

Antibacterial Activity of EndoSequence Root Repair Material and ProRoot MTA against Clinical Isolates of *Enterococcus faecalis*

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Abstract

Introduction: Endodontic repair materials such as mineral trioxide aggregate (MTA) are used for various endodontic procedures. An alternative material to MTA with purportedly improved handling properties is EndoSequence Root Repair Material, which is available as pre-mixed putty (ESP) or syringeable paste (ESS) and is described as possessing antibacterial activity during its setting reaction due to its highly alkaline pH. The aim of this *in vitro* study was to determine whether ESP and ESS possess antibacterial properties against a collection of *Enterococcus faecalis* strains recovered from root canal infections. The hypotheses tested were that (1) ESP and ESS possess antibacterial activity during their setting reaction, (2) there is no difference between ESP, ESS, and MTA in antibacterial activity, and (3) *E. faecalis* strains isolated from root canals differ in susceptibility to the materials. **Methods:** The direct contact test was used. ESP, ESS, and white MTA were preincubated at 37°C in >95% humidity for 30 minutes and 24 hours before 1-hour direct contact exposure to *E. faecalis* strains ($n = 10$). Absence of antibacterial carryover effect from the materials to the bacterial cultures was confirmed. Log₁₀ viable counts were compared by using analysis of variance with significance level at $P \leq .05$. **Results:** Combining data for all strains, the mean (\pm standard deviation) log₁₀ viable counts for ESP (4.55 ± 0.85), ESS (4.5 ± 0.95), and MTA (4.12 ± 1.26) were significantly lower than for untreated controls (7.40 ± 0.33) ($P < .0001$). The reduction in viable counts ranged from 1.86 ± 0.24 to 4.78 ± 0.42 , with no statistically significant differences between the materials or preincubation periods. One strain was significantly more susceptible than 4 other strains. **Conclusions:** ESP, ESS, and MTA had similar antibacterial efficacy against clinical strains of *E. faecalis*. Clinical strains varied in their susceptibility to the root repair materials. (*J Endod* 2011; ■:1–5)

Key Words

Antimicrobial activity, direct contact test, EndoSequence Root Repair putty, EndoSequence Root Repair syringeable paste, *Enterococcus faecalis*, ProRoot MTA, root canal isolates

Endodontic repair materials are used for various procedures that include pulp capping, apexification, root-end fillings, and perforation repairs. Successful placement of the materials is facilitated by optimal access to the repair site and trouble-free handling properties. For example, inadequately repaired perforations can lead to microbial leakage into the root canal and subsequent failure of the endodontic treatment (1, 2). Of the repair materials available, mineral trioxide aggregate (MTA) (Dentsply, Tulsa Dental Specialties, Tulsa, OK) possesses several advantageous properties that include good sealing capability, biocompatibility, and antibacterial activity (3, 4). A potential disadvantage, however, is that the setting time for white ProRoot MTA is reported to be 40 ± 2.9 minutes for initial set and 140 ± 2.6 minutes for final set (5), whereas the setting time for gray MTA averages 165 ± 5 minutes (6). Bioceramic materials with a shorter setting time and uniform consistency during placement might provide a useful alternative to MTA with improved handling characteristics.

EndoSequence Root Repair Material (ERRM) (Brasseler USA, Savannah, GA) is a bioceramic material delivered as pre-mixed moldable putty (ESP) or as preloaded syringeable paste (ESS) with delivery tips for intracanal delivery of the material. There are few data available on the material. In one study, cell cytotoxicity tests (methyl-thiazol-diphenyltetrazolium assays) with L929 cells showed no difference between either set or freshly mixed states of white MTA, gray MTA, and ERRM (7). According to the manufacturer, ERRM has a working time of 30+ minutes and possesses antibacterial properties during its setting reaction because of its highly alkaline pH. The cytotoxicity of the materials was found to be similar to that of ProRoot MTA and MTA-Angelus (8). A similar material manufactured as a sealer under the name iRootSP in Canada (Innovative BioCeramix Inc, Vancouver, Canada) has been shown to possess adequate apical sealing ability (9), mild cytotoxicity (10), and antibacterial activity for up to 7 days after placement (11). However, at this time, there appears to be no independent information on the antibacterial properties of the moldable putty product or comparisons between the putty and syringeable material.

Enterococci are gram-positive microorganisms that are part of the normal flora in the gastrointestinal tract of humans (12). *Enterococcus faecalis* can be recovered from the root canals of teeth with primary and secondary root canal infections (13, 14). Clinical strains demonstrate various virulence characteristics (15, 16), for example, gelatinase activity that might contribute to long-term survival of *E. faecalis* in obturated

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root canals (17). The species is commonly used to evaluate the antimicrobial efficacy of materials used in endodontic treatment, but frequently by using only a single strain from a nonendodontic source as opposed to multiple strains isolated from infected root canals. On the basis of the existence of variable characteristics of clinical strains of *E. faecalis* recovered from infected root canals (15, 16), it is feasible that strains differ in their susceptibility to the antimicrobial activity of root repair material.

The aim of this *in vitro* study was to determine whether the putty and syringeable forms of ERRM possess antibacterial properties comparable to MTA during their setting reaction against a collection of *E. faecalis* strains isolated from infected root canals. The hypotheses tested were that (1) ESP and ESS possess antibacterial activity during the setting reaction, (2) there is no difference between ESP, ESS, and MTA in antibacterial activity, and (3) *E. faecalis* strains isolated from root canals differ in susceptibility to the materials.

Materials and Methods

Bacterial Strains and Media

E. faecalis strains ($n = 10$) previously isolated from infected root canals were selected for antibacterial assays on the basis of diversity of phenotypic, genotypic, and biofilm formation characteristics (15, 17, 18) (Table 1). Strains were taken from -80°C stocks and plated onto Todd Hewitt Broth (Becton, Dickinson and Co, Sparks, MD) supplemented with 1.5% agar and incubated aerobically for 24 hours at 37°C . Expected colony and cell morphology and gram stain reaction were verified for each strain. For experiments, an isolated colony-forming unit (CFU) of each strain was suspended in 5 mL brain heart infusion (BHI) (Becton, Dickinson and Co) broth overnight. Then 0.5 mL of the suspension was added to 4.5 mL BHI broth and grown to an optical density of OD_{650} of 0.36, which was shown in pilot studies to correspond to approximately 3×10^7 CFU/mL.

Preparation of Materials for Antimicrobial Assays

EndoSequence Root Repair Material Putty (ESP), EndoSequence Root Repair Material Syringeable (ESS), and white ProRoot MTA (Dentsply, Tulsa Dental Specialties) were compared for antibacterial efficacy by using the direct contact test based on previous studies (19, 20), including controls for carryover effect (11).

A section of fixed area (3-mm diameter) and thickness (1 mm) on the side of a well in a vertically positioned 96-well microtiter plate was coated with an equal amount of material by using the tip of a mixing spatula. MTA was mixed and handled according to the manufacturer's

directions. ESP and ESS are provided premixed and, according to the manufacturer, require the presence of moisture to initiate the setting reaction. Initial observations were made that ESP and ESS in wells did not set during a 5-day period when stored aerobically at 37°C in $>95\%$ humidity. Subsequent pilot studies showed that when ESP and ESS in wells were covered with sterile distilled water (SDW) ($100 \mu\text{L}$ per well), initiation of the setting process occurred. After 30 minutes, ESP and ESS remained soft and penetrable by an endodontic explorer; by 24 hours, ESP and ESS had attained a hardened state impenetrable by an endodontic explorer instrument up to 50 g of pressure, after which the material crumbled. MTA (which was not covered with SDW) also remained unset at 30 minutes; at 24 hours, MTA was impenetrable to an explorer tip up to 25 g of pressure, after which the material crumbled. Therefore, to evaluate whether different stages of the setting reaction were associated with variations in antimicrobial activity, materials were preincubated for both 30 minutes and 24 hours aerobically at 37°C in $>95\%$ humidity before exposure to bacteria. During the preincubation period ESP and ESS were covered with $100 \mu\text{L}$ SDW, which was removed by sterile pipette at the end of the period.

Antimicrobial Assays with the Direct Contact Test

A $10\text{-}\mu\text{L}$ bacterial suspension was placed onto the surface of the ESP, ESS, and MTA. Strain suspensions ($10\text{-}\mu\text{L}$) placed in uncoated wells served as nonexposed (positive) controls. Materials incubated without bacteria served as negative controls. All samples were incubated aerobically for 1 hour at 37°C in $>95\%$ humidity; then $240 \mu\text{L}$ of BHI broth was added to each of the wells and gently mixed with a pipette for 1 minute. Serial dilutions were prepared in BHI broth and plated onto BHI agar. After aerobic incubation for 24–48 hours at 37°C , CFUs were enumerated, and CFU/mL was calculated. Experiments were performed in duplicate.

Controls for Carryover Effect

To investigate the potential for antimicrobial carryover effect of the materials, procedures were performed on the basis of Zhang et al (11). ESP, ESS, and MTA were preincubated for 30 minutes and 24 hours as described above, including coverage of ESP and ESS with SDW, which was aspirated after the incubation period. Fresh SDW ($10 \mu\text{L}$) was placed in direct contact with the materials. After incubation for 1 hour at 37°C , $240 \mu\text{L}$ of BHI broth was added to the wells and mixed gently with a pipette for 1 minute. After mixing, $10 \mu\text{L}$ of broth was transferred to $970 \mu\text{L}$ of BHI broth to which a $20\text{-}\mu\text{L}$ suspension of bacteria was added. As controls, the same amount of SDW was placed on the wall

TABLE 1. *E. faecalis* Strains Used in the Study

Strain and treatment	Phenotype	Biofilm scores*	Characteristics	
			Genotype	
GS1	Retreatment	Gel ⁺ , Tc ^r	0.053	Plasmid ⁺ , <i>gelE</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS2	Retreatment	Bac ⁺ , PR ⁺	0.160	Plasmid ⁺ , <i>gelE</i> , <i>ef184/fsr</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS4	Root canal	Gel ⁺ , PR ⁺	0.077	Plasmid ⁺ , <i>gelE</i> , <i>ef184/fsr</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS6	Primary	Gel ⁺ , PR ⁺	1.162	Plasmid ⁺ , <i>gelE</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS7	Primary	Gel ⁺	0.041	Plasmid ⁺ , <i>gelE</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS9	Root canal	Gel ⁺ , Bac ⁺	0.053	<i>gelE</i> , <i>esp</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS10	Root canal	Bac ⁺	0.076	Plasmid ⁺ , <i>gelE</i> , <i>ef184/fsr</i> , <i>esp</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS18	Primary	PR ⁺	0.089	Plasmid ⁺ , <i>gelE</i> , <i>ef184/fsr</i> , <i>esp</i> , <i>asa</i> , <i>ace</i> , <i>efaA</i>
GS25	Retreatment	—	0.051	Plasmid ⁺ , <i>gelE</i> , <i>ef184/fsr</i> , <i>asa</i> , <i>ace</i> , <i>cylA</i> , <i>efaA</i>
GS33	Retreatment	Bac ⁺ , PR ⁺	0.791	Plasmid ⁺ , <i>gelE</i> , <i>ef184/fsr</i> , <i>asa</i> , <i>ace</i> , <i>cylA</i> , <i>efaA</i>

ace, collagen binding antigen; *asa*, aggregation substance; Bac⁺, bacteriocin activity; *cylA*, cytolytic activator; *efaA*, endocarditis antigen; *esp*, enterococcal surface adhesin; Gel⁺, gelatinase activity; *gelE*, gelatinase; *ef184/fsr*, gelatinase-negative phenotype determinant; N/A, endodontic treatment details not available; Plasmid⁺, plasmid(s) present; PR⁺, clumping response to pheromone; Tc^r, tetracycline resistance.

*Refers to optical density readings at 570 nm in microtiter plate assays.

of the uncoated wells and processed as outlined above. Serial dilutions of test and control suspensions were processed for determination of CFU/mL as described above. The carryover tests were performed in triplicate. Viable counts of bacteria in controls were compared with those exposed to the material for each preincubation period; no difference in viable counts indicated the absence of a carryover effect.

Statistical Analysis

Viable counts were transformed to their \log_{10} values. Data were confirmed to be normally distributed by using Kolmogorov-Smirnov normality tests. Analysis of variance and Tukey multiple comparisons were used to evaluate (1) whether ESP, ESS, and MTA had antibacterial properties by comparing viable counts of exposed and nonexposed bacteria; (2) the antibacterial activity of ESP, ESS, and MTA during their setting period by comparing the reduction of viable counts of bacteria exposed to materials preincubated for 30 minutes or 24 hours before exposure to bacteria; and (3) variations in the susceptibility of different *E. faecalis* strains by comparing the reduction in viable counts after exposure to ESP, ESS, and MTA.

Prism 4.0a for Macintosh software (GraphPad Software Inc, La Jolla, CA) was used for statistical analyses. Significance was set at $P < .05$.

Results

Controls

E. faecalis was not recovered from any of the negative controls in direct contact tests. There was no evidence of carryover of the antibacterial effect from the materials to the bacterial cultures.

Antibacterial Activity of ESP, ESS, and MTA

Combining data for all strains, the mean (\pm standard deviation) \log_{10} viable counts for ESP (4.55 ± 0.85), ESS (4.5 ± 0.95), and MTA (4.12 ± 1.26) were significantly lower than for nonexposed controls (7.40 ± 0.33) ($P < .0001$). Differences between materials were not significant.

Antibacterial Activity during the Setting Reaction

The reduction of viable counts after preincubation for 30 minutes (2.90 ± 0.55 , ESP; 2.97 ± 0.73 , ESS; 3.44 ± 0.77 , MTA) and 24 hours (2.81 ± 0.47 , ESP; 2.93 ± 0.81 , ESS; 3.13 ± 0.94 , MTA) were not significantly different ($P > .05$) (Fig. 1).

Comparison of Susceptibility of Different Strains

\log_{10} reductions in viable counts for strains exposed to ESP, ESS, and MTA are shown in Table 2. The mean (\pm standard deviation) reduction in \log_{10} viable counts ranged from 2.17 ± 0.62 for *E. faecalis* GS33 to 3.36 ± 0.47 for *E. faecalis* GS2 when exposed to ESP, from 1.94 ± 0.44 for *E. faecalis* GS25 to 4.21 ± 1.42 for *E. faecalis* GS9 when exposed to ESS, and from 1.86 ± 0.24 for *E. faecalis* GS4 to 4.78 ± 0.42 for *E. faecalis* GS9 when exposed to MTA. The greatest reduction in viable counts occurred with GS9; this strain was significantly more susceptible than GS7, GS25, and GS33 ($P < .05$) and GS4 ($P < .01$) (Fig. 2). There were no differences between other strains.

Discussion

The results of this *in vitro* investigation showed that EndoSequence Root Repair premixed putty and syringeable paste, as well as white Pro-Root MTA, possess antibacterial properties against clinical strains of *E. faecalis* during their setting period. There was no difference between the materials in their antibacterial efficacy. Clinical strains

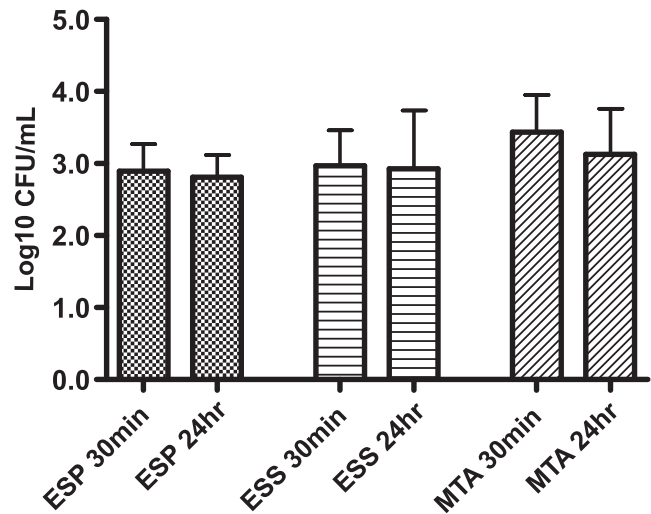


Figure 1. Reduction in viable counts of *E. faecalis* strains exposed to ESP, ESS, and MTA during setting of materials. Materials were preincubated at 37°C in $>95\%$ humidity for 30 minutes and 24 hours before 1-hour exposure to *E. faecalis* strains. There were no statistically significant differences between materials or preincubation periods ($P > .05$). Bars represent mean \pm standard deviation \log_{10} viable counts of combined data for 10 *E. faecalis* strains.

varied in the susceptibility to the materials tested. All hypotheses tested were accepted.

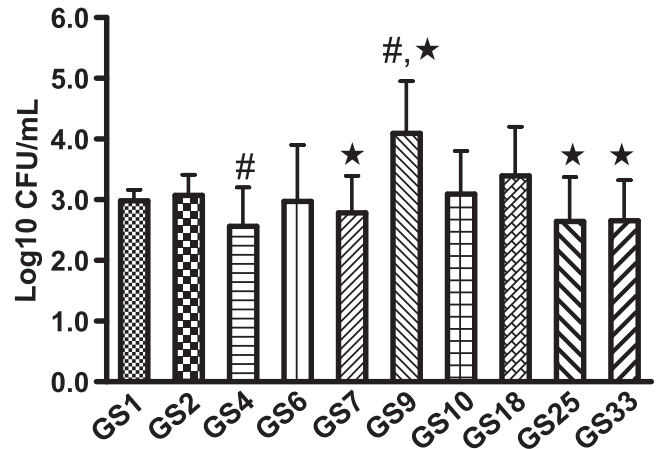
The rationale for selection of a 30-minute preincubation period during the setting process was based on the manufacturer's information that the material has a 30+-minute working time. Pilot studies showed that the materials were still undergoing setting at 24 hours; thus, this was included as an interim setting period for the material. For both time periods, the materials possessed similar antibacterial effects against the *E. faecalis* strains.

ERRM is a bioceramic material composed of calcium silicates, zirconium oxide, tantalum oxide, calcium phosphate monobasic, thickening agents, and proprietary fillers. EndoSequence has been manufactured to overcome some of the difficult handling characteristics of MTA. The materials have very different working properties; MTA has to be mixed with a sterile liquid to a desired consistency, and the EndoSequence materials are ready-to-use as packaged. Depending on the consistency desired, ERRM is manufactured in a syringeable form, which is flowable, and a putty form, which is firm and moldable. A difficulty encountered during the research was attaining a complete set of the EndoSequence materials. The manufacturer states that the moisture present in the dentinal tubules is adequate to allow the material to set. In pilot studies it was observed that the materials started to set only when completely covered by water. Interestingly, another study reported an inability of both putty and syringeable materials incubated at 37°C in 100% humidity to withstand a 500-g load until 168 hours (8). Similarly, it was recently reported that EndoSequence BC sealer required 108 hours (4.5 days) mixed with water to achieve an initial set, with the final set occurring at 168 hours (7 days) (21); this material differs from ESP and ESS only by the amount of thickening agents they contain. Further studies are needed to evaluate the setting reaction and the amount of moisture that is required for the putty and syringeable materials to set.

The direct contact test, as described by Weiss et al (20), is a quantitative and reproducible method designed to simulate the contact of the microorganism with the root repair materials in the root canal. This method allows for better control of confounding factors than the agar diffusion test (11, 20), as well as measurement of the bactericidal effect of the materials when controls for carryover are performed

TABLE 2. Mean (\pm standard deviation) \log_{10} Reduction in Viable Counts of *E. faecalis* Strains after Exposure to ESP, ESS, and MTA

	GS1	GS2	GS4	GS6	GS7	GS9	GS10	GS18	GS25	GS33	Overall
ESP	3.01 (0.41)	3.36 (0.47)	2.87 (1.05)	3.08 (0.60)	2.33 (0.64)	3.30 (1.62)	2.56 (0.84)	2.56 (0.24)	3.31 (1.19)	2.17 (0.62)	2.85 (0.86)
ESS	2.95 (0.31)	2.83 (0.68)	2.98 (0.75)	2.26 (0.48)	2.67 (1.33)	4.21 (1.22)	3.73 (0.71)	3.56 (0.40)	1.94 (0.44)	2.37 (0.37)	2.95 (0.94)
MTA	2.99 (0.53)	3.04 (0.74)	1.86 (0.24)	3.59 (1.66)	3.37 (1.59)	4.78 (0.42)	3.00 (0.43)	4.08 (1.03)	2.68 (1.89)	3.43 (1.19)	3.28 (1.24)

**Figure 2.** Reduction in viable counts of *E. faecalis* strains after 1-hour exposure to ESP, ESS, and MTA. Bars represent mean \pm standard deviation \log_{10} viable counts for all materials. #, *: GS9 was more susceptible than GS7, GS25, GS33 ($P < .05$) and GS4 ($P < .01$), respectively.

(11). However, a limitation of the method is that it does not allow evaluation of microorganisms under biofilm conditions. In the present study, harvesting of microorganisms was standardized to mid-exponential growth phase. Future studies could use models that include microorganisms in different phases of growth, since it has been shown that *E. faecalis* strains are more susceptible to endodontic medicaments during exponential growth phase compared with stationary and starved phases (22).

MTA is the current material of choice for root perforations, retrograde filling materials, and pulp capping (3, 4, 23). Previous studies have shown conflicting results regarding the antibacterial activity of MTA. For example, the antimicrobial activity of MTA has been limited in some studies (24–26), whereas it was effective against microorganisms including *E. faecalis* in other studies (27–30). In addition, antibacterial efficacy of MTA is dependent on concentrations and the type of preparation (31). The authors are not aware of any previous information regarding the antibacterial activity of both EPS and ESS. However, a sealer composed of similar materials but with a different amount of filler material for increased flow consistency demonstrated an antibacterial effect against an *E. faecalis* strain for up to 7 days in direct contact tests (11). This was attributed to the sealer's high pH, hydrophilicity, and active calcium hydroxide diffusion. In a recent study, intracanal placement of ESS and white MTA resulted in diffusion of hydroxyl ions across dentin (32). Although pH of materials was not measured in this study, it is feasible that the pH of the material during its setting reaction contributed to the antibacterial activity seen in the present study.

In conclusion, within the limitations of the present study, ERRM, both putty and syringeable forms, and white ProRoot MTA demonstrated similar antimicrobial properties during their setting reactions against 10 clinical strains of *E. faecalis*. Not all of the bacterial strains were equally susceptible to the materials, suggesting that the use of more than 1 strain in antimicrobial assays is advisable.

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The authors deny any conflicts of interest related to this study.

References

- Fuss Z, Trope M. Root perforations: classification and treatment choices based on prognostic factors. *Endod Dent Traumatol* 1996;12:255–64.
- Jew RC, Weine FS, Keene JJ Jr, Smulson MH. A histologic evaluation of periodontal tissues adjacent to root perforations filled with Cavit. *Oral Surg Oral Med Oral Pathol* 1982;54:124–35.
- Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review—part III: clinical applications, drawbacks, and mechanism of action. *J Endod* 2010;36:400–13.
- Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. *J Endod* 2010;36:16–27.
- Islam I, Chng HK, Yap AU. Comparison of the physical and mechanical properties of MTA and Portland cement. *J Endod* 2006;32:193–7.
- Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. *J Endod* 1995;21:349–53.
- AlAnezi AZ, Jiang J, Safavi KE, Spangberg LS, Zhu Q. Cytotoxicity evaluation of endo-sequence root repair material. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109:e122–5.
- Damas BA, Wheeler MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials. *J Endod* 2011;37:372–5.
- Zhang W, Li Z, Peng B. Assessment of a new root canal sealer's apical sealing ability. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:e79–82.
- Zhang W, Li Z, Peng B. ex vivo cytotoxicity of a new calcium silicate-based canal filling material. *Int Endod J* 2010;43:769–74.
- Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. *J Endod* 2009;35:1051–5.
- Facklam RR, Carvalho MS, Teixeira LM. History, taxonomy, biochemical characteristics, and antibiotic susceptibility testing of enterococci. In: Gilmore MS, ed. *The enterococci: pathogenesis, molecular biology and antibiotic resistance*. Washington, DC: American Society for Microbiology; 2002:1–54.
- 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.
- Sedgley C, Nagel A, Dahlen G, Reit C, Molander A. Real-time quantitative polymerase chain reaction and culture analyses of *Enterococcus faecalis* in root canals. *J Endod* 2006;32:173–7.
- Sedgley CM, Molander A, Flannagan SE, et al. Virulence, phenotype and genotype characteristics of endodontic *Enterococcus* spp. *Oral Microbiol Immunol* 2005;20:10–9.
- Reynaud af Geijersstam A, Culak R, Molenaar L, et al. Comparative analysis of virulence determinants and mass spectral profiles of Finnish and Lithuanian endodontic *Enterococcus faecalis* isolates. *Oral Microbiol Immunol* 2007;22:87–94.
- Sedgley CM. The influence of root canal sealer on extended intracanal survival of *Enterococcus faecalis* with and without gelatinase production ability in obturated root canals. *J Endod* 2007;33:561–6.
- Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic *Enterococcus faecalis*. *J Endod* 2007;33:815–8.
- Eldenz AU, Hadimli HH, Ataoglu H, Orstavik D. Antibacterial effect of selected root-end filling materials. *J Endod* 2006;32:345–9.
- Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. *Endod Dent Traumatol* 1996;12:179–84.
- Loushine BA, Bryan TE, Looney SW, et al. Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. *J Endod* 2011;37:673–7.
- 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.
- Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations. *J Endod* 2010;36:190–202.
- Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. *Braz Dent J* 2000;11:3–9.
- Miyagak DC, de Carvalho EM, Robazza CR, Chavasco JK, Levorato GL. in vitro evaluation of the antimicrobial activity of endodontic sealers. *Braz Oral Res* 2006;20:303–6.
- Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. *J Endod* 1995;21:403–6.
- Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Antibacterial effect of two mineral trioxide aggregate (MTA) preparations against *Enterococcus faecalis* and *Streptococcus sanguis* in vitro. *J Endod* 2006;32:1053–6.
- Sipert CR, Hussne RP, Nishiyama CK, Torres SA. in vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. *Int Endod J* 2005;38:539–43.
- Zhang H, Pappen FG, Haapasalo M. Dentin enhances the antibacterial effect of mineral trioxide aggregate and bioaggregate. *J Endod* 2009;35:221–4.
- Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, Rutledge RE. The anti-microbial effect against *Enterococcus faecalis* and the compressive strength of two types of mineral trioxide aggregate mixed with sterile water or 2% chlorhexidine liquid. *J Endod* 2007;33:844–7.
- Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, Simon JH, Rotstein I. MTA preparations from different origins may vary in their antimicrobial activity. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:e85–8.
- Hansen SW, Marshall JG, Sedgley CM. Comparison of intracanal EndoSequence Root Repair Material and ProRoot MTA to induce pH changes in simulated root resorption defects over 4 weeks in matched pairs of human teeth. *J Endod* 2011;37:502–6.