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ANTIBACTERIAL ACTIVITY OF TWO CALCIUM SILICATE-BASED ROOT CANAL SEALERS AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Aim: The purpose of the study was to compare between EndoSequence BC® and EndoSeal MTA® sealers regarding their antibacterial activity against Enterococcus faecalis. **Methods:** The "Direct contact test" was the method used to evaluate the antibacterial effect of the 2 tested sealers. Each sealer was dispensed at the bottom of 96-well plates. Three unfilled wells were used as control group. Samples were evaluated under three setting conditions: fresh sealer, one week-old, and one month-old. Aliquots of the bacterial suspension were placed on the tested sealers and in the control wells. After incubation for 1 hour, the liquid portion of the suspension evaporated providing direct exposure between the bacteria and the surface of sealers. BHI broth (245 µl) was added then to each well and the plates were gradually mixed for two minutes; 15 µl of the bacterial suspension was transferred from each well to corresponding wells in other plates. The kinetics of bacterial growth in each plate was followed by densitometric measurement every hour for 3 hours, using a spectrophotometer. **Results:** Endoseal showed the stronger antimicrobial effect in different setting conditions. Both sealers have extended antibacterial activity up to one month. **Conclusion:** Endoseal is the most effective in eliminating E. faecalis and may be the most useful sealer for preventing bacterial infection when treating root canals.

KEYWORDS: EndoSequence BC®; EndoSeal MTA®; Enterococcus faecalis; Bioceramics; Antibacterial activity.

INTRODUCTION

Bacterial infection into the root canals plays a major role in the initiation of pulp and periapical inflammation and is closely combined with the failure of endodontic therapy (Kakehashi et al., 1965). Because the root canal system varies in the anatomical criteria including fins, apical delta, ramifications isthmi, and accessory canals, complete eradication of the microbes from the root canal is impossible. In treating the root canal space, along with mechanical preparation, various intracanal irrigating solutions and medicaments, such as calcium hydroxide paste, sodium hydroxide, and chlorohexidine gel, are used in an attempts to remove microbes in the contaminated root canal, However, some microbes may survive inside the root canals (Ozcan el al., 2011). Therefore, a

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hermetic sealing of the root canal is mandatory to entomb any residual bacteria and ultimately kill them in the obturated root canals.

Endodontic sealers are used to overcome the limitations of gutta-percha (GP) cones and obturation techniques by filling the spaces between the GP core material and the dentinal wall. Hence, root canals sealers that possess superior sealing ability and antimicrobial activity would be clinically beneficial by preventing bacteria from re-entering the canal system and by inactivating bacteria already remaining in the canal system after root canal obturation.

Conventional root canal sealers are classified as zinc oxide eugenol (ZOE), epoxy resin (ER), or calcium hydroxide (CH) on the basis of their composition (Mutoh el al., 2013). Recently, calcium silicate-based cements with the addition of various oxide compounds have been developed for root sealing (Camilleri et al., 2005). These cements are known to have bioactive properties that stimulate tissue repairing and induction of biomineralization (Aminozarbian et al., 2012). For these reasons, the cement has been considered suitable for application as root canal sealer and have led to the development of root canal sealers. Antibacterial activity is also an important factor in investigating dental materials for application as root canal sealer because bacterial infection is closely associated with the failure of root canal treatment.

EndoSequence BC® sealer, (Brasseler USA, Savannah, GA) was the commonly used calcium silicate-based bioceramic sealer (Craig et al., 2013). It has the major advantage that it sets quicker and has favorable handling characteristics. According to the instructions of the manufacturer, this bioceramic material is formed of calcium silicates, calcium phosphates monobasic, zirconium oxides and tantalum oxides with fillers and thickening agents.

EndoSeal MTA® (Maruchi, Wonju, Korea) is a creamy, premixed, and ready for use injectable whitish hydraulic paste introduced for permanent root canal filling. EndoSeal MTA® is not soluble, radiopaque, and aluminum-free cement composed mainly of calcium silicate components, that need the existence of moist media to completely set and stabilizes (Zhang et al., 2009c).

Enterococcus faecalis has been detected in apical periodontitis lesions in root canal-treated teeth. It is usually observed in resistant and secondary endodontic infections in addition to Staphylococcus spp and Streptococcus mutans (Antunes et al., 2015). Some fresh sealers have been proved to kill E. faecalis effectively (Fuss et al., 1997). But, the antibacterial effect of these sealers has been found to reduce by time (Zhang et al., 2009a). The antimicrobial activity of root canal sealers has often been evaluated by using the agar diffusion method (ADT) or the direct contact method (DCT) (Kesler et al., 2013).

The aim of the current research was to compare between EndoSequence BC® sealer and EndoSeal MTA® sealer regarding their antibacterial activity against Enterococcus faecalis using the direct contact test.

MATERILAS AND METHODS

Antibacterial activity of the two materials was evaluated against facultative enterococcus faecalis. Bacteria were grown aerobically from frozen stock cultures in BHI broth at 37° C. 18 to 20 hours of culturing were used. Cells were harvested by centrifugation and resuspended in fresh BHI broth. Bacterial numbers were standardized to an optical density of 0.35 at a wavelength of 545 nm.

The " Direct contact test" was used to evaluate the antibacterial effect of the tested materials: EndoSequence BC® and EndoSeal MTA®. This test is based on measuring the turbidity of bacterial growth in 96-well plates.

Each of the tested materials was dispensed at the bottom of 96-well plates to a height of 1-2 mm and then adapted with a large plugger in triplicate. Three unfilled wells of the plate were used for the control

group. All the steps were performed under sterile environment in a laminar air flow cabinet.

The samples of each tested material were evaluated under three setting conditions as follows: fresh material, where the samples were immediately exposed to the bacterial suspension; one week-old, where the samples were allowed for setting at 37°C temperature and 100 % relative humidity in an incubator for one day then aged for one week in saline before testing; and one month-old, where the samples were allowed for setting at 37°C temperature and 100 % relative humidity in an incubator for 24 hours then aged for one month in saline before testing.

Aliquots of the bacterial suspension, 10 µl in volume, were placed on the tested materials and in the control wells. After incubation for 1 hour at 37°C temperature and 100 % relative humidity, the liquid portion of the suspension evaporated providing direct exposure between all the bacteria and the surface of the tested sealers. BHI broth (245 µl) was then added to each of the wells and the plates were mixed gently for two minutes; 15 µl of the bacterial suspension was transferred from each well of these plates to corresponding wells in other plates, where each well contained fresh medium (215 µl) and mixed again for two minutes (Eldeniz et al., 2006b).

The kinetics of bacterial growth in each plate was followed by continuous densitometric measurement every hour for three hours, using a microplate spectrophotometer at a wavelength of 620 nm. The microplate spectrophotometer is a machine used to measure the intensity of wavelengths in a spectrum of light compared with the intensity of light from a standardized source. The basic components of the spectrophotometer consist of: light source (lamp), prism or grating, collimator, measuring photo-detector and screen display. The development of the color is based on the concentration of the substance in the solution, and then this concentration can be measured by detecting the extension of light absorption at the suitable wavelength. The experiment was repeated ten times for each material under each condition for each reading point (i.e. n = 10 for each material at each setting condition for each reading point) to ensure reproducibility.

One way analysis of variance (one way ANOVA) was used to compare means of the optical density influenced by the type of the material (EndoSequence BC®, EndoSeal MTA® and control) under each setting condition (Fresh material, one week old and one month old) at each of the three reading points (1 h, 2 hs and 3 hs). This was followed by Tukey's post-hoc test for pair-wise comparisons of the experimental and control groups. Regression-analysis using the repeated-measures ANOVA test was used to study the effect of the Material, Setting condition and Reading on the antibacterial activity. These tests were followed by Tukey's post-hoc test for pair-wise comparisons of the experimental and control groups. Statistical analysis was performed with the Statistical Package for Scientific Studies (SPSS) 20.0. A significance level of 0.05 was used throughout all the statistical tests within this study.

RESULTS

The recorded mean and standard deviation values of the optical density of the bacterial growth for EndoSequence BC®, EndoSeal MTA® and the control group at each setting condition (fresh sealer, one week-old and one month-old) for every sealer at each of the three readings times (1 h, 2 hours and 3 hours) are summarized in table (1) and figure (1).

For the fresh sealer:

At the 1 hour reading, One-way ANOVA test revealed a statistically significant difference among the groups (p=0.01). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.035) as well as between EndoSeal MTA® and the control (p=0.001) while no significant difference was detected between EndoSequence BC® and the control (p=0.650).

At the 2 hours reading, One way ANOVA test showed a statistically significant difference among the groups (p=0.001). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.029), and a statistically significant difference existed between EndoSequence BC® and control (p=0.01) as well as between EndoSeal MTA® and the control (p=0.001).

At the 3 hours reading, One-way ANOVA test showed a statistically significant difference among the groups (p=0.001). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.011), and a statistically significant difference existed between EndoSequence BC® and control (p=0.01) as well as between EndoSeal MTA® and the control (p=0.001).

TABLE (1): The mean and standard deviation values of the optical density of Enterococcus faecalis with EndoSequence BC®, EndoSeal MTA® and control at the three setting conditions for each reading time.

Setting condition	**Reading Time**	**(EndoSequence) Mean ± SD (n=10)**	**(EndoSeal) Mean ± SD (n=10)**	**(Control) Mean ± SD (n=10)**
Fresh Sealer	**1 hour**	0.953 ± 0.023	0.750 ± 0.015	0.984 ± 0.033
	2 hours	0.999 ± 0.068	0.723 ± 0.023	1.455 ± 0.276
	3 hours	1.315 ± 0.333	0.773 ± 0.033	1.933 ± 0.153
One week	**1 hour**	0.882 ± 0.053	0.665 ± 0.085	0.855 ± 0.075
	2 hours	0.977 ± 0.078	0.733 ± 0.073	1.235 ± 0.133
	3 hours	1.109 ± 0.220	0.763 ± 0.075	1.555 ± 0.535
One Month	**1 hour**	0.953 ± 0.335	0.830 ± 0.033	1.555 ± 0.058
	2 hours	1.225 ± 0.022	0.725 ± 0.035	1.583 ± 0.344
	3 hours	1.320 ± 0.022	1.015 ± 0.255	1.943 ± 0.430

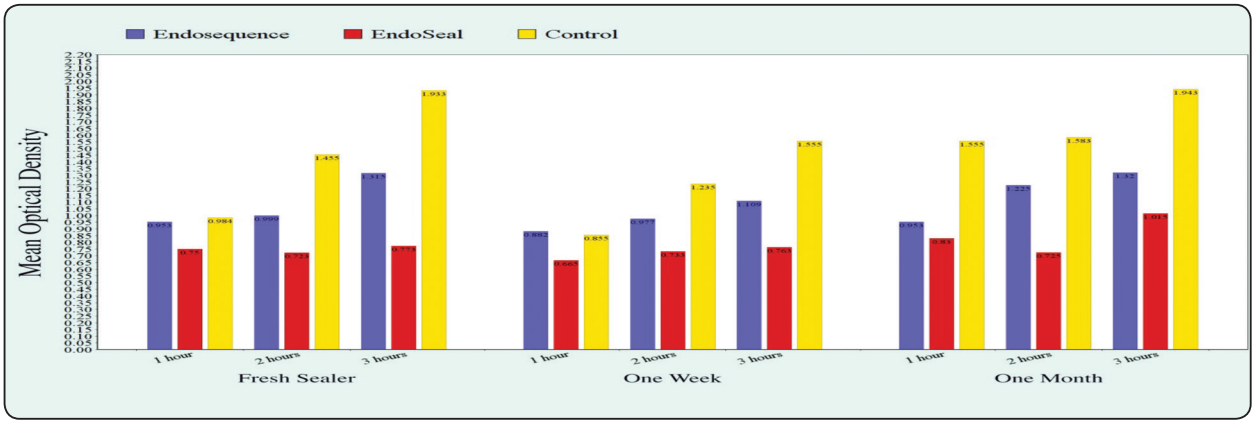

Figure (1): Bar chart showing the mean optical density of Enterococcus faecalis with the tested sealers and the control at the three setting conditions for each reading time.

After one week:

At the 1 hour reading, One-way ANOVA test revealed a statistically significant difference among the groups (p=0.033). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.026), as well as between EndoSeal MTA® and the control (p=0.01), while no significant difference was found between EndoSequence BC® and the control (p=0.280).

At the 2 hours reading, One way ANOVA test showed a statistically significant difference among the groups (p=0.001). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.003), and a statistically significant difference existed between EndoSequence BC® and control (p=0.01) as well as between EndoSeal MTA® and the control (p=0.001).

At the 3 hours reading, One-way ANOVA test showed a statistically significant difference among the groups (p=0.001). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.01), and a statistically significant difference existed between EndoSequence BC® and the control (p=0.01) as well as between EndoSeal MTA® and control (p=0.001).

After one month:

At the 1 hour reading, One-way ANOVA test showed a statistically significant difference among the groups (p=0.001). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.033), and a statistically significant difference existed between EndoSequence BC® and control (p=0.003) as well as between EndoSeal MTA® and the control (p=0.005).

At the 2 hours reading, One-way ANOVA test revealed a statistically significant difference among the groups (p=0.001, p<0.05). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.869, p<0.05), and a statistically significant difference existed between EndoSequence BC® and the control (p=0.001, p<0.05) as well as between EndoSeal MTA® and control (p=0.001, p<0.05).

At the 3 hours reading, One-way ANOVA test showed a statistically significant difference among the groups (p=0.001). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.011), and a statistically significant difference existed between EndoSequence BC® and control (p=0.01) as well as between EndoSeal MTA® and the control (p=0.001).

According the regression analysis using repeated measures ANOVA, there was a statistically significant effect of the Between-subject factors (Sealer and setting conditions) on the antibacterial activity (p=0.001). Also, the Within-subjects factors (Reading time) have a statistically significant effect on the antibacterial action (p=0.01). Tukey posthoc test showed a statistically significant difference between EndoSequence BC® and EndoSeal MTA® (p=0.036), and a statistically significant difference existed between EndoSequence BC® and control (p=0.001) as well as between EndoSeal MTA® and the control (p=0.001).

DISCUSSION

Control of the bacteria determines the success or failure of root canal therapy. Although, chemomechanical procedures, cleaning, and disinfectant treatment are performed to reduce the number of microbial flora when treating the root canals, some microbes often remain in the root canal system (Ozcan el al., 2011). Therefore; the root-filling materials with antibacterial activity are required and are advantageous. Recently, calcium silicate-based root canal materials have been developed, and their antibacterial activity has been evaluated (Jafari et al., 2016).

In the current study, the two sealers were evaluated against the following facultative bacterial species: Enterococcus faecalis. Although the aerobic and facultative microbes are commonly small constituents of the initial infections, it was observed by Sundqvist et al (1998) that they have been found in cases of flare-ups and endodontic failures. It was reported by Eldeniz et al (2006a) that Enterococcus faecalis are bacterial species resistant to several antimicrobial agents.

Enterococcus faecalis may grow as a monoinfection in the treated root canals in the absence of synergistic support from other organisms, can colonize the dentinal wall under stressful conditions like nutrient deficiency, chemomechanical instrumentation and intracanal medication with the help of adhesive substances that facilitate the adherence of the organism to the host collagen type I as mentioned by Love et al (2001); pH greater than 11.0 is needed to kill Enterococcus faecalis (McHugh et al., 2004).

In the present study the antibacterial effect of the tested sealers was assessed by the direct contact method. As reported by Shalhav et al (1997), it is a reproducible and quantitative measure which allows evaluation of water insoluble substances, continuous measurement of bacterial growth and could, also, be used for standardized aging studies. In the direct contact test, microorganisms are brought in direct contact with the tested samples for a controlled period of time to allow the measuring of the effect of close and direct contact between microbes and the tested substance on bacterial growth. It allows determining whether the data gathered reflects bactericidal or, just, bacteriostatic effect. This test was, also, used to overcome the disadvantages of the agar diffusion test, such as lack of the standardized inoculum density, the growth media, the agar viscosity and the storage condition of the agar plates. In addition, the agar diffusion test results were found to be highly affected by the diffusibility of the materials across the media; not only the antibacterial activity (Siqueira and De Uzeda 1997). The agar diffusion test, also, was found not to be an accurate method for testing the antimicrobial activity of calcium hydroxide-based agents; this substance has low solubility and can slowly be diffused in agar; (Eldeniz et al., 2006b).

Endoseal is a calcium silicate-based sealer and showed an antibacterial effect against E. faecalis in different setting conditions. Endoseal showed the stronger antimicrobial effect against bacteria in different setting conditions. However, Endosequence BC sealer, another calcium silicate-based sealer, showed weak antimicrobial activity against E. faecalis. Endosequence is a bioceramic sealer composed of calcium silicates, zirconium oxides, tantalum oxides, calcium phosphates monobasic thickening fillers, and proprietary agents. Both Endosequence BC and Endoseal sealers commonly exhibit antibacterial activity because of Ca (OH)2 (calcium hydroxide) reaction, which is bactericidal against bacteria through damage of bacterial membrane or DNA, and denaturation proteins (Siqueira and Lopes 1999).

Endoseal contained more types and more amount of the oxide compound known to have antimicrobial activity such as Na2O, MgO, Al2O3, SO2, and Fe2O than EndoSequence BC (Shin et al., 2018). Among oxide compounds, these oxide compounds damage the cell wall of Gram positive microorganisms and enhance the permeability of molecules into the cytoplasm through electrostatic interaction (Azam et al., 2012 and Hajipour et al., 2012). Finally, various oxide compounds with antimicrobial activity in Endoseal may damage the cell wall of bacteria and help the penetration of Ca (OH)2 into the cytosol, and then Ca (OH)2 may denature DNA and protein. Because Endosequence BC contained relatively low amount of oxide compounds with antimicrobial activity, Endosequence BC may weakly damage the cell walls of bacteria, and Ca (OH)2 may penetrate less. This indicates that calcium silicate-base sealers containing oxide compounds may show the strong antibacterial effect towards Gram negative and Gram positive microorganisms.

In the present research, it was found that both sealers have extended antibacterial activity up to one month, which was in accordance with Fridland and Rosado (2005), who reported that the high pH of MTA, ranging from 11.00 to 12.00, was maintained for 78 days. According to Wang et al (2012), calcium silicate-based sealers extended antimicrobial action could also be due to its biomineralization reaction caused by calcium silicates/phosphates ions within the sealer as well as the participation of mineralized dentine content. In the light of these studies, the antimicrobial activity of these sealers against to the microorganisms could be attributed to release of diffusible substances in terms of calcium silicates, calcium phosphates, and calcium hydroxides and to its high alkaline pH (Zhang et al., 2009b).

CONCLUSION

Within the limits of this current research, both sealers showed extended antibacterial activity up to one month. Endoseal continues to exhibit antibacterial activity during different setting conditions and may be the most effective in killing E. faecalis in the root canals. Finally, Endoseal could be the most useful sealer for preventing bacterial infection when treating the root canal.

REFERENCES

1. Aminozarbian MG, Barati M, Salehi I, Mousavi SB. Biocompatibility of mineral trioxide aggregate and three new endodontic cements: an animal study. Dent Res J 2012; 9:54-9.

2. Antunes HS, Rocas IN, Alves FR, et al. Total and specific bacterial levels in the apical root canal system of teeth with post-treatment apical periodontitis. J Endod 2015; 41: 1037–42.

3. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. Int J Nanomed 2012; 7:6003-9.

4. Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TR. The constitution of mineral trioxide aggregate. Dent Mater 2005; 21:297-303.

5. Craig S. Hirschberg, Niyati S. Patel, Loken M. Patel, Daniel E. Kadouri, Gary R. Hartwell. Comparison of sealing ability of MTA and EndoSequence Bioceramic Root Repair Material: A bacterial leakage study. Quintessence international 2013; 44:5; 157-62.

6. Eldeniz A U, Erdemir A, Hadimli H H, Belli S, Erganis O, Konya A and Kirikkale. Assessment of antibacterial activity of EndoRez. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 2006a; 102: 119-26.

7. Eldeniz A U, Hadimli H H, Ataoglu A and Orstavik. Antibacterial effect of selected root end filling materials. Journal of Endodontics 2006b; 32: 345-9.

8. Fuss Z, Weiss EI, Shalhav M. Antibacterial activity of calcium hydroxide-containing endodontic sealers on Enterococcus faecalis in vitro. Int Endod J 1997; 30: 397-402.

9. Hajipour MJ, Fromm KM, Ashkarran AA, et al. Antibacterial properties of nanoparticles. Trends Biotechnol 2012; 30:499-511.

10. Jafari F, Samadi Kafil H, Jafari S, Aghazadeh M, Momeni T. Antibacterial activity of MTA fillapex and AH 26 root canal sealers at different time intervals. Iran Endod J 2016; 11:192-7.

11. Shin J H, Lee D Y, Lee S H. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. Journal of Dental Sciences 2018; 13: 54-9.

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

13. Kesler Shvero D, Abramovitz I, Zaltsman N, et al. Towards antibacterial endodontic sealers using quaternary ammonium nanoparticles. Int Endod J 2013; 46:747-54.

14. Love R M. Enterococcus faecalis-a mechanism for its role in endodontic failure. International Endodontic Journal 2001; 34: 399-405.

15. McHugh C P, Zhang P, Michalek S and Eleaser P D. pH required to kill Enterococcus faecalis in vitro. Journal of Endodontics 2004; 30: 218-19.

16. Mutoh N, Satoh T, Watabe H, Tani-Ishii N. Evaluation of the biocompatibility of resin-based root canal sealers in rat periapical tissue. Dent Mater J 2013; 32:413-9.

17. Ozcan E, Eldeniz AU, Ari H. Bacterial killing by several root filling materials and methods in an ex vivo infected root canal model. Int Endod J 2011; 44:1102-9.

18. Shalhav M, Fuss Z and Weiss E I. In vitro antibacterial activity of a glass ionomer endodontic sealer. Journal of Endodontics 1997; 23: 519-22.

19. Siqueira J F and De Uzeda M. Intracanal medicament: evaluation of the antibacterial effect of chlorohexidine, metronidazole and calcium hydroxide associated with three vehicles. Journal of Endodontics 1997; 23: 167-69.

20. Siqueira Jr JF and Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999; 32:361-9.

21. Sundqvist G, Figdor D, Endo D, Persson S and Sjogren U. Microbiological analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 1998; 85: 86-93.

22. Wang Z, Shen Y, Haapasalo M. Dentin extents the antibacterial effect of endodontic sealers against enterococcuc faecalis biofilms J Endod. 2012; 40: 505-8.

23. Zhang H, Shen Y, Ruse ND, et al. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod 2009a; 35:1051-5.

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

25. 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. 2009c; 107(6): 79-82.