clinical strains of *E. faecalis*. However, clinical strains varied in their susceptibility to the root repair materials.
2012	Persoon IF, Hoogenkamp MA, Bury A, Wesselink PR, Hartog AF, Wever R, Crielaard	Effect of vanadium chloroperoxidase on *Enterococcus faecalis* biofilms	J Endod 2012;38: 72-4	*In vitro*. Vanadium chloroperoxidase (an enzyme derived from a fungus) had an antimicrobial effect on 24 hr *E. faecalis* biofilms (used four clinical strains). Might be useful as an antimicrobial dressing.
2012	Quah SY, Wu S, Lui JN, Sum CP, Tan KS	N-acetylcysteine inhibits growth and eradicates biofilm of *Enterococcus faecalis*	J Endod 2012;38: 81-5	*In vitro*. N-acetylcysteine was bactericidal against planktonic and biofilm forms of *E. faecalis* ATCC 29212. Antibacterial activity was unaffected by the presence of dentin.
2012	Jaramillo DE, Arriola A, Safavi K, Chavez de Paz LE	Decreased bacterial adherence and biofilm growth on surfaces coated with a solution of benzalkonium chloride	J Endod 2012;38: 821-5	*In vitro*. Benzalkonium chloride (BAK) is a cationic detergent expressing a high affinity to membrane proteins. Investigated its potential as an antibiofilm coating for root canal dentin. Concluded that BAK may be effective at the initial biofilm formation stage.
2013	Phee A, Bondy-Denomy J, Kishen A, Basrani B, Azarpazhooh A, Maxwell K	Efficacy of bacteriophage treatment on *Pseudomonas aeruginosa* biofilms	J Endod 2013;39: 364-9	*In vitro*. Investigated the potential for phage therapy (ie utilizing bacterial viruses) to degrade *P. aeruginosa* biofilms. Concluded that phage application reduced biomass of 24hr and 96hr PA14 biofilms grown on microplates but not when grown in the extracted tooth model.
2013	Pan J, Sun K, Liang Y, Sun P, Yang X, Wang J, et al.	Cold plasma therapy of a tooth root canal infected with *Enterococcus faecalis* biofilms in vitro	J Endod 2013;39: 105-10	*In vitro*. Cold plasma can generate high concentrations of highly reactive free radicals. Investigated its potential to inactivate *E. faecalis* biofilms in root canals. Concluded that plasma treatment of 8 or 10 minutes had antimicrobial activity, and destroyed biofilm structure.

8.8 "Conventional" needle delivery irrigation

Do irrigants reach the apical part of the canal and reduce microbial load?

Clinical studies

- Inaccessible biofilms in complex root canal systems of mandibular first molar roots cannot be removed by contemporary instruments and irrigation alone in one-visit treatment (Nair et al. 2005)
- Apical enlargement to Profile #8 plus 1% NaOCl rendered 13 cuspid/bicuspids bacteria-free *in vivo* (Card et al. 2002)

***In vitro* studies on mechanical efficacy of irrigation to remove labeled bacteria from root canals**:

- Using a 21-gauge needle solution reach the apex of a #80 canal, a 23-gauge needle to #50, a 25-gauge needle to #45, and a 30-gauge needle to #20 in simulated canals. Little fluid exchange and displacement of particles beyond the tip of the needle in simulated canals (Chow 1983)
- Volume: 6ml significantly more effective than 3ml (Sedgley et al. 2004)
- Needle depth: 1mm more effective than 5mm from apex (Sedgley et al. 2005)
- Preparation size: Larger apical instrumentation removed more bacteria than small apical instrumentation (McGurkin-Smith et al. 2005). Reduced efficacy of irrigation with smaller diameter preparation 36. No difference in irrigation efficacy between the two larger sizes 60 and 77 (Falk and Sedgley 2005).
- Canal curvature: Irrigation was significantly less effective in curved canals prepared to size 27/.04 compared to 46/.04 (Nguy and Sedgley 2006)
- Irrigation with single side-port safety needles was significantly more effective than double port and hypodermic needles (Vinothkumar et al. 2007)

***In vitro* studies on mechanical efficacy of irrigation**:

- Irrigation needles should be placed to within 1 mm from working length to ensure fluid exchange (Boutsioukis et al. 2009)
- Fluid flow is dependent on needle tip design: irrigant did not reach apex with side-vented needles (Shen et al. 2010); open-ended compared to close-ended needles resulted in more irrigant replacement in front but also higher apical pressure (Boutsioukis et al. 2010)
- Greatest extrusion with slotted needle irrigation and in teeth with apical preparation size 35.06 vs 50.06 (Mitchell et al. 2011)

1983	Chow TW	Mechanical effectiveness of root canal irrigation	J Endod 1983;11:475-9	*In vitro* study. Largest sized needle to reach the apex of various sized simulated canals was investigated. 21-G needle reached the apex of #80 canal, 23-G needle to #50, 25-G needle to #45, 30-G needle to #20. There is little fluid exchange and displacement of particles beyond the tip of the needle.
2002	Card SJ, Sigurdsson A, Orstavik D, Trope M	The effectiveness of increased apical enlargement in reducing intracanal bacteria	J Endod 2002;28:779-83	Clinical study. Instrumented cuspid (n=2) and bicuspid canals (n=11) were bacteria-free after using 1% NaOCl and 0.04 taper ProFile rotary files to a #8 size. For molars, 22/27 mesial roots of molars were also bacteria-free. Further preparation using Lightspeed to apical #60 24/27 molars were bacteria-free.

2004	Sedgley C, Applegate B, Nagel A, Hall D	Real-time imaging and quantification of bioluminescent bacteria in root canals *in vitro*	J Endod 2004;30:893-8	*In vitro* study. Used a nondestructive method to quantify root-canal bacteria over sequential treatment procedures using real-time imaging in conjunction with the bioluminescent reporter strain *Pseudomonas fluorescen*s 5RL. 6ml significantly more effective than 3ml in removal of bacteria.
2005	Sedgley CM, Nagel A, Hall D, Applegate B	Influence of irrigant needle depth in removing root canal bacteria using real-time imaging of biolumin-escent bacteria *in vitro*	Int Endod J 2005;38:97-104	The mechanical efficacy of 6mL of irrigant in reducing intracanal bacteria was significantly greater when delivered 1mm compared to 5mm from working length.
2005	McGurkin-Smith R, Trope M, Caplan D, Sigurdsson A	Reduction of intracanal bacteria using GT rotary instrumentation, 5.25% NaOCl, EDTA, and $Ca(OH)_2$	J Endod 2005;31:359-63	Clinical study. Studied bacterial reduction using Profile GT files and a strict irrigation protocol utilizing 5.25% NaOCl and EDTA in 31 subjects with apical periodontitis. GT protocol significantly reduced the number of bacteria in the canal but failed to render the canal bacteria free in >half of the cases. Large apical instrumentation removed more bacteria than small apical instrumentation
2005	Nair PN, Henry S, Cano V, Vera J	Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment	OOOOE 2005;99:231-52	Clinical study. Used light and transmission electron microscopy to study mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Concluded that inaccessible biofilms in complex root canal systems of mandibular first molar roots cannot be removed by contemporary instruments and irrigation alone in one-visit treatment
2005	Falk K, Sedgley CM	The influence of preparation size on the mechanical efficacy of root canal irrigation *in vitro*	J Endod 2005;31:742-5	*In vitro* study. Compared irrigation efficacy in the same canals sequentially prepared to sizes 36, 60 and 77. Irrigation 1mm from WL was significantly less effective in canals prepared to size 36. There was no difference between sizes 60 and 77.
2006	Nguy D, Sedgley CM	The influence of canal curvature on the mechanical efficacy of root canal irrigation *in vitro* using real-time imaging of biolum-inescent bacteria	J Endod 2006;32:1077-80	*In vitro* study. Using bioluminescent bacteria, quantitatively compared irrigation efficacy in canals with different curvature: (1) 4°-8°, (2) 15°-19°, (3) 24°-28° sequentially prepared to sizes 27/.04, 36.04 and 46/.04. Irrigation was significantly less effective in 24 to 28 degrees curvature canals prepared to size 27/.04 compared to 46/.04 ($p < 0.007$)
2007	Vinothkumar TS, Kavitha S, Lakshminarayanan L, Gomathi NS, Kumar V	Influence of irrigating needle-tip designs in removing bacteria inoculated into instrumented root canals measured using single-tube luminometer	J Endod 2006;32:1077-80	*In vitro* study. Using bioluminescent bacteria, quantitatively compared irrigation efficacy in canals using needles with different tips design: single side-port, double side-port and hypodermic needles. Irrigation was significantly more effective using single-side-port (Kruskall-Wallis). No difference between double side-port and hypodermic needles

2009	Boutsioukis C, Lambrianidis T, Kastrinakis E	Irrigant flow within a prepared root canal using various flow rates: a Computational Fluid Dynamics study	Int Endod J 2009;42:144-55	*In vitro* study. Irrigant replacement was limited to 1-1.5 mm apical to the needle tip for all flow rates tested. Concluded that irrigation needles should be placed to within 1 mm from working length to ensure fluid exchange.
2010	Shen Y, Gao Y, Qian W, Ruse ND, Zhou X, Wu H, Haapasalo M	Three-dimensional numeric simulation of root canal irrigant flow with different irrigation needles	J Endod 2010 May;36:884-9	*In vitro*. Compared 27-gauge notched and side-vented open-ended needles, placed at 3 and 5 mm from apex of simulated straight root canal in a plastic block. Evaluated fluid flow using computational fluid dynamics. When 3 mm from apex, irrigant reached, or almost reached, the apex. When 5 mm from apex, irrigant did not reach apex with side-vented needles.
2010	Boutsioukis C, Verhaagen B, Versluis M, Kastrinakis E, Wesselink PR, van der Sluis LW	Evaluation of irrigant flow in the root canal using different needle types by an unsteady computational fluid dynamics model	J Endod 2010 May;36:875-9	*In vitro*. Compared 30-gauge open-ended and closed-ended (notched) needles. Evaluated fluid flow using computational fluid dynamics. Flow pattern of the open-ended needles was different from the close-ended needles, resulting in more irrigant replacement in front of the open-ended needles but also higher apical pressure.
2011	Mitchell RP, Baumgartner JC, Sedgley CM	Apical extrusion of sodium hypochlorite using different root canal irrigation systems	J Endod. 2011 Dec;37(12):1677-81	*In vitro*. Compared EndoActivator, EndoVac, Rispi Sonic /MicroMega 1500, passive ultrasonic irrigation, and syringe irrigation with a slot-tipped needle (SN), in a randomized crossover design. Greatest extrusion with SN irrigation and in teeth with apical preparation size 35.06 vs 50.06.

8.9 Simultaneous Irrigation/Evacuation

Clinical study:

- No differences between EndoVac and conventional irrigation in number of culture positive samples (Pawar et al. 2012)

***In vitro* studies**:

- Less debris observed at 1 mm from working length using negative pressure irrigant delivery (EndoVac) compared with 30 gauge ProRinse needle irrigation. Used similar times of exposure to 5.25% NaOCl. Higher volume of irrigant used in EndoVac group (42 ml versus 16 ml) (Nielsen and Baumgartner 2007)
- No differences between conventional, EndoActivator and EndoVac in *E. faecalis* removal (Brito et al. 2009)

2007	Nielsen BA, Baumgartner JC	Comparison of the EndoVac system to needle irrigation of root canals	J Endod 2007;33:611-5	*In vitro*. Compared Endovac (negative pressure irrigant delivery) and 30 gauge ProRinse irrigating needle delivery of 5.25% NaOCl to debride root canals. Delivery time standardized in both groups. Less debris observed at 1 mm from working length using EndoVac compared with needle irrigation. However, higher volume of irrigant was used in EndoVac group (42mL) compared to needle irrigation (16mL)

2009	Brito PR, Souza LC, Machado de Oliveira JC, Alves FR, De-Deus G, Lopes HP, Siqueira JF Jr	Comparison of the effectiveness of three irrigation techniques in reducing intracanal *Enterococcus faecalis* populations: an *in vitro* study	J Endod 2009;35: 1422-7	*In vitro*. Extracted teeth contaminated with *E. faecalis*. Compared CFUS after (1) conventional irrigation with NaviTip needles inserted up to 3 mm short of WL; (2) same as (1), but supplemented with final irrigant activation by EndoActivator; and (3) irrigation with EndoVac. Reported no sig differences between the 3 techniques.
2012	Pawar R, Alqaied A, Safavi K, Boyko J, Kaufman B	Influence of an apical negative pressure irrigation system on bacterial elimination during endodontic therapy: a prospective randomized clinical study	J Endod 2012;38:11 77-81	Clinical study. 48 patients assigned to either EndoVac or standard needle irrigation (control) with 0.5% NaOCl irrigation. Intracanal samples cultured anaerobically. No significant difference between EndoVac and control in number of culture-positive samples.

8.10 Ultrasonic and sonic activation

Review of ultrasonics in endodontics: van der Sluis et al. 2007
Clinical studies:

- Ultrasonic technique eliminated the bacteria from the canals more efficiently than hand instrumentation (Sjögren and Sundqvist 1987)
- Ultrasonic irrigation for 1 min following instrumentation resulted in a significant reduction in debris and biofilm (Burleson et al. 2007) and microorganisms (Carver et al. 2007)
- No sig difference between sonic (EndoActivator) and control (conventional syringe) groups to eliminate cultivable bacteria from canals (Huffaker et al. 2010)
- 50 patients assigned to either passive ultrasonic or standard needle irrigation with 1% NaOCl. Intracanal samples cultured anaerobically. No significant difference groups in number of culture-positive samples (Beus et al. 2012).

***In vitro* studies:**

- Ultrasonic agitation significantly more effective than needle irrigation and EndoVac irrigation at removing intracanal bacteria. Ultrasonic, EndoActivator, F-File, and sonic agitation were similar in their ability to remove bacteria in a plastic simulated canal (Townsend and Maki 2009)
- Cavitation bubbles produced using high-intensity focused ultrasound can be used as a potential method to deliver antibacterial nanoparticles into the dentinal tubules to enhance root canal disinfection (Shrestha et al. 2009)
- Better tissue-dissolving effects with ultrasonic than sonic irrigant activation (Al-Jadaa et al. 2009)
- Conventional irrigation and passive ultrasonic irrigation with 1% NaOCl were effective at completely removing intraradicular *E. faecalis* biofilms (Bhuva et al. 2010)

- Improved root canal debris removal has been described when using the sonic devices: EndoActivator (Kanter et al. 2011), Sonicare CanalBrush (Salman et al. 2010), Vibringe (Rodig et al. 2010) and (multisonic) GentleWave System (LM) (Molina et al. 2015).

1987	Sjögren U, Sundqvist G	Bacteriologic evaluation of ultrasonic root canal instrumentation	OOO 1987;63: 366-70	Clinical study. Ultrasonically activated filing technique eliminated bacteria from canals more efficiently than hand instrumentation alone *in vivo*. NaOCl (0.5%) used as irrigant
2007	Burleson A, Nusstein J, Reader A, Beck M	The *in vivo* evaluation of hand/rotary/ultra sound instrumentation in necrotic, human mandibular molars	J Endod 2007;33: 782-7	Clinical study. Prospective, randomized, single-blinded study. Compared biofilm/necrotic debridement efficiency of a hand/rotary vs hand/rotary/1 min ultrasound technique in mesial roots of necrotic lower molars. Teeth examined histologically after extraction. Cleanliness at 1-, 2- and 3-mm levels for the h/r and h/r/ultra techniques, were respectively: Canals, 80% vs 95%, 92% vs 99%, 95% vs 100%; Isthmuses, 33% vs 83%, 31% vs 86%, 45% vs 91%
2007	Carver K, Nusstein J, Reader A, Beck M	*In vivo* antibacterial efficacy of ultrasound after hand and rotary instrumentation in human mandibular molars	J Endod 2007;33:10 38-43	Clinical study.P rospective, randomized, single-blinded study. Culture methods. Used 31 mesial root canals of necrotic mandibular molars. Compared antibacterial efficacy of a hand/rotary technique (in 16 canals)versus hand/ rotary/1 min ultrasound technique (in 15 canals). Reported reduction in CFU count (p=0.0006) and positive cultures (p=0.0047) with addition of 1 min ultrasonic irrigation. Logistic regression analysis 1 min ultrasonic irrigation 7 times more likely to yield a negative culture.
2007	van der Sluis LW, Versluis M, Wu MK, Wesselink PR	Passive ultrasonic irrigation of the root canal: a review of the literature	In Endod J 2007;40:41 5-26	Review. Ultrasonic irrigation of the root canal can be performed with or without simultaneous ultrasonic instrumentation. When canal shaping is not simultaneously undertaken is called passive ultrasonic irrigation (PUI) and uses a small file or smooth wire (size 10-20) oscillating freely in the root canal to induce acoustic microstreaming. Role of cavitation during PUI inconclusive.
2009	Shrestha A, Fong SW, Khoo BC, Kishen A	Delivery of anti-bacterial nanoparticles into dentinal tubules using high-intensity focused ultrasound	J Endod 2009;35;10 28-33	Cavitation bubbles produced using high-intensity focused ultrasound can be used as a potential method to deliver antibacterial nanoparticles into the dentinal tubules to enhance root canal disinfection
2009	Townsend C, Maki J	An *in vitro* comparison of new irrigation and agitation techniques to ultrasonic agitation in removing bacteria from a simulated root canal	J Endod 2009;35;10 40-3	In a plastic simulated canal, ultrasonic agitation was significantly more effective than needle irrigation and EndoVac irrigation at removing intracanal bacteria. Ultrasonic, EndoActivator, F-File, and sonic agitation are similar in their ability to remove bacteria in a plastic simulated canal.

2009	Al-Jadaa A, Paque F, Attin T, Zehnder M	Acoustic hypochlorite activation in simulated curved canals	J Endod 2009;35: 1408-11	*In vitro*. In simulated canals passive ultrasonic irrigation (Endosonore) had better tissue-dissolving effects than sonic irrigant activation (EndoActivator).
2010	Bhuva B, Patel S, Wilson R, Niazi S, Beighton D, Mannocci F	The effectiveness of passive ultrasonic irrigation on intraradicular *E. faecalis* biofilms in extracted single-rooted human teeth	Int Endod J 2010 Mar;43:241-50	*In vitro*. SEM. In extracted teeth both conventional syringe irrigation and passive ultrasonic irrigation with 1% NaOCl were effective at completely removing intraradicular *E. faecalis* biofilms.
2010	Salman MI, Baumann MA, Hellmich M, Roggendorf MJ, Termaat S	SEM evaluation of root canal debridement with Sonicare CanalBrush irrigation	Int Endod J 2010;43:363-9	*In vitro*. Described improved root canal debris removal using Sonicare CanalBrush. No info on antimicrobial effect.
2010	Rodig T, Bozkurt M, Konietschke F, Hulsmann M	Comparison of the Vibringe system with syringe and passive ultrasonic irrigation in removing debris from simulated root canal irregularities	J Endod 2010;36:1410-3	*In vitro*. Described improved root canal debris removal using Vibringe over syringe irrigation, but not as effective as passive ultrasonic irrigation. No info on antimicrobial effect.
2010	Huffaker SK, Safavi K, Spangberg LS, Kaufman B	Influence of a passive sonic irrigation system on the elimination of bacteria from root canal systems: a clinical study	J Endod 2010;36:1315-8	Clinical study. Reported no sig difference between sonic (EndoActivator) and control (conventional syringe) groups to eliminate cultivable bacteria from canals. A second session and intervisit $Ca(OH)_2$ disinfection eliminated cultivable bacteria from significantly more teeth than a single session of treatment.
2011	Kanter V, Weldon E, Nair U, Varella C, Kanter K, et al.	A quantitative and qualitative analysis of ultrasonic versus sonic endodontic systems on canal cleanliness and obturation	OOORE 2011;112 :809-13	*In vitro*. Described improved root canal debris removal using EndoActivator over ultrasonice and syringe irrigation. No info on antimicrobial effect.
2012	Beus C, Safavi K, Stratton J, Kaufman B	Comparison of the effect of two endodontic irrigation protocols on the elimination of bacteria from root canal system: a prospective, randomized clinical trial	J Endod 2012;38:1479-83	Clinical study. 50 patients assigned to either PUI or standard needle irrigation (NUI) with 1% NaOCl. Intracanal samples cultured anaerobically. No significant difference between PUI and control in number of culture-positive samples at second visit.
2015	Molina B, Glickman G, Vandrangi P, Khakpour M.	Evaluation of Root Canal Debridement of Human Molars Using the GentleWave System	J Endod 2015;41:1701-5	*In vitro*. Described improved root canal debris removal using GentleWave System. ["Multisonic energy (energy generated by multiple wavelengths of sound over a broad range of frequencies)"]. No info on antimicrobial effect.

8.11 Lasers

***In vitro* studies:**

- Neither Er,Cr:YSGG laser nor rotary instrumentation eliminated endodontic infection (Jha et al. 2006)
- Nd:YAG laser irradiation is not an alternative but a possible supplement to existing protocols for canal disinfection (Bergmans et al. 2006, Eldeniz et al. 2007)
- Photodynamic therapy (PDT): up to 80% reduction of CFU (Fimple et al. 2008)
- PDT significantly reduced residual bacteria within the root canal system (Ng et al. 2011)
- PDT using toluidine blue O and a low-energy light-emitting diode lamp has the potential to be used as an adjunctive antimicrobial procedure in conventional endodontic therapy (Rios et al. 2011)
- Laser irradiation systems (Nd:YAG, KTP) as well as photoactivated disinfection were less effective than NaOCl in reducing *E. faecalis*, both in aqueous suspension and in an infected tooth model (Meire et al. 2009)
- Er,Cr:YSGG laser eliminated bacteria from straight but not all curved canals (Dewsnup et al. 2010)
- Streaming caused by the collapse of the laser induced bubble is main cleaning mechanism of laser-activated irrigation (deGroot et al. 2009)
- PIPS: Photon-initiated photoacoustic streaming of irrigant by using pulsed erbium:YAG laser. PIPS generated more negative bacterial samples than conventional and ultrasonic activation (Peters et al. 2011)

Systematic review:

- Insufficient evidence for whether lasers can be recommended as an adjunct to chemo-mechanical disinfection of infected root canals (Fransson et al. 2013)

2006	Jha D, Guerrero A, Ngo T, Helfer A, Hasselgren G	Inability of laser and rotary instrumentation to eliminate root canal infection	JADA 2006;137:67-70	*In vitro*. Evaluated the antibacterial effectiveness of laser instrumentation and rotary instrumentation in dentin shavings of anterior, single-rooted teeth infected with *E. faecalis*. Neither Er,Cr:YSGG laser nor rotary instrumentation was able to eliminate endodontic infection
2006	Bergmans L, Moisiadis P, Teughels W, Van Meerbeek B, Quirynen M, Lambrechts P	Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens *ex vivo*	Int Endod J 2006;39:547-57	*In vitro*. Observed CFU and bacterial structural changes using conventional SEM and environmental SEM on inoculated dentin surfaces, following indirect and direct Nd:YAG laser irradiation. Concluded that Nd:YAG laser irradiation is not an alternative but a possible supplement to existing protocols for canal disinfection. *E. faecalis* strains grown as biofilms on dentin discs could not be eradicated upon direct laser exposure.
2006	Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S, Foschi	Photodynamic therapy for endodontic disinfection	J Endod 2006:32:979-84	*In vitro*. Bacteria in root canals were sensitized with methylene blue (MB) and exposed to red light of 665 nm with an energy fluence of 30 J/cm2. MB eliminated all bacterial species with the exception of *E. faecalis* (53% killing). The same concentration of MB with red light (222 J/cm2) was able to eliminate 97% of *E.*

	F, Doucette S, Bammann LL, Fontana CR, Doukas AG, Stashenko PP			*faecalis* biofilm in canals using an optical fiber with multiple cylindrical diffusers that uniformly distributed light at 360 degrees. PDT may be an adjunctive procedure to kill residual bacteria in the root canal system after standard endodontic treatment.
2007	Eldeniz AU, Ozer F, Hadimli HH, Erganis O	Bactericidal efficacy of Er,Cr:YSGG laser irradiation against *Enterococcus faecalis* compared with NaOCl irrigation: an *ex vivo* pilot study	Int Endod J 2007;40 39:112-9	*In vitro*. In teeth with straight roots the Er,Cr:YSGG laser reduced the viable microbial population in root canals with small and large apical foramina but did not eradicate all bacteria. 3% NaOCl inhibited the growth of *E. faecalis* and effectively sterilized all root canals.
2008	Fimple JL, Fontana CR, Foschi F, Ruggiero K, Song X, Pagonis TC, Tanner AC, Kent R, Doukas AG, Stashenko PP, Soukos NS	Photodynamic treatment of endodontic polymicrobial infection *in vitro*	J Endod 2008;34: 728-34	*In vitro*. Uses photosensitizer solutions (e.g. methylene blue, tolonium chloride) and low-power laser light. [Solution binds to microbial cell. Laser light applied (via plastic flexible optical fiber) activates dye. Free radicals produced destroy cell] Grew multispecies biofilms (4 species) in root canals Photodynamic therapy : up to 80% reduction of CFU
2009	Meire MA, De Prijck K, Coenye T, Nelis HJ, De Moor RJ	Effectiveness of different laser systems to kill *Enterococcus faecalis* in aqueous suspension and in an infected tooth model	Int Endod J 2009;42: 351-9	*In vitro*. Laser irradiation systems (Nd:YAG, KTP) as well as photo activated disinfection (PAD) were less effective than NaOCl in reducing *E. faecalis*, both in aqueous suspension and in an infected tooth model.
2009	de Groot SD, Verhaagen B, Versluis M, Wu MK, Wesselink PR, van der Sluis LW	Laser-activated irrigation within root canals: cleaning efficacy and flow visualization	Int Endod J 2009 Dec;42:1 077-83	*In vitro*. Used high speed imaging to observe fluid dynamics. At 20sec laser-activated irrigation (LAI) was sig more effective than passive ultrasonic irrigation (PUI) or hand irrigation in removing dentin debris from apical part of the root canal. Streaming caused by the collapse of the laser induced bubble is main cleaning mechanism of LAI.
2010	Dewsnup N, Pileggi R, Haddix J, Nair U, Walker C, Varella CH	Comparison of bacterial reduction in straight and curved canals using Er,Cr:YSGG laser treatment versus a traditional irrigation technique with NaOCl	J Endod 2010 Apr;36:7 25-8	*In vitro*. Extracted teeth. Conventional irrigation techniques using 6.15% NaOCl eliminated bacteria in straight and curved canals. Er,Cr:YSGG laser eliminated bacteria from straight canals but not all curved canals.
2011	Ng R, Singh F, Papamanou DA, Song X, Patel C, Holewa C, Patel N, Klepac-Ceraj	Endodontic photodynamic therapy ex vivo	J Endod. 2011 Feb;37(2):217-22	Used 52 extracted teeth with pulpal necrosis and PARL. Compared viable counts after chemomechanical debridement with or without PDT. Bacterial species within dentinal tubules were detected in 17 of 22 (77.3%) and 15 of 29 (51.7%) canals in the CMD and

	V, Fontana CR, Kent R, Pagonis TC, Stashenko PP, Soukos N			CMD+PDT groups, respectively (p=0.034). PDT significantly reduced residual bacteria within the root canal system.
2011	Rios A, He J, Glickman GN, Spears R, Schneiderman ED, Honeyman AL	Evaluation of photodynamic therapy using a light-emitting diode lamp against *Enterococcus faecalis* in extracted human teeth	J Endod 2011 Jun;37(6):856-9	*In vitro*. Evaluated the antimicrobial effect of PDT using toluidine blue O (TBO) and a low-energy light-emitting diode (LED) lamp in extracted teeth infected with *E. faecalis*. Results: bacterial survival rate of NaOCl/TBO/light group (0.1%) was significantly lower (P < .005) than the NaOCl (0.66%) and TBO/light groups (2.9%).
2011	Peters OA, Bardsley S, Fong J, Pandher G, Divito E	Disinfection of root canals with photon-initiated photoacoustic streaming	J Endod 2011;37:1008-12.	*In vitro*. PIPS uses pulsed erbium:YAG laser to create photon-initiated photoacoustic streaming in irrigant. 60 extracted premolars prepared to apical #20.07 and infected with oral bacteria. More negative bacterial samples were generated using PIPS compared to conventional and ultrasonic activation. No difference in percentage reduction of microorganisms (96.6%-99.5%).
2013	Fransson H, Larsson KM, Wolf E	Efficacy of lasers as an adjunct to chemo-mechanical disinfection of infected root canals: a systematic review	Int Endod J 2013;46:296-307	Systematic review: insufficient evidence for whether lasers can be recommended as an adjunct to chemo-mechanical disinfection of infected root canals

8.12 File systems

Clinical studies:

Clinical studies using real-time qPCR that have shown that different file systems do not differ in their ability to reduce microbial load in root canals

- No difference between Hand NiTi using alternated rotation motion or rotary BioRace (Rôças and Siqueira 2013)
- No difference between Twisted fileAdaptive and Self Adjusting file for retreatment cases (Rodrigues et al. 2014)

***In vitro* studies:**

There are many *in vitro* studies comparing different file systems in their ability to reduce microbial load (usually *E. faecalis*) in root canals of decoronated teeth. Most show no significant difference between file systems. For example:

- No difference between Reciproc and BioRaCe (Alves et al. 2012)
- No difference between K-file and ProTaper (Nakamura et al. 2013)
- No difference between SAF, Twisted File and Reciproc (Siqueira et al. 2013)
- No difference between Wave One and One Shape (Nabeshima et al. 2014)

2012	Alves FR, Rôças IN, Almeida BM, Neves MA, Zoffoli J, Siqueira JF, Jr	Quantitative molecular and culture analyses of bacterial elimination in oval-shaped root canals by a single-file instrumentation technique	Int Endod J 2012;45: 871-7	*In vitro*. Compared use of either a single Reciproc instrument or the BioRaCe instrument series to reduce *E. faecalis*. Measured by real-time qPCR and culture. No sig difference seen provided the width of apical preparation, volume of irrigants and duration of irrigation were kept similar.
2013	Nakamura VC, Cai S, Candeiro GT, Ferrari PH, Caldeira CL, Gavini G	Ex vivo evaluation of the effects of several root canal preparation techniques and irrigation regimens on a mixed microbial infection	Int Endod J 2013;46: 217-24	*In vitro*. Compared use of manual (K-type) or rotary (Protaper Universal) instruments in mandibular premolars to reduce mixed flora of *E. faecalis* and *Candida albicans*. Both manual and rotary technique significantly reduced microorganisms with no significant difference between them.
2013	Siqueira JF, Jr., Alves FR, Versiani MA, Rôças IN, Almeida BM, Neves MA, et al.	Correlative bacteriologic and micro-computed tomographic analysis of mandibular molar mesial canals prepared by self-adjusting file, reciproc, and twisted file systems	J Endod 2013;39: 1044-50	*In vitro*. Evaluated the disinfecting and shaping ability of SAF, Twisted File and Reciproc by means of correlative bacteriologic (using *E. faecalis*) and micro-computed tomographic (mumuCT) analysis. Found no sig difference between the the 3 instrumentation systems in disinfecting and shaping performance in the preparation of mesial canals of mandibular molars.
2013	Rôças IN, Lima KC, Siqueira JF, Jr	Reduction in bacterial counts in infected root canals after rotary or hand nickel-titanium instrumentation--a clinical study	Int Endod J 2013;46: 681-7	Clinical study. Root canals from 40 single-rooted teeth instrumented using either hand NiTi instruments in the alternated rotation motion technique or rotary BioRaCe instruments. Irrigant was 2.5% NaOCl. Intergroup comparison showed no sig difference between the two techniques. Used real-time qPCR
2014	Nabeshima CK, Caballero -Flores H, Cai S, Aranguren J, Borges Britto ML, Machado ME	Bacterial removal promoted by 2 single-file systems: Wave One and One Shape	J Endod 2014;40: 1995-8	*In vitro*. In DB canals of maxillary molars, single-file systems Wave One and One Shape significantly reduced *E. faecalis* numbers with no significant difference between systems
2014	Rodrigues RC, Antunes HS, Neves MA, Siqueira JF, Jr., Rôças IN	Infection Control in Retreatment Cases: In Vivo Antibacterial Effects of 2 Instrumentation Systems	J Endod 2015;41: 1600-5	Clinical study. Root canals from 48 single-rooted teeth needing retreatment were instrumented using either Self-Adjusting File or Twisted File Adaptive. Irrigant was 2.5% NaOCl with or without PUI. Both SAF and TFA instrumentation resulted in significant intracanal bacterial reduction with no sig differences between SAF and TFA with or without PUI. Used real-time qPCR

ENDODONTIC MICROBIOLOGY REFERENCES

Abdullah M, Ng YL, Gulabivala K, Moles DR, Spratt DA. Susceptibilties of two *Enterococcus faecalis* phenotypes to root canal medications. J Endod 2005;31:30-6

Abou-Rass M, Bogen G. Microorganisms in closed periapical lesions. Int Endod J 1998;31:39-47

Al-Ahmad A, Maier J, Follo M, Spitzmüller B, Wittmer A, Hellwig E, Hübner J, Jonas D. Food-borne enterococci integrate into oral biofilm: an in vivo study. J Endod. 2010 Nov;36(11):1812-9.

Al-Ahmad A, Ameen H, Pelz K, Karygianni L, Wittmer A, Anderson AC, et al. Antibiotic resistance and capacity for biofilm formation of different bacteria isolated from endodontic infections associated with root-filled teeth. J Endod 2014;40:223-30.

Al-Azemi A, Fielder MD, Abuknesha RA, Price RG. Effects of chelating agent and environmental stresses on microbial biofilms: relevance to clinical microbiology. J Appl Microbiol 2011;110:1307-13.

Allard U, Stromberg U, Stromberg T. Endodontic treatment of experimentally induced apical periodontitis in dogs. Endod Dent Traumatol 1987;3:240-4

Al-Jadaa A, Paque F, Attin T, Zehnder M. Acoustic hypochlorite activation in simulated curved canals. J Endod. 2009 Oct;35(10):1408-11.

Alves J, Walton R, Drake D. Coronal leakage: endotoxin penetration from mixed bacterial communities through obturated, post-prepared root canals. J Endod 1998;24:587-91

Alves FR, Rocas IN, Almeida BM, Neves MA, Zoffoli J, Siqueira JF, Jr. Quantitative molecular and culture analyses of bacterial elimination in oval-shaped root canals by a single-file instrumentation technique. Int Endod J 2012;45:871-7

Anderson AC, Hellwig E, Vespermann R, Wittmer A, Schmid M, Karygianni L, et al. Comprehensive analysis of secondary dental root canal infections: a combination of culture and culture-independent approaches reveals new insights. PLoS One 2012;7:e49576

Appelbe OK, Sedgley CM. Effects of prolonged exposure to alkaline pH on *Enterococcus faecalis* survival and specific gene transcripts. Oral Microbiol Immunol 2007;22:169-74

Azarpazhooh A, Fillery ED. Prion disease: the implications for dentistry. J Endod 2008;34:1158-66.

Baca P, Junco P, Arias-Moliz MT, González-Rodríguez MP, Ferrer-Luque CM. Residual and antimicrobial activity of final irrigation protocols on *Enterococcus faecalis* biofilm in dentin. J Endod. 2011 Mar;37(3):363-6.

Badr AE, Omar N, Badria FA. A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. Int Endod J. 2011 Jan;44(1):51-8.

Bae KS, Baumgartner JC, Shearer TR, David LL Occurrence of *Prevotella nigrescens* and *Prevotella intermedia* in infections of endodontic origin. J Endod 1997;23:620-3

Baik JE, Jang KS, Kang SS, Yun CH, Lee K, Kim BG, Kum KY, Han SH. Calcium hydroxide inactivates lipoteichoic acid from *Enterococcus faecalis* through deacylation of the lipid moiety. J Endod. 2011 Feb;37(2):191-6.

Baik JE, Ryu YH, Han JY, Im J, Kum KY, Yun CH, Lee K, Han SH. Lipoteichoic acid partially contributes to the inflammatory responses to *Enterococcus faecalis*. J Endod 2008;34:975-82

Baker NE, Liewehr FR, Buxton TB, Joyce AP. Antibacterial efficacy of calcium hydroxide, iodine potassium iodide, betadine, and betadine scrub with and without surfactant against *E. faecalis in vitro*. OOOOE 2004;98:359-64

Barbin LE, Saquy PC, Guedes DF, Sousa-Neto MD, Estrela C, Pécora JD. Determination of para-chloroaniline and reactive oxygen species in chlorhexidine and chlorhexidine associated with calcium hydroxide. J Endod 2008;34:1508-14.

Barnard D, Davies J, Figdor D. Susceptibility of *Actinomyces israelii* to antibiotics, sodium hypochlorite and calcium hydroxide. Int Endod J 1996;29:320-6

Barrieshi KM, Walton RE, Johnson WT, Drake DR. Coronal leakage of mixed anaerobic bacteria after obturation and post space preparation. OOOOE 1997;84:310-4

Basrani B, Santos JM, Tjaderhane L, Grad H, Gorduysus O, Huang J, Lawrence HP, Friedman S. Substantive antimicrobial activity in chlorhexidine-treated human root dentin. OOOOE 2002;94:240-5

Basrani BR, Manek S, Fillery E. Using diazotization to characterize the effect of heat or sodium hypochlorite on 2.0% chlorhexidine. J Endod. 2009 Sep;35(9):1296-9.

Basrani BR, Manek S, Mathers D, Fillery E, Sodhi RN. Determination of 4-chloroaniline and its derivatives formed in the interaction of sodium hypochlorite and chlorhexidine by using gas chromatography. J Endod. 2010 Feb;36(2):312-4.

Basrani BR, Manek S, Sodhi RNS, Fillery E, Manzur A. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod 2007;33:966-9

Bate AL, Ma JK, Pitt Ford TR. Detection of bacterial virulence genes associated with infective endocarditis in infected root canals. Int Endod J 2000;33:194-203

Baumgartner JC, Falkler WA Jr, Beckerman T. Experimentally induced infection by oral anaerobic microorganisms in a mouse model. Oral Microbiol Immunol 1992;7:253-6

Baumgartner JC, Falkler WA JR, Bernie. RS, Suzuki JB. Serum IgG reactive with oral anaerobic microorganisms associated with infections of endodontic origin. Oral Microbiol Immunol 1992;7:106-10

Baumgartner JC, Falkler WA Jr. Bacteria in the apical 5 mm of infected root canals. J Endod 1991;17:380-3

Baumgartner JC, Heggers JP, Harrison JW. Incidence of bacteremias related to endodontic procedures II. Surgical endodontics. J Endod 1977;3:399-402

Baumgartner JC, Heggers JP, Harrison JW. The incidence of bacteremias related to endodontic procedures I. Nonsurgical endodontics. J Endod 1976;2:135-40

Baumgartner JC, Johal S, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/BioPure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. J Endod 2007;33:48-51

Baumgartner JC, Khemaleelakul S, Xia T. Identification of spirochetes (Treponemes) in endodontic infections. J Endod 2003;29:794-7

Baumgartner JC, Watkins BJ, Bae KS, Xia T. Association of black-pigmented bacteria with endodontic infections. J Endod 1999;25:413-5

Baumgartner JC, Watts CM, Xia T. Occurrence of *Candida albicans* in infections of endodontic origin. J Endod 2000;26:695-8

Baumgartner JC, Xia T. Antibiotic susceptibility of bacteria associated with endodontic abscesses. J Endod 2003;29:44-7

Behrend GD, Cutler CW, Gutmann JL. An *in vitro* study of smear layer removal and microbial leakage along root-canal fillings. Int Endod J 1996;29:99-107

Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odont Revy 1974;25:347-58

Bergmans L, Moisiadis P, Teughels W, Van Meerbeek B, Quirynen M, Lambrechts P. Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens ex vivo. Int Endod J 2006;39:547-57

Bergmans L, Moisiadis P, Van Meerbeek B, Quirynen M, Lambrechts P. Microscopic observation of bacteria: review highlighting the use of environmental SEM. Int Endod J 2005;38:775-88

Beus C, Safavi K, Stratton J, Kaufman B. Comparison of the effect of two endodontic irrigation protocols on the elimination of bacteria from root canal system: a prospective, randomized clinical trial. J Endod 2012;38:1479-83.

Bhuva B, Patel S, Wilson R, Niazi S, Beighton D, Mannocci F. The effectiveness of passive ultrasonic irrigation on intraradicular *Enterococcus faecalis* biofilms in extracted single-rooted human teeth. Int Endod J. 2010 Mar;43(3):241-50.

Blanquet-Grossard F, Sazdovitch V, Jean A, Deslys JP, Dormont D, Hauw JJ, Marion D, Brown P, Cesbron JY Prion protein is not detectable in dental pulp from patients with Creutzfeldt-Jakob disease. J Dent Res 2000;79:700

Bogen G, Slots J. Black-pigmented anaerobic rods in closed periapical lesions. Int Endod J 1999;32:204-10

Boutsioukis C, Lambrianidis T, Kastrinakis E. Irrigant flow within a prepared root canal using various flow rates: a Computational Fluid Dynamics study. Int Endod J 2009;42:144-55.

Boutsioukis C, Verhaagen B, Versluis M, Kastrinakis E, Wesselink PR, van der Sluis LW. Evaluation of irrigant flow in the root canal using different needle types by an unsteady computational fluid dynamics model. J Endod. 2010 May;36(5):875-9.

Brito PR, Souza LC, Machado de Oliveira JC, Alves FR, De-Deus G, Lopes HP, Siqueira JF Jr. Comparison of the effectiveness of three irrigation techniques in reducing intracanal *Enterococcus faecalis* populations: an in vitro study. J Endod. 2009 Oct;35(10):1422-7.

Brundin M, Figdor D, Roth C, Davies JK, Sundqvist G, Sjögren U. Persistence of dead-cell bacterial DNA in ex vivo root canals and influence of nucleases on DNA decay in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Dec;110(6):789-94.

Brundin M, Figdor D, Sundqvist G, Sjogren U. Starvation response and growth in serum of *Fusobacterium nucleatum*, *Peptostreptococcus anaerobius*, *Prevotella intermedia*, and *Pseudoramibacter alactolyticus*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Jul;108(1):129-34.

Brundin M, Figdor D, Johansson A, Sjogren U. Preservation of bacterial DNA by human dentin. J Endod 2014;40:241-5

Bryce G, O'Donnell D, Ready D, Ng YL, Pratten J, Gulabivala K. Contemporary root canal irrigants are able to disrupt and eradicate single- and dual-species biofilms. J Endod. 2009 Sep;35(9):1243-8.

Buck RA, Cai J, Eleazer PD, Staat RH, Hurst HE. Detoxification of endotoxin by endodontic irrigants and calcium hydroxide. J Endod 2001;27:325-7

Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod. 2008 Feb;34(2):181-5.

Burleson A, Nusstein J, Reader A, Beck M. The in vivo evaluation of hand/rotary/ultrasound instrumentation in necrotic, human mandibular molars. J Endod 2007;33:782-7

Bystrom A, Happonen R-P, Sjögren U, Sunqvist G. Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. Endod Dent Traumatol 1987;3:58-63

Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. OOO 1983;55:307-12

Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8

Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int J Endod 1985;18:35-40

Card SJ, Sigurdsson A, Orstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod 2002;28:779-83

Carr GB, Schwartz RS, Schaudinn C, Gorur A, Costerton JW. Ultrastructural examination of failed molar retreatment with secondary apical periodontitis: an examination of endodontic biofilms in an endodontic retreatment failure. J Endod. 2009 Sep;35(9):1303-9.

Carver K, Nusstein J, Reader A, Beck M. *In vivo* antibacterial efficacy of ultrasound after hand and rotary instrumentation in human mandibular molars. J Endod 2007;33:1038-43.

Chavez de Paz LE. Development of a multispecies biofilm community by four root canal bacteria. J Endod 2012;38:318–23

Chavez de Paz L, Svensater G, Dahlén G, Bergenholtz G. *Streptococci* from root canals in teeth with apical periodontitis receiving endodontic treatment. OOOOE 2005;100:232-41

Chavez de Paz L. Redefining the persistent infection in root canals: possible role of biofilm communities. J Endod 2007;33:652-62

Chavez de Paz LE, Bergenholtz G, Dahlen G, Svensater G. Response to alkaline stress by root canal bacteria in biofilms. Int Endod J 2007;40:344-55

Chavez de Paz LE, Molander A, Dahlén G. Gram-positive rods prevailing in teeth with apical periodontitis undergoing root canal treatment. Int Endod J 2004;37:579-87

Chavez de Paz LE, Bergenholtz G, Svensater G. The effects of antimicrobials on endodontic biofilm bacteria. J Endod 2010;36:70-7.

Chavez de Paz Villanueva LE. *Fusobacterium nucleatum* in endodontic flare-ups. OOOOE 2002;93:179-83

Chen V, Chen Y, Li H, Kent K, Baumgartner JC, Machida CA. Herpesviruses in abscesses and cellulitis of endodontic origin. J Endod 2009;35:182-8.

Cheung GS, Ho MW. Microbial flora of root canal-treated teeth associated with asymptomatic periapical radiolucent lesions. Oral Microbiol Immunol 2001;16:332-7

Chhour KL, Nadkarni MA, Byun R, Martin FE, Jacques NA, Hunter N. Molecular analysis of microbial diversity in advanced caries. J Clin Microbiol 2005;43:843-9

Chivatxaranukul P, Dashper SG, Messer HH. Dentinal tubule invasion and adherence by *Enterococcus faecalis*. Int Endod J 2008;41:873-82.

Chow TW. Mechanical effectiveness of root canal irrigation. J Endod 1983;11:475-9

Christensen CE, McNeal SF, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. J Endod 2008;34:449-52

Chu FC, Tsang CS, Chow TW, Samaranayake LP. Identification of cultivable microorganisms from primary endodontic infections with exposed and unexposed pulp space. J Endod 2005;31:424-9

Chugal N, Wang JK, Wang R, He X, Kang M, Li J, Zhou X, Shi W, Lux R. Molecular characterization of the microbial flora residing at the apical portion of infected root canals of human teeth. J Endod. 2011 Oct;37(10):1359-64.

Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod 2006;32:434-7

Cvek M, Nord CE, Hollender L. Antimicrobial effect of root canal debridement in teeth with immature root. A clinical and microbiological study. Odontol Revy 1976;27:1-10

Dahle UR, Tronstad L, Olsen I. Spirochaetes in oral infections. Endod Dent Traumatol 1993;9:87-94

Dahlén G, Bergenholtz G. Endotoxic activity in teeth with necrotic pulps. J Dent Res 1980;59:1033-40

Dahlén G, Magnuson BC, Möller AJ. Histological and histochemical study of the influence of lipopolysaccharide extracted from *Fusobacterium nucleatum* on the periapical tissues in the monkey *Macaca fascicularis*. Arch Oral Biol 1981;26:591-8

Dahlén G, Samuelsson W, Molander A, Reit C. Identification and antimicrobial susceptibility of enterococci isolated from the root canal. Oral Microbiol Immunol 2000;15:309-12

de Groot SD, Verhaagen B, Versluis M, Wu MK, Wesselink PR, van der Sluis LW. Laser-activated irrigation within root canals: cleaning efficacy and flow visualization. Int Endod J. 2009 Dec;42(12):1077-83.

de Oliveira LD, Jorge AO, Carvalho CA, Koga-Ito CY, Valera MC. *In vitro* effects of endodontic irrigants on endotoxins in root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:135-42

de Souza CA, Teles RP, Souto R, Chaves MA, Colombo AP. Endodontic therapy associated with calcium hydroxide as an intracanal dressing: microbiologic evaluation by the checkerboard DNA-DNA hybridization technique. J Endod 2005;31:79-83

Debelian GJ, Olsen I, Tronstad L. Anaerobic bacteremia and fungemia in patients undergoing endodontic therapy: an overview. Ann Periodontol 1998;3:281-7

Debelian GJ, Olsen I, Tronstad L. Bacteremia in conjunction with endodontic therapy. Endod Dent Traumatol 1995;11:142-9

Debelian GJ, Olsen I, Tronstad L. Observation of *Saccharomyces cerevisiae* in blood of patient undergoing root canal treatment. Int Endod J 1997;30:313-7

Del Carpio-Perochena AE, Bramante CM, Duarte MA, Cavenago BC, Villas-Boas MH, Graeff MS, Bernardineli N, de Andrade FB, Ordinola-Zapata R. Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin. J Endod. 2011 Aug;37(8):1134-8.

Delivanis PD, Fan VSC. The localization of blood-borne bacteria in instrumented unfilled and overinstrumented canals. J Endod 1984;10:521-4

Delivanis PD, Snowden RB, Doyle RJ. Localization of blood-borne bacteria in instrumented unfilled root canals. OOO 11981;52:430-2

Deng DM, Hoogenkamp MA, Exterkate RA, Jiang LM, van der Sluis LW, Ten Cate JM, Crielaard W. Influence of *Streptococcus mutans* on *Enterococcus faecalis* biofilm formation. J Endod. 2009 Sep;35(9):1249-52.

Dewsnup N, Pileggi R, Haddix J, Nair U, Walker C, Varella CH. Comparison of bacterial reduction in straight and curved canals using erbium, chromium:yttrium-scandium-gallium-garnet laser treatment versus a traditional irrigation technique with sodium hypochlorite. J Endod. 2010 Apr;36(4):725-8.

Didilescu AC, Rusu D, Anghel A, Nica L, Iliescu A, Greabu M, Bancescu G, Stratul SI. Investigation of six selected bacterial species in endo-periodontal lesions. Int Endod J. 2012;45:282-93

Dougherty WJ, Bae KS, Watkins BJ, Baumgartner JC. Black-pigmented bacteria in coronal and apical segments of infected root canals. J Endod 1998;24:356-8

Duggan J, Sedgley CM. Biofilm formation of oral and endodontic Enterococcus faecalis. J Endod 2007;33:815-8

Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. J Endod 2006;32:527-31

Dwyer TG, Torabinejad M. Radiographic and histologic evaluation of the effect of endotoxin on the periapical tissues of the cat. J Endod 1981;7:31-5

Egan MW, Spratt DA, Ng YL, Lam JM, Moles DR, Gulabivala K. Prevalence of yeasts in saliva and root canals of teeth associated with apical periodontitis. Int Endod J 2002;35:321-9

Eldeniz AU, Ozer F, Hadimli HH, Erganis O. Bactericidal efficacy of Er,Cr:YSGG laser irradiation against *Enterococcus faecalis* compared with NaOCl irrigation: an ex vivo pilot study. Int Endod J 2007;40:112-9

Elkins DA, Torabinejad M, Schmidt RE, Rossi JJ, Kettering JD. Polymerase chain reaction detection of human immunodeficiency virus DNA in human periradicular lesions. J Endod 1994;20:386-8

Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. Int Endod J 2002;35:221-8

Fabricius L, Dahlén G, Holm S, Möller AJR. Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res 1982;90:200-6

Fabricius L, Dahlén G, Ohman AE, Möller AJR. Predominant indigenous oral bacteria isolated from infected root canals after varied times of closure. Scand J Dent Res 1982;90:134-44

Falk K, Sedgley CM. The influence of preparation size on the mechanical efficacy of root canal irrigation *in vitro*. J Endod 2005;31:742-5

Ferreira DC, Paiva SS, Carmo FL, Rôças IN, Rosado AS, Santos KR, Siqueira JF Jr. Identification of herpesviruses types 1 to 8 and human papillomavirus in acute apical abscesses. J Endod. 2011 Jan;37(1):10-6.

Figdor D, Davies J. Cell surface structures of *Actinomyces israelii*. Aust Dent J 1997;42:125-8

Fimple JL, Fontana CR, Foschi F, Ruggiero K, Song X, Pagonis TC, Tanner AC, Kent R, Doukas AG, Stashenko PP, Soukos NS. Photodynamic treatment of endodontic polymicrobial infection in vitro. J Endod 2008;34:728-34

Finnegan S, Percival SL. EDTA: An Antimicrobial and Antibiofilm Agent for Use in Wound Care. Adv Wound Care (New Rochelle) 2015;4:415-21.

Fish EW. Bone infection. JADA 1939;26: 691-712

Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010 Aug 2.[Epub ahead of print]

Foschi F, Cavrini F, Montebugnoli L, Stashenko P, Sambri V, Prati C. Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients. Oral Microbiol Immunol 2005;20:289-95

Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R, Hazlett K, Radolf JD. PCR-based identification of bacteria associated with endodontic infections. J Clin Microbiol 2002;40:3223-31

Fouad AF, Zerella J, Barry J, Spangberg LS. Molecular detection of *Enterococcus* species in root canals of therapy-resistant endodontic infections. OOOOE 2005;99:112-8

Fransen JN, He J, Glickman GN, Rios A, Shulman JD, Honeyman A. Comparative assessment of ActiV GP/glass ionomer sealer, Resilon/Epiphany, and gutta-percha/AH plus obturation: a bacterial leakage study. J Endod 2008;34:725-7

Fransson H, Larsson KM, Wolf E. Efficacy of lasers as an adjunct to chemo-mechanical disinfection of infected root canals: a systematic review. Int Endod J 2013;46:296-307

Gatti JJ, Dobeck JM, Smith C, Socransky SS, Skobe Z. Bacteria of asymptomatic periradicular endodontic lesions identified by DNA-DNA hybridization. Endod Dent Traumatol 2000;16:197-204

Gharbia SE, Haapasalo M, Shah HN, Kotiranto A, Lounatmaa K, Pearce MA, Devine DA. Characterization of *Prevotella intermedia* and *Prevotella nigrescens* isolates from periodontic and endodontic infections. J Periodontol 1994;65:56-61

Giardino L, Pontieri F, Savoldi E, Tallarigo F. *Aspergillus mycetoma* of the maxillary sinus secondary to overfilling of a root canal. J Endod 2006;32:692-4

Gier RE, Mitchell DF. Anachoretic effect of pulpitis. J Dent Res 1968 ;47:564-70

Gill DS, Tredwin CJ, Gill SK, Ironside JW. The transmissible spongiform encephalopathies (prion diseases): a review for dental surgeons. Int Dent J 2001;51:439-46

Glick M, Trope M, Bagasra O, Pliskin ME. Human immunodeficiency virus infection of fibroblasts of dental pulp in seropositive patients. OOO 1991;71:733-6

Gnau HL, Goodell GG, Imamura GM. Rapid chairside sterilization of endodontic files using 6% sodium hypochlorite. J Endod. 2009 Sep;35(9):1253-4.

Gomes BP, Ferraz CC, Garrido FD, Rosalen PL, Zaia AA, Teixeira FB, de Souza-Filho FJ. Microbial susceptibility to calcium hydroxide pastes and their vehicles. J Endod 2002;28:758-61

Gomes BP, Jacinto RC, Pinheiro ET, Sousa EL, Zaia AA, Ferraz CC, Souza-Filho FJ. *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia* and *Prevotella nigrescens* in endodontic lesions detected by culture and by PCR. Oral Microbiol Immunol 2005;20:211-5

Gomes BP, Jacinto RC, Pinheiro ET, Sousa EL, Zaia AA, Ferraz CC, Souza-Filho FJ. Molecular analysis of *Filifactor alocis*, *Tannerella forsythia*, and *Treponema denticola* associated with primary endodontic infections and failed endodontic treatment. J Endod 2006;32:937-40

Gomes BP, Martinho FC, Vianna ME. Comparison of 2.5% sodium hypochlorite and 2% chlorhexidine gel on oral bacterial lipopolysaccharide reduction from primarily infected root canals. J Endod. 2009 Oct;35(10):1350-3.

Gomes BP, Montagner F, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Polymerase chain reaction of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* in primary endodontic infections. J Endod 2007;33:1049-52.

Gomes BP, Endo MS, Martinho FC. Comparison of endotoxin levels found in primary and secondary endodontic infections. J Endod 2012;38:1082-6

Gomes BP, Sato E, Ferraz CC, Teixeira FB, Zaia AA, Souza-Filho FJ. Evaluation of time required for recontamination of coronally sealed canals medicated with calcium hydroxide and chlorhexidine. Int Endod J 2003;36:604-9

Gomes BPFA, Lilley JD, Drucker DB. Association of endodontic symptoms and signs with particular combinations of specific bacteria. Int J Endod 1996;29:69-75

Gomes C, Fidel S, Fidel R, de Moura Sarquis MI. Isolation and taxonomy of filamentous fungi in endodontic infections. J Endod. 2010 Apr;36(4):626-9.

Grossman LI. Penicillin treatment of pulpless teeth. J Endod 1946;1:30-2

Grossman LI. Polyantibiotic treatment of pulpless teeth. J Am Dent Assoc 1951;43:265-78

Grossman LI. Treatment of pulpless teeth with a concentrated sulfonamide solution. J Am Dent Assoc 1945;32:1432-6

Haapasalo M, Qian W, Portenier I, Waltimo T. Effects of dentin on the antimicrobial properties of endodontic medicaments. J Endod 2007;33:917-25

Haapasalo M, Ranta H, Ranta K, Shah H. Black-pigmented *Bacteroides* spp. in human apical periodontitis. Infect Immun 1986;53:149-53

Hahn CL, Falkler WA Jr, Minah GE. Correlation between thermal sensitivity and microorganisms isolated from deep carious dentin. J Endod 1993;19:26-30

Hancock III HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population. OOOOE 2001;91;579-86

Happonen R-P. Periapical actinomycosis: A follow-up study of 16 surgically treated cases. Endod Dent Traumatol 1986;2:205-9

Hashioka K, Suzuki K, Yoshida T, Nakane A, Horiba N, Nakamura H. Relationship between clinical symptoms and enzyme-producing bacteria isolated from infected root canals. J Endod 1994;20:75-7

Hashioka K, Yamasaki M, Nakane A, Horiba N, Nakamura H. The relationship between clinical symptoms and anaerobic bacteria from infected root canals. J Endod 1992;18:558-61

Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA. An *in vitro* evaluation of the ability of ozone to kill a strain of *Enterococcus faecalis*. Int Endod J 2005;38:22-9

Henry M, Reader A, Beck M. Effect of penicillin on postoperative endodontic pain and swelling in symptomatic necrotic teeth. J Endod 2001;27:117-23

Hernádi K, Szalmás A, Mogyorósi R, Czompa L, Veress G, Csoma E, Márton I, Kónya J. Prevalence and activity of Epstein-Barr virus and human cytomegalovirus in symptomatic and asymptomatic apical periodontitis lesions. J Endod. 2010 Sep;36(9):1485-9.

Ho YC, Chang YC. Effects of a bacterial lipid byproduct on human pulp fibroblasts *in vitro*. J Endod 2007;33:437-41

Hoelscher AA, Bahcall JK, Maki JS. *In vitro* evaluation of the antimicrobial effects of a root canal sealer-antibiotic combination against *Enterococcus faecalis*. J Endod 2006;32:145-7

Horiba N, Maekawa Y, Abe Y, Ito M, Matsumoto T, Nakamura H. Correlations between endotoxin and clinical symptoms or radiolucent areas in infected root canals. OOO 1991;71:492-5

Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, Iwaku M. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J 1996 Mar;29(2):125-30

Huffaker SK, Safavi K, Spangberg LS, Kaufman B. Influence of a passive sonic irrigation system on the elimination of bacteria from root canal systems: a clinical study. J Endod. 2010 Aug;36(8):1315-8.

Huth KC, Quirling M, Maier S, Kamereck K, Alkhayer M, Paschos E, Welsch U, Miethke T, Brand K, Hickel R. Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J 2009;42:3-13.

Huynh T, Kapur RV, Kaplan CW, Cacalano N, Kinder Haake S, Shi W, Sieling P, Jewett A. The role of aggregation in Fusobacterium nucleatum- induced immune cell death. J Endod. 2011 Nov;37(11):1531-5.

Itoh T, Nakamura H, Kishi J, Hayakawa T. The activation of matrix metalloproteinases by a whole-cell extract from *Prevotella nigrescens*. J Endod 2009;35:55-9.

Iwu C, Macfarlane TW, MacKenzie. D, Stenhouse D. The microbiology of periapical granulomas. OOO 1990;69:502-5

Jacinto RC, Gomes BP, Ferraz CC, Zaia AA, Filho FJ. Microbiological analysis of infected root canals from symptomatic and asymptomatic teeth with periapical periodontitis and the antimicrobial susceptibility of some isolated anaerobic bacteria. Oral Microbiol Immunol 2003;18:285-92

Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ. Incidence and antimicrobial susceptibility of *Porphyromonas gingivalis* isolated from mixed endodontic infections. Int Endod J 2006;39:62-70

Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ. Quantification of endotoxins in necrotic root canals from symptomatic and asymptomatic teeth. J Med Microbiol 2005;54:777-83

Jaramillo DE, Arriola A, Safavi K, Chavez de Paz LE. Decreased bacterial adherence and biofilm growth on surfaces coated with a solution of benzalkonium chloride. J Endod 2012;38:821-5

Jha D, Guerrero A, Ngo T, Helfer A, Hasselgren G. Inability of laser and rotary instrumentation to eliminate root canal infection. JADA 2006;137:67-70.

Jiang YT, Xia WW, Li CL, Jiang W, Liang JP. Preliminary study of the presence and association of bacteria and archaea in teeth with apical periodontitis. Int Endod J. 2009 Dec;42(12):1096-103.

Johnson EM, Flannagan SE, Sedgley CM. Coaggregation interactions between oral and endodontic Enterococcus faecalis and bacterial species isolated from persistent apical periodontitis. J Endod 2006;32:946-50

Jung I, Choi B, Kum K, Yoo Y, Yoon T, Lee S, Lee C. Identification of oral spirochetes at the species level and their association with other bacteria in endodontic infections. OOOOE 2001;92:329-34

Jung IY, Choi BK, Kum KY, Roh BD, Lee SJ, Lee CY, Park DS. Molecular epidemiology and association of putative pathogens in root canal infection. J Endod 2000;26:599-604

Jung IY, Lee SJ, Hargreaves KM. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. J Endod 2008 Jul;34(7):876-87

Jungermann GB, Burns K, Nandakumar R, Tolba M, Venezia RA, Fouad AF. Antibiotic resistance in primary and persistent endodontic infections. J Endod. 2011 Oct;37(10):1337-44.

Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. OOO 1965:20:340-9

Kakoli P, Nandakumar R, Romberg E, Arola D, Fouad AF. The effect of age on bacterial penetration of radicular dentin. J Endod 2009;35:78-81.

Kampfer J, Gohring TN, Attin T, Zehnder M. Leakage of food-borne *Enterococcus faecalis* through temporary fillings in a simulated oral environment. Int Endod J 2007;40:471-7

Kanter V, Weldon E, Nair U, Varella C, Kanter K, Anusavice K, et al. A quantitative and qualitative analysis of ultrasonic versus sonic endodontic systems on canal cleanliness and obturation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:809-13

Kantz WE, Henry CA. Isolation and classification of anaerobic bacteria from intact chambers of non-vital teeth in man. Arch Oral Biol 1974;19:91-6.

Kaufman B, Spangberg L, Barry J, Fouad AF. *Enterococcus* spp. in endodontically treated teeth with and without periradicular lesions. J Endod 2005;31:851-6.

Kayaoglu G, Erten H, Orstavik D. Growth at high pH increases *Enterococcus faecalis* adhesion to collagen. Int Endod J 2005;38:389-96

Keenan JV, Farman AG, Fedorowicz Z, Newton JT. A Cochrane systematic review finds no evidence to support the use of antibiotics for pain relief in irreversible pulpitis. J Endod 2006;32:87-92

Khemaleelakul S, Baumgartner JC, Pruksakom S. Autoaggregation and coaggregation of bacteria associated with acute endodontic infections. J Endod 2006;32:312-8

Khemaleelakul S, Baumgartner JC, Pruksakorn S. Identification of bacteria in acute endodontic infections and their antimicrobial susceptibility. OOOOE 2002;94:746-55

Kiryu T, Hoshino E, Iwaku M. Bacteria invading periapical cementum. J Endod 1994;20:169-72

Kishen A, Shi Z, Shrestha A, Neoh KG. An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. J Endod 2008;34:1515-20.

Kishen A, Sum CP, Mathew S, Lim CT. Influence of irrigation regimens on the adherence of *Enterococcus faecalis* to root canal dentin. J Endod 2008;34:850-4

Kobayashi T, Hayashi A, Yoshikawa R, Okuda K, Hara K. The microbial flora from root canals and periodontal pockets of non-vital teeth associated with advanced periodontitis. Int Endod J 1990;23:100-6

Kowalski WJ, Kasper EL, Hatton JF, Murray BE, Nallapareddy SR, Gillespie MJ. *Enterococcus faecalis* adhesin, ace, mediates attachment to particulate dentin. J Endod 2006;32:634-7

Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on *Enterococcus faecalis*. J Endod 2007;33:28-30

Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endod 2007;33:1473-6.

Langeland K, Rodrigues H, Dowden W. Periodontal disease, bacteria, and pulpal histopathology. OOO 1974;37:257-70

Law A, Messer H. An evidence-based analysis of the antibacterial effectiveness of intracanal medicaments. J Endod 2004;30:689-94

LeCorn DW, Vertucci FJ, Rojas MF, Progulske-Fox A, Belanger M. In vitro activity of amoxicillin, clindamycin, doxycycline, metronidazole, and moxifloxacin against oral *Actinomyces*. J Endod 2007;33:557-60

Lee JK, Baik JE, Yun CH, Lee K, Han SH, Lee W, Bae KS, Baek SH, Lee Y, Son WJ, Kum KY. Chlorhexidine gluconate attenuates the ability of lipoteichoic acid from *Enterococcus faecalis* to stimulate toll-like receptor 2. J Endod 2009;35:212-5.

Leonardo MR, Rossi MA, Silva LA, Ito IY, Bonifacio KC. SEM evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth. J Endod 2002;28:815-8

Leung KP, Fukushima H, Nesbitt WE, Clark WB. *Prevotella intermedia* fimbriae mediate hemagglutination. Oral Microbiol Immunol 1996;11:42-50

Lewis MA, MacFarlane TW, McGowan DA. Quantitative bacteriology of acute dento-alveolar abscesses. J Med Microbiol 1986;21:101-4

Li H, Chen V, Chen Y, Baumgartner JC, Machida CA. Herpesviruses in endodontic pathoses: association of Epstein-Barr virus with irreversible pulpitis and apical periodontitis. J Endod 2009;35:23-9.

Li L, Hsiao WW, Nandakumar R, Barbuto SM, Mongodin EF, Paster BJ, Fraser-Liggett CM, Fouad AF. Analyzing endodontic infections by deep coverage pyrosequencing. J Dent Res. 2010 Sep;89(9):980-4.

Lindeboom JA, Frenken JW, Valkenburg P, van den Akker HP. The role of preoperative prophylactic antibiotic administration in periapical endodontic surgery: a randomized, prospective double-blind placebo-controlled study. Int Endod J 2005;38:877-81

Liu H, Wei X, Ling J, Wang W, Huang X. Biofilm formation capability of *Enterococcus faecalis* cells in starvation phase and its susceptibility to sodium hypochlorite. J Endod. 2010 Apr;36(4):630-5.

Lovato KF, Sedgley CM. Antibacterial activity of endosequence root repair material and proroot MTA against clinical isolates of Enterococcus faecalis. J Endod. 2011 Nov;37(11):1542-6.

Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med 2002; 13:171-83

Lui JN, Sae-Lim V, Song KP, Chen NN. *In vitro* antimicrobial effect of chlorhexidine-impregnated gutta percha points on *Enterococcus faecalis*. Int Endod J 2004;37:105-13

Lundstrom JR, Williamson AE, Villhauer AL, Dawson DV, Drake DR. Bactericidal activity of stabilized chlorine dioxide as an endodontic irrigant in a polymicrobial biofilm tooth model system. J Endod. 2010 Nov;36(11):1874-8.

Ma J, Wang Z, Shen Y, Haapasalo M. A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. J Endod. 2011 Oct;37(10):1380-5.

Ma Z, Wang Y, Zhu X, Zhang C, Li S, Jin L, Shen Y, Haapasalo M. Role of polymorphonuclear neutrophils in the clearance of *Enterococcus faecalis* derived from saliva and infected root canals. J Endod. 2011 Mar;37(3):346-52.

MacDonald JB, Hare GC, Wood AWS. The bacteriologic status of the pulp chambers in intact teeth found to be nonvital following trauma. OOO 1957;10:318-22

Malkhassian G, Manzur AJ, Legner M, Fillery ED, Manek S, Basrani BR, Friedman S. Antibacterial efficacy of MTAD final rinse and two percent chlorhexidine gel medication in teeth with apical periodontitis: a randomized double-blinded clinical trial. J Endod. 2009 Nov;35(11):1483-90.

Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod 2007;33:114-8

Martin FE, Nadkarni MA, Jacques NA, Hunter N. Quantitative microbiological study of human carious dentine by culture and real-time PCR: association of anaerobes with histopathological changes in chronic pulpitis. J Clin Microbiol 2002;40:1698-704

Martinho FC, Gomes BP. Quantification of endotoxins and cultivable bacteria in root canal infection before and after chemomechanical preparation with 2.5% sodium hypochlorite. J Endod 2008;34:268-72

Mathew S, Yaw-Chyn L, Kishen A. Immunogenic potential of *Enterococcus faecalis* biofilm under simulated growth conditions. J Endod. 2010 May;36(5):832-6.

McGurkin-Smith R, Trope M, Caplan D, Sigurdsson A. Reduction of intracanal bacteria using GT rotary instrumentation, 5.25% NaOCl, EDTA, and $Ca(OH)_2$. J Endod 2005;31:359-63

Meire MA, De Prijck K, Coenye T, Nelis HJ, De Moor RJ. Effectiveness of different laser systems to kill Enterococcus faecalis in aqueous suspension and in an infected tooth model. Int Endod J. 2009 Apr;42(4):351-9.

Melker KB, Vertucci FJ, Rojas MF, Progulske-Fox A, Belanger M. Antimicrobial efficacy of medicated root canal filling materials. J Endod 2006;32:148-51

Metzger Z, Better H, Abramovitz I. Immediate root canal disinfection with ultraviolet light: an *ex vivo* feasibility study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:425-33.

Metzger Z, Lin YY, Dimeo F, Ambrose WW, Trope M, Arnold RR. Synergistic pathogenicity of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in the mouse subcutaneous chamber model. J Endod 2009;35:86-94.

Mitchell RP, Baumgartner JC, Sedgley CM. Apical extrusion of sodium hypochlorite using different root canal irrigation systems. J Endod. 2011 Dec;37(12):1677-81.

Mohammadi Z, Abbott PV. On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology. Int Endod J. 2009 Jul;42(7):555-67. Review.

Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1-7

Molander A, Reit C, Dahlén G. The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. Endod Dent Traumatol 1999;15:205-9

Möller AJ. Microbiological examination of root canals and periapical tissues of human teeth. Thesis Odontol Tidskr 1966; spec 74

Möller AJR, Fabricius L Dahlén G, Ohman Ae, Heydon G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981:89:475-84

Montagner F, Jacinto RC, Signoretti FG, Gomes BP. *Treponema* species detected in infected root canals and acute apical abscess exudates. J Endod. 2010 Nov;36(11):1796-9.

Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capacity of sodium hypochlorite. Int Endod J 1982;15:187-96

Moraes SR, Siqueira JF, Colombo AP, Rôças IN, Ferreira M, Domingues R. Comparison of the effectiveness of bacterial culture, 16S rDNA directed polymerase chain reaction, and checkerboard DNA-DNA hybridization for detection of *Fusobacterium nucleatum* in endodontic infections. J Endod 2002;28:86-9

Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res 2002 ;81:761-6

Nabeshima CK, Caballero-Flores H, Cai S, Aranguren J, Borges Britto ML, Machado ME. Bacterial removal promoted by 2 single-file systems: Wave One and One Shape. J Endod 2014;40:1995-8

Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod 2004;30:778-81

Nagle D, Reader A, Beck M, Weaver J. Effect of systemic penicillin on pain in untreated irreversible pulpitis. OOOOE 2000;90:636-40

Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. OOOOE 2005;99:231-52

Nair PN, Sjögren U, Figdor D, Sundqvist G. Persistent periapical radiolucencies of root-filled human teeth, failed endodontic treatments, and periapical scars. OOOOE 1999 ;87:617-27

Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249-81

Nair PNR, Sjögren U, Krey G, Kahnberg K-E, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod 1990;16:580-8

Nakajo K, Nakazawa F, Iwaku M, Hoshino E. Alkali-resistant bacteria in root canal systems. Oral Microbiol Immunol 2004;19:390-4

Nakamura VC, Cai S, Candeiro GT, Ferrari PH, Caldeira CL, Gavini G. Ex vivo evaluation of the effects of several root canal preparation techniques and irrigation regimens on a mixed microbial infection. Int Endod J 2013;46:217-24

Nandakumar R, Madayiputhiya N, Fouad AF. Proteomic analysis of endodontic infections by liquid chromatography-tandem mass spectrometry. Oral Microbiol Immunol. 2009 Aug;24(4):347-52.

Newberry BM, Shabahang S, Johnson N, Aprecio RM, Torabinejad M. The antimicrobial effect of biopure MTAD on eight strains of *Enterococcus faecalis*: an in vitro investigation. J Endod 2007;33:1352-4.

Ng R, Singh F, Papamanou DA, Song X, Patel C, Holewa C, Patel N, Klepac-Ceraj V, Fontana CR, Kent R, Pagonis TC, Stashenko PP, Soukos NS. Endodontic photodynamic therapy ex vivo. J Endod. 2011 Feb;37(2):217-22.

Ng YL, Spratt D, Sriskantharajah S, Gulabivala K. Evaluation of protocols for field decontamination before bacterial sampling of root canals for contemporary microbiology techniques. J Endod 2003;29:317-20

Nguy D, Sedgley C. The influence of canal curvature on the mechanical efficacy of root canal irrigation in vitro using real-time imaging of bioluminescent bacteria. J Endod 2006;32:1077-80.

Nielsen BA, Craig Baumgartner J. Comparison of the EndoVac system to needle irrigation of root canals. J Endod 2007;33:611-5

Ning Y, Hu X, Ling J, Du Y, Liu J, Liu H, et al. Candida albicans survival and biofilm formation under starvation conditions. Int Endod J 2013;46:62-70

Noda M, Inoue S, Komatsu H. A comparison of methods for detecting bacteria in root canal exudates. J Endod 1999;25:187-9

Noiri Y, Ehara A, Kawahara T, Takemura N, Ebisu S. Participation of bacterial biofilms in refractory and chronic periapical periodontitis. J Endod 2002;28:679-83

Noiri Y, Katsumoto T, Azakami H, Ebisu S. Effects of Er:YAG laser irradiation on biofilm-forming bacteria associated with endodontic pathogens in vitro. J Endod 2008;34:826-9

Nowicki JB, Sem DS. An in vitro spectroscopic analysis to determine the chemical composition of the precipitate formed by mixing sodium hypochlorite and chlorhexidine. J Endod 2011;37:983-8.

Ogawa AT, Brasil de Souza Tde A, de Uzeda M, Jankevicius JV, Jankevicius SI. Characterization of proteolytic activities of *Fusobacterium nucleatum*. J Endod 2006;32:521-3

Oguntebi B, Slee AM, Tanzer JM, Langeland K. Predominant microflora associated with human dental periapical abscesses. J Clin Microbiol 1982;15:964-66

Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990;6:142-9

Orstavik D, Kerekes K, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. Int Endod J 1991;24:1-7

Ozdemir HO, Buzoglu HD, Calt S, Stabholz A, Steinberg D. Effect of ethylenediaminetetraacetic acid and sodium hypochlorite irrigation on *Enterococcus faecalis* biofilm colonization in young and old human root canal dentin: in vitro study. J Endod 2010;36:842-6.

Özok AR, Wu MK, Luppens SBI, Wesselink PR. Comparison of growth and susceptibility to sodium hypochlorite of mono- and dual-species biofilms of *Fusobacterium nucleatum* and *Peptostreptococcus (micromonas) micros*. J Endod 2007;33:819-22

Pan J, Sun K, Liang Y, Sun P, Yang X, Wang J, et al. Cold plasma therapy of a tooth root canal infected with Enterococcus faecalis biofilms in vitro. J Endod 2013;39:105-10

Paquette L, Legner M, Fillery ED, Friedman S. Antibacterial efficacy of chlorhexidine gluconate intracanal medication in vivo. J Endod 2007;33:788-95

Parmar D, Hauman CH, Leichter JW, McNaughton A, Tompkins GR. Bacterial localization and viability assessment in human *ex vivo* dentinal tubules by fluorescence confocal laser scanning microscopy. Int Endod J 2011;44:644-51.

Pawar R, Alqaied A, Safavi K, Boyko J, Kaufman B. Influence of an apical negative pressure irrigation system on bacterial elimination during endodontic therapy: a prospective randomized clinical study. J Endod 2012;38:1177-81.

Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root filled teeth with chronic apical periodontitis. Int J Endod 2001;34:429-34

Persoon IF, Hoogenkamp MA, Bury A, Wesselink PR, Hartog AF, Wever R, Crielaard W. Effect of Vanadium Chloroperoxidase on *Enterococcus faecalis* Biofilms. J Endod. 2012 Jan;38(1):72-4.

Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J 2002;35:13-21

Peters LB, Wesselink PR, Buijs JF, van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod 2001;27:76-81.

Peters LB, Wesselink PR, Moorer WR. The fate and role of bacteria left in dentinal tubules. Int J Endod 1995;28:95-9

Peters LB, Wesselink PR, van Winkelhoff AJ. Combinations of bacterial species in endodontic infections. Int J Endod 2002;35:698-702

Peters LB, Wesselink PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms. Int J Endod 2002;35:660-7

Peters OA, Bardsley S, Fong J, Pandher G, Divito E. Disinfection of root canals with photon-initiated photoacoustic streaming. J Endod 2011;37:1008-12.

Phee A, Bondy-Denomy J, Kishen A, Basrani B, Azarpazhooh A, Maxwell K. Efficacy of bacteriophage treatment on Pseudomonas aeruginosa biofilms. J Endod 2013;39:364-9.

Pinheiro ET, Anderson MJ, Gomes BP, Drucker DB. Phenotypic and genotypic identification of enterococci isolated from canals of root-filled teeth with periapical lesions. Oral Microbiol Immunol 2006;21:137-44

Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J 2003;36:1-11

Plotino G, Pameijer CH, Maria Grande N, Somma F. Ultrasonics in endodontics: a review of the literature. J Endod 2007;33:81-95

Portenier I, Haapasalo H, Rye A, Waltimo T, Orstavik D, Haapasalo M. Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin. Int Endod J 2001;34:184-8

Portenier I, Waltimo T, Orstavik D, Haapasalo M. Killing of *Enterococcus faecalis* by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentine powder or BSA. J Endod 2006;32:138-41

Portenier I, Waltimo T, Orstavik D, Haapasalo M. The susceptibility of starved, stationary phase, and growing cells of *Enterococcus faecalis* to endodontic medicaments. J Endod 2005;31:380-6

Provenzano JC, Siqueira JF, Jr., Rocas IN, Domingues RR, Paes Leme AF, Silva MR. Metaproteome analysis of endodontic infections in association with different clinical conditions. PLoS One 2013;8:e76108.

Provenzano JC, Rocas IN, Tavares LF, Neves BC, Siqueira JF, Jr. Short-chain Fatty Acids in Infected Root Canals of Teeth with Apical Periodontitis before and after Treatment. J Endod 2015;41:831-5.

Quah SY, Wu S, Lui JN, Sum CP, Tan KS. N-Acetylcysteine inhibits growth and eradicates biofilm of *Enterococcus faecalis*. J Endod. 2012 Jan;38(1):81-5.

Rasimick BJ, Nekich M, Hladek MM, Musikant BL, Deutsch AS. Interaction between chlorhexidine digluconate and EDTA. J Endod 2008;34:1521-3.

Rechenberg DK, Thurnheer T, Zehnder M. Potential systematic error in laboratory experiments on microbial leakage through filled root canals: an experimental study. Int Endod J. 2011 Sep;44(9):827-35.

Reynaud af Geijersstam A, Sorsa T, Stackelberg S, Tervahartiala T, Haapasalo M. Effect of *E. faecalis* on the release of serine proteases elastase and cathepsin G, and collagenase-2 (MMP-8) by human polymorphonuclear leukocytes (PMNs). Int Endod J 2005;38:667-77

Reynaud Af Geijersstam AH, Ellington MJ, Warner M, Woodford N, Haapasalo M. Antimicrobial susceptibility and molecular analysis of *Enterococcus faecalis* originating from endodontic infections in Finland and Lithuania. Oral Microbiol Immunol 2006;21:164-8

Ribeiro AC, Matarazzo F, Faveri M, Zezell DM, Mayer MP. Exploring bacterial diversity of endodontic microbiota by cloning and sequencing 16S rRNA. J Endod 2011 Jul;37(7):922-6.

Richards D, Davies JK, Figdor D. Starvation survival and recovery in serum of *Candida albicans* compared with *Enterococcus faecalis*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Jul;110(1):125-30.

Richardson N, Mordan NJ, Figueiredo JA, Ng YL, Gulabivala K. Microflora in teeth associated with apical periodontitis: a methodological observational study comparing two protocols and three microscopy techniques. Int Endod J. 2009 Oct;42(10):908-21.

Ricucci D, Bergenholtz G. Bacterial status in root-filled teeth exposed to the oral environment by loss of restoration and fracture or caries – a histobacteriological study of treated cases. Int Endod J 2003;36:787-802

Ricucci D, Grondahl K, Bergenholtz G. Periapical status of root-filled teeth exposed to the oral environment by loss of restoration or caries. OOOOE 2000;90:354-9

Ricucci D, Pascon EA, Ford TR, Langeland K. Epithelium and bacteria in periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:239-49

Ricucci D, Siqueira JF Jr, Bate AL, Pitt Ford TR. Histologic investigation of root canal-treated teeth with apical periodontitis: a retrospective study from twenty-four patients. J Endod. 2009 Apr;35(4):493-502.

Ricucci D, Siqueira JF Jr. Apical actinomycosis as a continuum of intraradicular and extraradicular infection: case report and critical review on its involvement with treatment failure. J Endod 2008;34:1124-9

Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod. 2010 Aug;36(8):1277-88.

Ricucci D, Siqueira JF, Jr., Lopes WS, Vieira AR, Rocas IN. Extraradicular infection as the cause of persistent symptoms: a case series. J Endod 2015;41:265-73.

Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM. *In vivo* evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. J Endod 1982;8:200-4

Rios A, He J, Glickman GN, Spears R, Schneiderman ED, Honeyman AL. Evaluation of photodynamic therapy using a light-emitting diode lamp against *Enterococcus faecalis* in extracted human teeth. J Endod 2011 Jun;37(6):856-9.

Robinson HBG, Boling LR. The anachoretic effect in pulpitis bacteriologic studies. JADA 1941;28:268-82

Rôças IN, Baumgartner JC, Xia T, Siqueira JF Jr. Prevalence of selected bacterial named species and uncultivated phylotypes in endodontic abscesses from two geographic locations. J Endod 2006;32:1135-8

Rôças IN, Siqueira JF Jr, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. J Endod 2004;30:315-20

Rôças IN, Siqueira JF Jr. Occurrence of two newly named oral treponemes - *Treponema parvum* and *Treponema putidum* - in primary endodontic infections. Oral Microbiol Immunol 2005;20:372-5

Rôças IN, Siqueira JF Jr. Comparison of the in vivo antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. J Endod. 2011 Feb;37(2):143-50.

Rôças IN, Siqueira JF Jr. Distribution of *Porphyromonas gingivalis* fimA genotypes in primary endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Mar;109(3):474-8.

Rôças IN, Siqueira JF Jr. *In vivo* antimicrobial effects of endodontic treatment procedures as assessed by molecular microbiologic techniques. J Endod. 2011 Mar;37(3):304-10.

Rocas IN, Siqueira JF, Jr. Detection of antibiotic resistance genes in samples from acute and chronic endodontic infections and after treatment. Arch Oral Biol 2013;58:1123-8.

Rocas IN, Lima KC, Assuncao IV, Gomes PN, Bracks IV, Siqueira JF, Jr. Advanced Caries Microbiota in Teeth with Irreversible Pulpitis. J Endod 2015;41:1450-5

Rocas IN, Siqueira JF, Jr. Characterization of microbiota of root canal-treated teeth with posttreatment disease. J Clin Microbiol 2012;50:1721-4.

Rocas IN, Siqueira JF Jr. Prevalence of new candidate pathogens *Prevotella baroniae*, *Prevotella multisaccharivorax* and as-yet-uncultivated *Bacteroidetes* clone X083 in primary endodontic infections. J Endod. 2009 Oct;35(10):1359-62.

Rôças IN, Siqueira JF, Santos KRN, Coelho AMA."Red complex" (*Bacteroides forsythus, Porphyromonas gingivalis* and *Treponema denticola)* in endodontic infections: a molecular approach. OOOOE 2001:91: 468-71

Rocas IN, Lima KC, Siqueira JF, Jr. Reduction in bacterial counts in infected root canals after rotary or hand nickel-titanium instrumentation--a clinical study. Int Endod J 2013;46:681-7

Rocha CT, Rossi MA, Leonardo MR, Rocha LB, Nelson-Filho P, Silva LA. Biofilm on the apical region of roots in primary teeth with vital and necrotic pulps with or without radiographically evident apical pathosis. Int Endod J 2008;41:664-9

Rodig T, Bozkurt M, Konietschke F, Hulsmann M. Comparison of the Vibringe system with syringe and passive ultrasonic irrigation in removing debris from simulated root canal irregularities. J Endod 2010;36:1410-3.

Rodrigues RC, Antunes HS, Neves MA, Siqueira JF, Jr., Rocas IN. Infection Control in Retreatment Cases: In Vivo Antibacterial Effects of 2 Instrumentation Systems. J Endod 2015;41:1600-5

Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie. D, Coldero L, Bagg J. Molecular identification of microorganisms from endodontic infections. J Clin Microbiol 2001;39:3282-9

Rosenthal S, Spangberg L, Safavi K. Chlorhexidine substantivity in root canal dentin. OOOOE 2004;98:488-92

Rossi-Fedele G, Roberts AP. A preliminary study investigating the survival of tetracycline resistant *Enterococcus faecalis* after root canal irrigation with high concentrations of tetracycline. Int Endod J 2007;40:772-7.

Ruff ML, McClanahan SB, Babel BS. In vitro antifungal efficacy of four irrigants as a final rinse. J Endod 2006;32:331-3

Rupf S, Kannengiesser S, Merte K, Pfister W, Sigusch B, Eschrich K. Comparison of profiles of key periodontal pathogens in periodontium and endodontium. Endod Dent Traumatol 2000;16:269-75

Saber MH, Schwarzberg K, Alonaizan FA, Kelley ST, Sedghizadeh PP, Furlan M, et al. Bacterial flora of dental periradicular lesions analyzed by the 454-pyrosequencing technology. J Endod 2012;38:1484-8.

Sabeti M, Daneshmand A, Simon JH, Slots J. Cytomegalovirus-infected inflammatory cells in dental periapical lesions. Oral Microbiol Immunol. 2009;24:434-6.

Safavi KE, Nichols FC. Alteration of biological properties of bacterial lipopolysaccharide by calcium hydroxide treatment. J Endod. 1994 Mar;20(3):127-9.

Safavi KE, Nichols FC. Effect of calcium hydroxide on bacterial lipopolysaccharide. J Endod. 1993 Feb;19(2):76-8

Sakamoto M, Rôças IN, Siqueira JF Jr, Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. Oral Microbiol Immunol 2006;21:112-22

Saleh IM, Ruyter IE, Haapasalo M, Orstavik D. Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers *in vitro*. Int Endod J 2004;37:193-8

Salman MI, Baumann MA, Hellmich M, Roggendorf MJ, Termaat S. SEM evaluation of root canal debridement with Sonicare CanalBrush irrigation. Int Endod J 2010;43:363-9

Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis. Int Endod J 2007;40:2-10

Sathorn C, Parashos P, Messer HH. How useful is root canal culturing in predicting treatment outcome? J Endod 2007;33:220-5

Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. Int Endod J 1996;29:118–24.

Sato T, Hoshino E. Uematsu H, Kota K, Iwaku M, Noda T. Bactericidal efficacy of a mixture of ciprofloxacin, metronidazole, minocycline and rifampicin against bacteria of carious and endodontic lesions of human deciduous teeth in vitro. Microb Ecol Health Dis 1992;5:171-7

Sato Y, Kishi J, Suzuki K, Nakamura H, Hayakawa T. Sonic extracts from a bacterium related to periapical disease activate gelatinase A and inactivate tissue inhibitor of metalloproteinases TIMP-1 and TIMP-2. Int Endod J. 2009;42(12):1104-11.

Schafer E, Bossmann K. Antimicrobial efficacy of chlorhexidine and two calcium hydroxide formulations against *Enterococcus faecalis*. J Endod 2005;31:53-6

Schein B, Schilder H. Endotoxin content in endodontically involved teeth. J Endod 1975;1:19-21

Schirrmeister JF, Liebenow AL, Braun G, Wittmer A, Hellwig E, Al-Ahmad A. Detection and eradication of microorganisms in root-filled teeth associated with periradicular lesions: an *in vivo* study. J Endod 2007;33:536-40

Schirrmeister JF, Liebenow AL, Pelz K, Wittmer A, Serr A, Hellwig E, Al-Ahmad A. New bacterial compositions in root-filled teeth with periradicular lesions. J Endod 2009;35:169-74.

Schneider K, Korkmaz Y, Addicks K, Lang H, Raab WH. Prion protein (PrP) in human teeth: an unprecedented pointer to PrP's function. J Endod 2007;33:110-3

Schonfeld SE, Greening AB, Glick DH, Frank AL, Simon JH, Herles SM. Endotoxic activity in periapical lesions. OOO 1982;53:82-7

Sedgley C, Applegate B, Nagel A, Hall D. Real-time imaging and quantification of bioluminescent bacteria in root canals *in vitro*. J Endod 2004;30:893-8

Sedgley C, Buck G, Appelbe O. Prevalence of *Enterococcus faecalis* at multiple oral sites in endodontic patients using culture and PCR. J Endod 2006;32:104-9

Sedgley CM, Lee EH, Martin MJ, Flannagan SE. Antibiotic resistance gene transfer between *Streptococcus gordonii* and *Enterococcus faecalis* in root canals of teeth *ex vivo*. J Endod 2008;34:570-4

Sedgley CM, Lennan SL, Appelbe OK. Survival of *Enterococcus faecalis* in root canals *ex vivo*. Int Endod J 2005;38:735-42

Sedgley CM, Lennan SL, Clewell DB. Prevalence, phenotype and genotype of oral enterococci. Oral Microbiol Immunol 2004;19:95-101

Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, Dahlén G. Virulence, phenotype and genotype characteristics of endodontic *Enterococcus* spp. Oral Microbiol Immunol 2005;20:10-9

Sedgley CM, Nagel A, Hall D, Applegate B. Influence of irrigant needle depth in removing root canal bacteria using real-time imaging of bioluminescent bacteria *in vitro*. Int Endod J 2005;38:97-104

Sedgley CM, Nagel AC, Dahlén G, Reit C, Molander A. Real-time quantitative PCR and culture analysis of *Enterococcus faecalis* in root canals. J Endod 2006;32:173-7

Sedgley CM, Nagel AC, Shelburne CE, Clewell DB, Appelbe O, Molander A. Quantitative real-time PCR detection of oral *Enterococcus faecalis* in humans. Arch Oral Biol 2005;50:575-83

Sedgley CM. The influence of root canal sealer and gelatinase activity on extended survival of Enterococcus faecalis in obturated root canals in vitro. J Endod 2007;33:561-6

Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endod Dent Traumatol 1995;11:6-9

Senges C, Wrbas KT, Altenburger M, Follo M, Spitzmüller B, Wittmer A, Hellwig E, Al-Ahmad A. Bacterial and Candida albicans adhesion on different root canal filling materials and sealers. J Endod. 2011 Sep;37(9):1247-52.

Seol JH, Cho BH, Chung CP, Bae KS. Multiplex polymerase chain reaction detection of black-pigmented bacteria in infections of endodontic origin. J Endod 2006;32:110-4

Shabahang S, Torabinejad M. Effect of MTAD on *Enterococcus faecalis*-contaminated root canals of extracted human teeth. J Endod 2003;29:576-9

Shen Y, Gao Y, Qian W, Ruse ND, Zhou X, Wu H, Haapasalo M. Three-dimensional numeric simulation of root canal irrigant flow with different irrigation needles. J Endod. 2010 May;36(5):884-9.

Shen Y, Qian W, Chung C, Olsen I, Haapasalo M. Evaluation of the effect of two chlorhexidine preparations on biofilm bacteria in vitro: a three-dimensional quantitative analysis. J Endod. 2009 Jul;35(7):981-5

Shen Y, Stojicic S, Haapasalo M. Antimicrobial efficacy of chlorhexidine against bacteria in biofilms at different stages of development. J Endod. 2011 May;37(5):657-61.

Shen Y, Stojicic S, Qian W, Olsen I, Haapasalo M. The synergistic antimicrobial effect by mechanical agitation and two chlorhexidine preparations on biofilm bacteria. J Endod. 2010 Jan;36(1):100-4.

Shovelton DS. The presence and distribution of microorganisms within non-vital teeth. Brit Dent J 1964;117:101-7

Shrestha A, Fong SW, Khoo BC, Kishen A. Delivery of antibacterial nanoparticles into dentinal tubules using high-intensity focused ultrasound. J Endod. 2009 Jul;35(7):1028-33.

Shrestha A, Kishen A. Antibiofilm efficacy of photosensitizer-functionalized bioactive nanoparticles on multispecies biofilm. J Endod 2014;40:1604–10

Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26:751-5

Shur AL, Sedgley CM, Fenno JC. The antimicrobial efficacy of 'MGP' gutta-percha *in vitro*. Int Endod J 2003;36:616-21

Singla MG, Garg A, Gupta S. MTAD in endodontics: an update review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112:e70-6

Siqueira JF Jr, Alves FR, Rôças IN. Pyrosequencing analysis of the apical rootcanal microbiota. J Endod. 2011 Nov;37(11):1499-503.

Siqueira JF Jr, Magalhaes KM, Rôças IN. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. J Endod 2007;33:667-72

Siqueira JF Jr, Rôças IN, Alves FR, Santos KR. Selected endodontic pathogens in the apical third of infected root canals: a molecular investigation. J Endod 2004;30:638-43

Siqueira JF Jr, Rôças IN, Oliveira JC, Santos KR. Molecular detection of black-pigmented bacteria in infections of endodontic origin. J Endod 2001;27:563-6

Siqueira JF Jr, Rôças IN, Paiva SS, Guimarães-Pinto T, Magalhães KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:122-3

Siqueira JF Jr, Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. OOOOE 2004;97:85-94

Siqueira JF Jr, Rôças IN. Catonella morbi and Granulicatella adiacens: new species in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:259-64

Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-1301

Siqueira JF Jr, Rocas IN. Community as the unit of pathogenicity: an emerging concept as to the microbial pathogenesis of apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Jun;107(6):870-8.

Siqueira JF Jr, Rôças IN. Detection of *Filifactor alocis* in endodontic infections associated with different forms of periradicular diseases. Oral Microbiol Immunol 2003;18:263-5

Siqueira JF Jr, Rôças IN. Polymerase chain reaction detection of *Propionibacterium propionicus* and *Actinomyces radicidentis* in primary and persistent endodontic infections. OOOOE 2003;96:215-22

Siqueira JF, Jr., Alves FR, Versiani MA, Rocas IN, Almeida BM, Neves MA, et al. Correlative bacteriologic and micro-computed tomographic analysis of mandibular molar mesial canals prepared by self-adjusting file, reciproc, and twisted file systems. J Endod 2013;39:1044-50

Siqueira JF Jr, Rôças IN. *Pseudoramibacter alactolyticus* in primary endodontic infections. J Endod 2003;29:735-8

Siqueira JF Jr, Rôças IN. *Treponema socranskii* in primary endodontic infections as detected by nested PCR. J Endod 2003;29:244-7

Siqueira JF Jr. Microbial causes of endodontic flare-ups. Int Endod J 2003;36:453-63

Siqueira JF Jr. Taxonomic changes of bacteria associated with endodontic infections. J Endod 2003;29:619-23

Siqueira JF, Jung IY, Rôças IN, Lee CY. Differences in prevalence of selected bacterial species in primary endodontic infections from two distinct geographic locations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:641-7

Siqueira JF, Paiva SS, Rôças IN. Reduction in the cultivable bacterial populations in infected root canals by a chlorhexidine-based antimicrobial protocol. J Endod 2007;33:541-7

Siqueira JF, Rôças IN, Andrade AFB, Uzeda M. *Peptostreptococcus micros* in primary endodontic infections as detected by 16S rDNA-based polymerase chain reaction. J Endod 2003;29:111-3
Siqueira JF, Rôças IN, Souto R, De Uzeda M, Colombo AP. Checkerboard DNA-DNA hybridization analysis of endodontic infections. OOOOE 2000;89:744-8
Siqueira JF, Rôças IN, Souto R, De Uzeda M, Colombo AP. Microbiological evaluation of acute periradicular abscesses by DNA-DNA hybridization. OOOOE 2001;92:451-7
Siqueira JF, Rôças IN. *Dialister pneumosintes* can be a suspected endodontic pathogen. OOOOE 2002;94:494-8
Siqueira JF. Endodontic infections: concepts, paradigms, and perspectives. OOOOE 2002;94:281-93
Siqueira Junior J, Magalhaes FA, Lima KC, de Uzeda M. Pathogenicity of facultative and obligate anaerobic bacteria in monoculture and combined with either *Prevotella intermedia* or *Prevotella nigrescens*. Oral Microbiol Immunol 1998;13:368-72
Siqueira Junior JF, Rôças IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. J Endod 2000;26:331-4
Siqueira Junior JF, Rôças IN. Simultaneous detection of *Dialister pneumosintes* and *Filifactor alocis* in endodontic infections by 16S rDNA-directed multiplex PCR. J Endod 2004;30:851-4
Siren EK, Haapasalo MPP, Ranta K, Salmi P, Kerusuo EN. Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation. Int J Endod 1997;30:91-5
Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. J Endod 2005;31:669-71
Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297-306
Sjögren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int J Endod 1991;24:119-25
Sjögren U, Happonen RP, Kahnberg KE, Sundqvist G. Survival of *Arachnia propionica* in periapical tissue. Int J Endod 1988;21:277-82
Sjögren U, Sundqvist G. Bacteriologic evaluation of ultrasonic root canal instrumentation. OOO 1987;63:366-70
Skucaite N, Peciuliene V, Vitkauskiene A, Machiulskiene V. Susceptibility of endodontic pathogens to antibiotics in patients with symptomatic apical periodontitis. J Endod. 2010 Oct;36(10):1611-6.
Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? OOOOE 2003;96:327-31
Smith A, Dickson M, Aitken J, Bagg J. Contaminated dental instruments. J Hosp Infect 2002;51:233-5
Soares JA, Roque de Carvalho MA, Cunha Santos SM, Mendonca RM, Ribeiro-Sobrinho AP, Brito-Junior M, et al. Effectiveness of chemomechanical preparation with alternating use of sodium hypochlorite and EDTA in eliminating intracanal Enterococcus faecalis biofilm. J Endod 2010;36:894-8.
Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE. "Checkerboard" DNA-DNA hybridization. Biotechniques 1994;17:788-92
Son HH, Lim S, Shon W, Kim HS, Lee W. Effects of sonicated *Enterococcus faecalis* extracts on interleukin-2 and interleukin-4 production by human T cells. J Endod 2004;30:701-3
Sonntag D, Peters OA. Effect of prion decontamination protocols on nickel-titanium rotary surfaces. J Endod 2007;33:442-6
Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S, Foschi F, Doucette S, Bammann LL, Fontana CR, Doukas AG, Stashenko PP. Photodynamic therapy for endodontic disinfection. J Endod. 2006 Oct;32(10):979-84
Stojicic S, Shen Y, Haapasalo M. Effect of the source of biofilm bacteria, level of biofilm maturation, and type of disinfecting agent on the susceptibility of biofilm bacteria to antibacterial agents. J Endod 2013:39:473–7
Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. Int Endod J 2012;45:363-71.
Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. J Endod. 2010 Sep;36(9):1558-62
Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8

Subramanian K, Mickel AK. Molecular analysis of persistent periradicular lesions and root ends reveals a diverse microbial profile. J Endod 2009;35:950-7.
Sunde PT, Olsen I, Gobel UB, Theegarten D, Winter S, Debelian GJ, et al. Fluorescence in situ hybridization (FISH) for direct visualization of bacteria in periapical lesions of asymptomatic root-filled teeth. Microbiology 2003;149:1095-102.
Sunde PT, Olsen I, Debelian GJ, Tronstad L. Microbiota of periapical lesions refractory to endodontic therapy. J Endod 2002 ;28:304-10
Sunde PT, Olsen I, Lind PO, Tronstad L. Extraradicular infection: a methodological study. Endod Dent Traumatol 2000;16:84-90
Sunde PT, Tronstad L, Eribe ER, Lind PO, Olsen I. Assessment of periradicular microbiota by DNA-DNA hybridization. Endod Dent Traumatol 2000;16:191-6
Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment. OOOOE 1998;85:86-93
Sundqvist G, Johansson E, Sjögren U. Prevalence of black-pigmented *Bacteroides* species in root canal infections. J Endod 1989;15:13-9
Sundqvist G, Reuterving C-O. Isolation of *Actinomyces israelii* from periapical lesion. J Endod 1980;6: 602-6
Sundqvist G. Associations between microbial species in dental root canal infections. Oral Microbiol Immunol 1992;7:257-62
Sundqvist G. Taxonomy, ecology,and pathogenicity of the root canal flora. OOO 1994;78:522-30
Sundqvist GK, Eckerbom MI, Larsson AP, Sjögren UT. Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. Infect Immun 1979;25:685-93
Tanner ACR, Visconti RA, Holdeman LV, Sundqvist G, Socransky SS. Similarity of *Wolinella recta* strains isolated from periodontal pockets and root canals. J Endod 1982;8:294-300
Tanomaru JM, Leonardo MR, Tanomaru Filho M, Bonetti Filho I, Silva LA. Effect of different irrigation solutions and calcium hydroxide on bacterial LPS. Int Endod J 2003;36:733-9
Tavares WL, Neves de Brito LC, Teles RP, Massara ML, Ribeiro Sobrinho AP, Haffajee AD, Socransky SS, Teles FR. Microbiota of deciduous endodontic infections analysed by MDA and Checkerboard DNA-DNA hybridization. Int Endod J. 2011 Mar;44(3):225-35.
Tay FR, Mazzoni A, Pashley DH, Day TE, Ngoh EC, Breschi L. Potential iatrogenic tetracycline staining of endodontically treated teeth via NaOCl/MTAD irrigation: a preliminary report. J Endod 2006;32:354-8
Teles AM, Manso MC, Loureiro S, Silva R, Madeira IG, Pina C, et al. Effectiveness of two intracanal dressings in adult Portuguese patients: a qPCR and anaerobic culture assessment. Int Endod J 2014;47:32-40
Tervit C, Paquette L, Torneck CD, Basrani B, Friedman S. Proportion of healed teeth with apical periodontitis medicated with two percent chlorhexidine gluconate liquid: a case-series study. J Endod. 2009 Sep;35(9):1182-5.
Thomas JE, Sem DS. An in vitro spectroscopic analysis to determine whether para-chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine. J Endod. 2010 Feb;36(2):315-7.
Tong Z, Zhou L, Kuang R, Lv H, Qu T, Ni L. In vitro evaluation of MTAD and nisin in combination against common pathogens associated with root canal infection. J Endod 2012;38:490-4.
Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S. A new solution for the removal of the smear layer. J Endod 2003;29:170-5
Townsend C, Maki J. An in vitro comparison of new irrigation and agitation techniques to ultrasonic agitation in removing bacteria from a simulated root canal. J Endod. 2009 Jul;35(7):1040-3.
Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. Endod Dent Traumatol 1987;3:86-90
Trope M. Regenerative potential of dental pulp. J Endod. 2008 Jul;34(7 Suppl):S13-7.
Tziafas D. Experimental bacterial anachoresis in dog dental pulps capped with calcium hydroxide. J Endod 1989;15:591-5
Upadya M, Shrestha A, Kishen A. Role of efflux pump inhibitors on the antibiofilm efficacy of calcium hydroxide, chitosan nanoparticles, and light-activated disinfection. J Endod. 2011 Oct;37(10):1422-6.
van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J 2007;40:415-26
Vianna ME, Conrads G, Gomes BP, Horz HP. Identification and quantification of archaea involved in primary

endodontic infections. J Clin Microbiol 2006;44:1274-82
Vianna ME, Horz HP, Conrads G, Zaia AA, Souza-Filho FJ, Gomes BP. Effect of root canal procedures on endotoxins and endodontic pathogens. Oral Microbiol Immunol 2007;22:411-8.
Vianna ME, Horz HP, Gomes BP, Conrads G. In vivo evaluation of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue. Int Endod J 2006;39:484-92
Vickerman MM, Brossard KA, Funk DB, Jesionowski AM, Gill SR. Phylogenetic analysis of bacterial and archaeal species in symptomatic and asymptomatic endodontic infections. J Med Microbiol 2007;56:110-8
Vinothkumar TS, Kavitha S, Lakshminarayanan L, Gomathi NS, Kumar V. Influence of irrigating needle-tip designs in removing bacteria inoculated into instrumented root canals measured using single-tube luminometer. J Endod 2007;33:746-8
Violich DR, Chandler NP. The smear layer in endodontics - a review. Int Endod J. 2010 Jan;43(1):2-15.
Waltimo T, Brunner TJ, Vollenweider M, Stark WJ, Zehnder M. Antimicrobial effect of nanometric bioactive glass 45S5. J Dent Res 2007;86:754-7
Waltimo T, Kuusinen M, Jarvensivu A, Nyberg P, Vaananen A, Richardson M, Salo T, Tjaderhane L. Examination on *Candida* spp. in refractory periapical granulomas. Int Endod J 2003;36:643-7
Waltimo TM, Orstavik D, Siren EK, Haapasalo MP. *In vitro* susceptibility of *Candida albicans* to four disinfectants and their combinations. Int Endod J 1999;32:421-9
Waltimo TMT, Siren EK, Torkko HLK, Olsen I, Haapasalo MPP. Fungi in therapy-resistant apical periodontitis. Int J Endod 1997;30: 96-101
Walton RE, Ardjmand K. Histological evaluation of the presence of bacteria in induced periapical lesions in monkeys. J Endod. 1992 May;18(5):216-27
Walton RE, Chiappinelli J. Prophylactic penicillin: effect on posttreatment symptoms following root canal treatment of asymptomatic periapical pathosis. J Endod 1993;19:466-70
Wang CS, Arnold RR, Trope M, Teixeira FB. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. J Endod 2007;33:1283-9
Wang L, Dong M, Zheng J, Song Q, Yin W, Li J, Niu W. Relationship of biofilm formation and *gelE* gene expression in *Enterococcus faecalis* recovered from root canals in patients requiring endodontic retreatment. J Endod. 2011 May;37(5):631-6.
Wang Q, Zhou XD, Zheng QH, Wang Y, Tang L, Huang DM. Distribution of *Porphyromonas gingivalis* fimA genotypes in chronic apical periodontitis associated with symptoms. J Endod. 2010 Nov;36(11):1790-5.
Weiger R, Manncke B, Werner H, Lost C. Microbal flora of sinus tracts and root canals of non-vital teeth. Endod Dent Traumatol 1995;11:15-9
White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod 1997;23:229-31
Williams BL, McCann GF, Schoenknecht FD. Bacteriology of dental abscesses of endodontic origin. J Clin Microbiol 1983;18:770-4
Williams JM, Trope M, Caplan DJ, Shugars DC. Detection and quantitation of *E. faecalis* by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment. J Endod 2006;32:715-21
Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*. J Endod 2009;35:95-7
Windley W 3rd, Teixeira F, Levin L, Sigurdsson A, Trope M. Disinfection of immature teeth with a triple antibiotic paste. J Endod 2005;31:439-43
Winkler KC, van Amerongen J. Bacteriologic results from 4000 root canal cultures. OOO 1959; 12:857-75
Wittgow WC, Sabistan CB Jr. Microorganisms from pulp chambers of intact teeth with necrotic pulps. J Endod 1975;1:168-71
Xia T, Baumgartner JC. Occurrence of *Actinomyces* in infections of endodontic origin. J Endod 2003;29:549-52
Yamashita JC, Tanomaru Filho M, Leonardo MR, Rossi MA, Silva LA. Scanning electron microscopic study of the cleaning ability of chlorhexidine as a root-canal irrigant. Int Endod J 2003;36:391-4
Yared GM, Bou Dagher FE. Influence of apical enlargement on bacterial infection during treatment of apical periodontitis. J Endod 1994;20:535-7

Yildirim S, Yapar M, Kubar A, Slots J. Human cytomegalovirus, Epstein-Barr virus and bone resorption-inducing cytokines in periapical lesions of deciduous teeth. Oral Microbiol Immunol 2006;21:107-11

Yoshida M, Fukushima H, Yamamoto K, Ogawa K, Toda T. Correlation between clinical symptoms and microorganisms isolated from root canals of teeth with periapical pathosis. J Endod 1987;13:24-8

Young G, Turner S, Davies JK, Sundqvist G, Figdor D. Bacterial DNA persists for extended periods after cell death. J Endod 2007;33:1417-20

Zamany A, Safavi K, Spangberg LS. The effect of chlorhexidine as an endodontic disinfectant. OOOOE 2003;96:578-81

Zapata RO, Bramante CM, de Moraes IG, Bernardineli N, Gasparoto TH, Graeff MS, Campanelli AP, Garcia RB. Confocal laser scanning microscopy is appropriate to detect viability of Enterococcus faecalis in infected dentin. J Endod 2008;34:1198-201.

Zehnder M, Guggenheim B. The mysterious appearance of enterococci in filled root canals. Int Endod J. 2009 Apr;42(4):277-87.

Zehnder M, Söderling E, Salonen J, Waltimo T. Preliminary evaluation of bioactive glass S53P4 as an endodontic medication in vitro. J Endod 2004;30:220-4.

Zeldow BJ, Ingle JI. Correlation of the positive culture to the prognosis of endodontically treated teeth: a clinical study JADA 1963; 66:9-13

Zerella JA, Fouad AF, Spangberg LS. Effectiveness of a calcium hydroxide and chlorhexidine digluconate mixture as disinfectant during retreatment of failed endodontic cases. OOOOE 2005;100:756-61

Zhang H, Pappen FG, Haapasalo M. Dentin enhances the antibacterial effect of mineral trioxide aggregate and bioaggregate. J Endod 2009;35:221-4

Zhang K, Tay FR, Kim YK, Mitchell JK, Kim JR, Carrilho M, Pashley DH, Ling JQ. The effect of initial irrigation with two different sodium hypochlorite concentrations on the erosion of instrumented radicular dentin. Dent Mater. 2010 Jun;26(6):514-23.

Zoletti GO, Siqueira JF Jr, Santos KR. Identification of *Enterococcus faecalis* in root-filled teeth with or without periradicular lesions by culture-dependent and-independent approaches. J Endod 2006;32:722-6

Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. J Endod 2010;36:793-6.