

In vitro antimicrobial efficiency of different root canal sealers against *Enterococcus faecalis*

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ABSTRACT

Objective: The antibacterial effectiveness of four different sealers AH Plus, EndoRez, mineral trioxide aggregate (MTA) Fillapex, iRoot SP against *Enterococcus faecalis* was evaluated by time kill assay method *in vitro*. **Materials and Methods:** Four sealers are used in this study: An epoxy resin-based sealer, AH Plus (Dentsply, Maillefer, Switzerland), a polymethacrylate resin-based sealer, EndoRez (Ultradent, South Jordan, UT) and two calcium silicate-based sealers, MTA Fillapex (Angelus Solucxoes Odontologicas, Londrina, Brazil), iRoot SP (Innovative BioCreamix Inc., Vancouver, Canada). Each sealer was mixed according to manufacturer's instructions. Five mg of each sealer was added to sterile tubes separately and evaluated at 20 min, 24 h, 7 days, and 30 days. Two tubes were used as positive and negative. **Results:** At the 20th min AH Plus and iRoot SP were bactericidal, MTA Fillapex, and EndoRez were ineffective at the 20th min. At the 1st day MTA Fillapex was ineffective and rest of the sealers was bacteriostatic. At the 7th day, only MTA Fillapex showed bactericidal effect, AH Plus, iRoot SP and EndoRez were bacteriostatic. At the 30th day, MTA Fillapex was still bactericidal, AH Plus, iRoot SP, and EndoRez were still bacteriostatic. **Conclusion:** All root canal sealers tested were effective against *E. faecalis*. Fresh iRoot SP and fresh AH Plus had bactericidal action against *E. faecalis*. EndoRez has bacteriostatic behavior against *E. faecalis*. MTA Fillapex was the only sealer that could be bacteriocidal at 7th and 30th day.

Key words

Antibacterial activity, *Enterococcus faecalis*, mineral trioxide aggregate Fillapex, root canal sealer, time kill assay

INTRODUCTION

The main objective of endodontic treatment is to eliminate bacteria from the root canal system and to prevent them from infecting or re-infecting the root canal or the peri-apical tissues.^[1] The radicular space is a complex system with accessory, lateral, and furcation canals, with many dentinal tubules that are potential ways of entry to the radicular space.^[2] The most commonly used methods for microbial control include instrumentation, antimicrobial irrigation, intracanal dressing, adequate filling, and coronal restoration.^[3]

Microorganisms may be present not only throughout the pulp chamber but also in areas inaccessible to

instrumentation and disinfection, such as lateral canals, apical ramifications, crevices, and dentinal tubules. Therefore, the focus of root canal treatment must be complete, three-dimensional obturation of chemomechanically prepared root canal space followed by placement of a coronal restoration that produces optimal sealing of the access opening.^[2] Microorganisms may be destroyed by the antimicrobial activity of the sealer.^[4-6] The root canal sealers are mandatory for three-dimensional obturation. Root canal sealers should adhere to the core material and fill the irregularities between the core material and the root canal dentin.^[7,8] It is stated that, the root canal sealers show antibacterial activity that may contribute to the destruction of intracanal microorganisms and all sealers exhibit highest toxicity and antibacterial activity when freshly mixed that decreases during setting.^[9,10]

To evaluate the antibacterial activities of root canal sealers different methods like agar diffusion test, direct contact test, time kill assay (TKA) have been used.^[10-12] The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medications.^[3,13] This method allows direct comparisons of

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Quick Response Code:	Website: www.ejgd.org
	DOI: 10.4103/2278-9626.112311

root canal sealers against the test microorganisms.^[13] It is stated that this system could be affected by the diffusibility of the tested materials and the results obtained with this system do not reflect the true antimicrobial potential of the tested materials.^[14] The TKA is a method used to assess the ability of a fixed concentration of an antimicrobial agent to destroy a bacterial isolate under controlled conditions.^[10,15]

Enterococcus faecalis is one of the most commonly seen microorganism recovered from the root canals of teeth with failed root treatment^[16] and has also been implicated in persistent root canal infections.^[17,18] Its pathogenicity ranges from life threatening diseases in compromised individuals to less severe conditions, such as infection of obturated root canals with chronic apical periodontitis. In *in vitro* studies *E. faecalis* has been shown to invade dentinal tubules.^[19-22]

iRoot SP root canal sealer (Innovative BioCreamix Inc., Vancouver, Canada), has recently been introduced to the market. According to the manufacturer (http://www.veriodent.com/pb/wp_a0eb3a9d/wp_a0eb3a9d.html), iRoot SP is a convenient, premixed, ready-to-use injectable white hydraulic cement paste developed for permanent root canal filling and sealing applications. iRoot SP is an insoluble, radiopaque, and aluminum-free material based on a calcium silicate composition, which requires the presence of water to set and harden.^[12]

Another recently introduced root canal sealer is a mineral trioxide aggregate (MTA) based root canal sealer, MTA fillapex (Angelus Solucxoes Odontologicas, Londrina, Brazil). According to manufacturer, it consists of resins, silica, and MTA and it has high radioopacity, low solubility, and low expansion during setting and it promotes deposition of hard-tissue (http://www.angelus.ind.br/en/endodontics/mta_fillapex/).

The aim of this study was to evaluate the time depended pharmacodynamic activities of AH Plus, EndoRez, MTA Fillapex, iRoot SP against *E. faecalis* using the TKA method. The null hypothesis was that antimicrobial activity of all the root canal sealers against *E. faecalis* decrease by time.

MATERIALS AND METHODS

Four sealers are used in this study: An epoxy resin-based sealer, AH Plus (Dentsply, Maillefer, Switzerland), a polymethacrylate resin-based sealer, EndoRez (Ultradent, South Jordan, UT) and two calcium silicate-based sealers, MTA Fillapex (Angelus Solucxoes Odontologicas, Londrina, Brazil) and iRoot SP (Innovative BioCreamix Inc., Vancouver, Canada) [Table 1].

Preparation of sealers

Sealers were mixed according to manufacturers' instructions. Four different sterile tubes were prepared

Table 1: Composition of the sealers

Sealers	Compositions
AH Plus	Diepoxide, calcium tungstate, zirconium oxide, aerosol, 1-adamantane amine, diamine (3 (4),8 (9)-bis (aminomethyl) tricyclo-(5.2.1.02,6)decane), dibenzylidiamine, aminoadamantane, pigments
i Root SP	Zirconium oxide, calcium silicates, calcium phosphate, calcium hydroxide filler, and thickening agents
MTA Fillapex	Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, Mineral trioxide aggregate, pigments
EndoRez	30% urethane dimethacrylate, zinc oxide, barium sulfate, pigments

for each sealer and equal amount of the sealer (5 mg ± 0.1) was added into 10 × 90 mm sterile glass tube (Isotherm, Türkiye) by using a sterile wood swab. For the standardization of the samples, each sealer was added to the bottom of the tubes at a height of approximately 10 mm. Four of the tubes were used within 20 min after mixing of the sealers and the next four tubes were used at 24 h after mixing of the sealer, four tubes at 7 days after mixing of the sealer and four tubes at 30 days after mixing of the sealers. Samples were stored in at 35°C up to the study.

Time kill assay

E. faecalis, American Type of Culture Collection 29212 was used in this study. Inoculum was prepared by suspending *E. faecalis* colonies from blood agar into sterile to match a 0.5 McFarland Standard (approximately 10⁸ colony forming units (CFU)/mL) using a spectrometer (Becton Dickinson, USA) at a wave length 625 nm. A 100 µL of bacterial suspension (approximately 10⁸ CFU/mL) was taken with a micropipette. Then micropipette was used to inoculate this suspension into the tube containing 900 µL Tryptic soy broth (Oxoid, UK), resulting in a final inoculum of approximately 10⁷ CFU/mL. Then, the tube was vortexed and incubated at 35°C for 24 h.^[23] A 100 µL samples were taken with micropipette from 107 CFU/mL bacteria suspension, and inoculate at 0 (20th min), 1, 6, and 24 h and inoculated into another four tubes containing 900 µL Tryptic soy broth and sealer. The main tube was allowed to continue the incubation up to next sample time (1, 6, and 24 h, respectively). Two more tubes containing 900 µL of Tryptic soy broth were used as controls; one without bacteria for a sterility control and the other with bacteria for a growth control.

One hundred microliter samples were taken from each dilution tube and used to inoculate two 5% sheep blood agar plates (BioMerieux, France). Purity control was done with the subculture onto sheep blood agar plates. Colonies were counted following 18 h of incubation at 35°C the counts from plates showing 30-300 CFUs were averaged.^[23] The average counts were converted to actual CFU per mL by multiplying the average of raw counts by

dilution factors. CFU per milliliter (*y* axis) versus time (*x* axis) was plotted on a semi-log paper.

Pharmacodynamic analysis

In vitro model time-kill curves were determined by plotting mean colony counts (log₁₀ CFU/mL) from each tube versus time. Bactericidal activity (99.9% kill) was defined as a 3-log₁₀ CFU/mL reduction in the colony count from the original inoculum. Bacteriostatic activity (99.9% growth inhibition) was defined as a 2-log₁₀ CFU/mL reduction and indifference was defined as 1 log₁₀ CFU/mL reduction in the original inoculums.

RESULTS

Antibacterial activities of the four sealers using a TKA are shown in Figure 1. Freshly mixed sealers showed differences in their activity against *E. faecalis*. At the 20th min, 1st, 7th, and 30th days and at the 20th min AH Plus and I Root SP were bactericidal, MTA Fillapex and EndoRez were ineffective at the 20th min. At the 1st day MTA Fillapex was ineffective and rest of the sealers was bacteriostatic. At the 7th day, only MTA Fillapex showed bactericidal effect, AH Plus, iRoot SP, and EndoRez were bacteriostatic. At the 30th day, MTA Fillapex was still bactericidal, AH Plus, iRoot SP, and EndoRez were still bacteriostatic [Figure 1].

DISCUSSION

Microorganisms infecting the root canal dentine might adhere superficially to the dentinal wall or penetrate deeper into the dentinal tubules.^[24,25] Microorganisms that are placed deeper into the dentinal tubules could be killed by the antibacterial effects of the root canal sealers.^[26] An ideal endodontic sealer should be dimensionally stable, biocompatible and should have well sealing ability, long-lasting antibacterial efficiency.^[12,27-29] Antibacterial activity of sealers might help to eliminate the residual microorganisms that have survived the chemomechanical instrumentation and consequently improve the success of the endodontic treatment.^[12] Although chemomechanical instrumentation is mandatory for the disinfection of the root canal system, some residual microorganisms could survive^[17,21] and antibacterial activity of the root canal sealers would help to eliminate these surviving residual microorganisms.^[1,11-13]

E. faecalis is one of the most commonly isolated microorganisms from refractory periapical periodontitis^[30] and it is one of the most drug resistant bacteria and has ability to survive up to 12 months even under nutrient-deprived conditions in the root canal after routine root canal therapy.^[4,18,31,32] Because of *E. faecalis* persistent behavior we used it as a test microorganism.

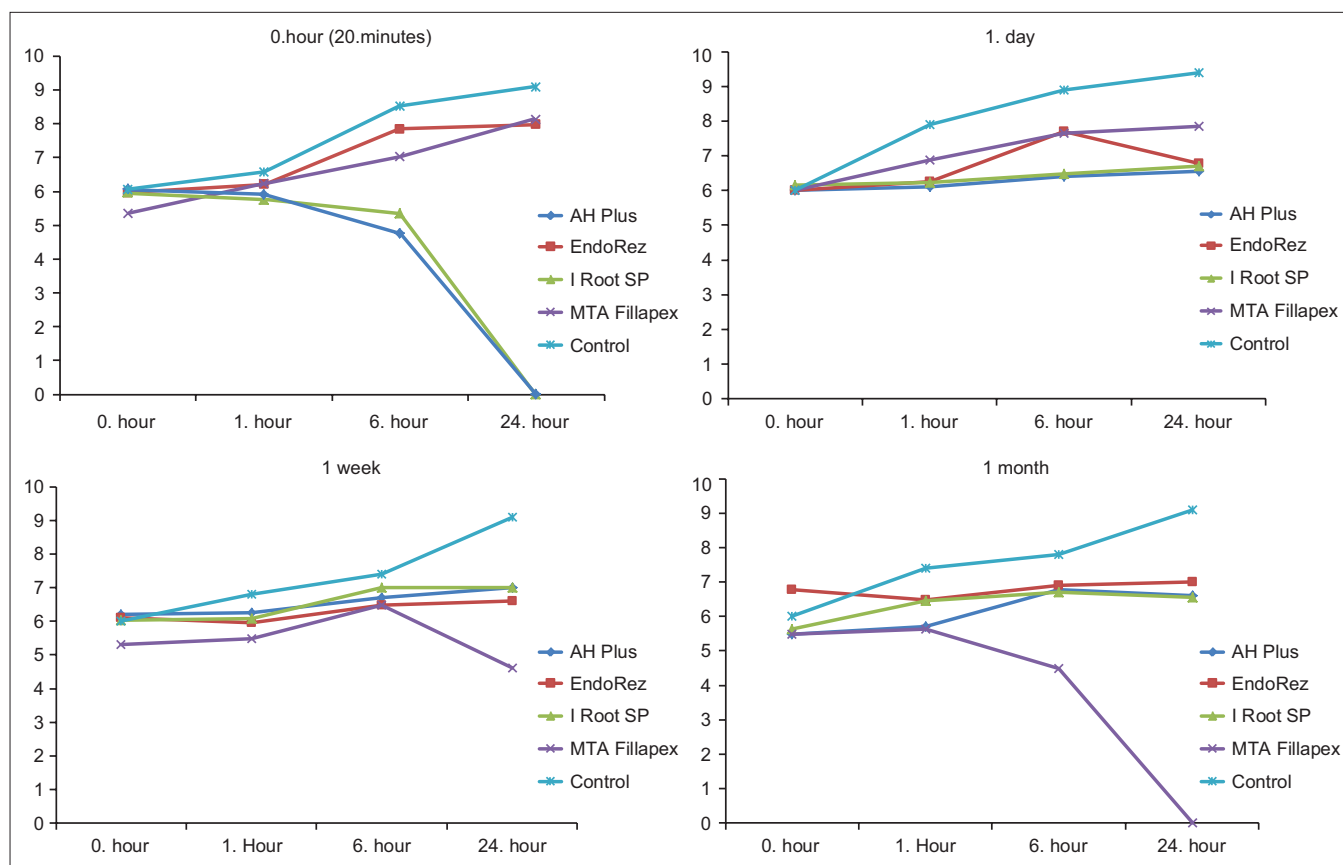


Figure 1: Antibacterial activities of the four sealers with time kill assay method at different time intervals

The antimicrobial activity of root canal sealers has been tested previously using various methods. These include agar diffusion tests,^[3,33-35] direct contact tests^[12,36,37] and TKA method.^[110] The TKA method is a method used to assess the antimicrobial activity of a measured amount of a antimicrobial agent against a constant amount of bacterial isolate at different time intervals.^[15] The kill rate is determined by measuring the number of viable bacteria at different time intervals by a graphic design called time-kill curve and it gives the opportunity to evaluate the antibacterial activity of sealers and classify them according to bacteriostatic or bactericidal action.^[4,10]

AH Plus was improved by its manufacturer from AH26 and has been reported as releasing almost no formaldehyde.^[38] In the present study, AH Plus was found to be bactericidal at the 20th min but at the 1st, 7th and 30th day it was found to be bacteriostatic. Zhang *et al.*^[16] and Sagsen *et al.*^[14] also reported that freshly mixed AH Plus had high antimicrobial activity. The antibacterial activity of AH Plus could be related to epoxy resin and amines ingredients.^[39] Our results were similar with Zhang *et al.*^[12] and Kayaoğlu *et al.*^[26] who reported that freshly mixed AH Plus killed *E. faecalis* effectively.

EndoRez (Ultradent, South Jordan, UT) is a hydrophilic, dual-cured sealer containing zinc oxide, barium sulphate, resins and pigments in a matrix of urethane dimethacrylate resin.^[40] In the present study, EndoRez did not show antibacterial activity at 20 min. At the 1st day, EndoRez was bacteriostatic and at the 7th and 30th days it was still bacteriostatic. Our results were similar as the study of Zhang *et al.*^[12] that reported EndoRez was bacteriostatic at the 1st, 7th, and 30th days. However, contrary to Zhang *et al.*,^[12] according to our results at the minute EndoRez was ineffective. At the 20th min our results were similar to Eldeniz *et al.*^[41] who found that EndoRez did not show any antibacterial activity.

I Root SP is a newly introduced endodontic sealer based on calcium silicate.^[42] In the present study, I Root SP was found to be bactericidal at the 20th min. At the 1st, 7th, and 30th day I Root SP was still found to be bacteriostatic. Zhang *et al.*^[12] reported that I Root SP showed bactericidal action at the 2nd min of contact when freshly mixed and after 1 day of setting I Root SP killed all the bacteria at the 20th min but at the 7th day I Root SP was ineffective. In the present study, freshly mixed samples showed bactericidal activity but at the 7th and 30th days we found that I Root SP was bacteriostatic contrary to Zhang *et al.*^[12] The sealer contains calcium silicate cement, calcium phosphate and calcium oxide.^[42] The antibacterial effect of I Root SP sealer might be a combination of high pH, hydrophilicity, and active calcium hydroxide diffusion.^[12]

MTA Fillapex is another newly introduced resin silicate and calcium silicate based root canal sealer. In the present study, MTA Fillapex was found to be ineffective

at the first 20 min. At the 1st day it was bacteriostatic and at 7th and 30th day MTA Fillapex was bactericidal. On the contrary to our results Morgental *et al.*^[43] stated that MTA Fillapex was effective before setting but not effective 7 days after setting. Such discrepancies are probably due to methodology. The antimicrobial activity of MTA was reported by Torabinejad *et al.*,^[44] who detected its efficiency against some facultative bacteria; however, no activity was found against *E. faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* or against anaerobic bacteria. However, Stowe *et al.*^[45] assessed the antimicrobial properties of MTA and found that it inhibited the growth of both *E. faecalis* and *Streptococcus sanguis*. MTA contains calcium oxide, which forms calcium hydroxide on contact with water, which gives antibacterial property to MTA.^[39,44,46] MTA Fillapex contains calcium silicate cement and with the moisture from dentin the hydration reactions of calcium silicates begins and calcium silicate hydrogel and calcium hydroxide exists which gives the high PH and antibacterial property to MTA Fillapex.^[12] Our results could be related to calcium silicate and MTA and resin ingredient of the MTA Fillapex.

In conclusion, the results of the present study showed that only fresh I Root SP and AH Plus had bactericidal action against *E. faecalis* and both sealers had continued to have bacteriostatic behavior at 1st, 7th, and 30th days. EndoRez had bacteriostatic behavior during the study except freshly mixed samples. MTA Fillapex was the only sealer that could be bactericidal at 7th and 30th day. Within the limitation of the present study it is essential not to forget the importance of chemomechanic preparation in endodontic practice and further investigations are needed for evaluation of the antimicrobial efficiencies of the root canal sealers.

ACKNOWLEDGMENT

We would like to thank Angelus for their support with MTA Fillapex.

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How to cite this article: Ustun Y, Sagsen B, Durmaz S, Percin D. *In vitro* antimicrobial efficiency of different root canal sealers against *Enterococcus faecalis*. *Eur J Gen Dent* 2013;2:134-8.

Source of Support: Nil, **Conflict of Interest:** None declared.