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ANTIBACTERIAL EFFICACY OF MINERAL TRIOXIDE AGGREGATE COMBINED WITH NANO-SILVER ADDITIVES

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ABSTRACT

Introduction The aim of the study was to evaluate the antibacterial effect of Mineral Trioxide Aggregate (MTA) mixed with saline and Mineral Trioxide Aggregate mixed with silver nanoparticles solution; 25 ppm (25AG) and 12 ppm (12 AG) at three setting conditions using the direct contact test.

Methodology Each of the tested materials was placed at the bottom of 96-well plates then immediately exposed to the bacterial suspension or exposed after 3 days and after 7 days of setting. Aliquots of the bacterial suspension were placed on the tested materials and in the control wells for 1 hour to ensure direct contact between all the bacteria and the surface of the tested materials. BHI broth was then added to each of the wells; 15 µl of the bacterial suspension was transferred to corresponding wells in other plates, containing fresh culture medium. The kinetics of bacterial growth was measured every two hours for six hours, using spectrophotometer at a wavelength of 620.

Results There was a statistically significant effect of the material on the antibacterial activity ($p=0.001$, $p<0.05$), where the 25 AG showed the highest antibacterial effect.

Conclusion The addition of Nano-silver solution to MTA seemed to increase its antibacterial effect against *Enterococcus faecalis and Pseudomonas aeruginosa*.

INTRODUCTION

Endodontic surgery is an alternative to dental extraction to save the tooth when conventional retreatment had failed or is impossible to be made. One of the main goals of the endodontic surgery is to improve the apical seal and this could be done by placing the root-end filling material in a well-prepared root-end cavity [1].

An ideal root-end filling material should adhere and adapt to the dentinal walls of the prepared root-end cavity, prevent the leakage of the microorganisms and their byproducts into the periapical tissues, be insoluble in tissue fluids, be biocompatible, be dimensionally stable, be easy to manipulate, not be affected by moisture and should have an antibacterial effect [2-5].

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Mineral trioxide aggregate (MTA), has been introduced in endodontics in 1993 for its advantageous properties of biocompatibility; especially its ability to enhance cementogenesis, promoting healing and good sealing ability. MTA has many clinical applications: it could be used in the repair of perforations, in the management of open apices as an apical barrier, in vital pulp therapy and in filling of prepared root-end cavities [6-9]. Another commercial form of MTA was introduced in the Brazilian market in 2001 (MTA-Angelus) as an alternative to ProRoot™ MTA. MTA-Angelus contains 80% Portland cement and 20% bismuth oxide with no calcium sulfate (gypsum) in an attempt to reduce the setting time [10]. Inspite of its advantageous properties, there are controversy in the result of the antibacterial studies [11].

Several investigations used additives to improve the handling characteristics and physicochemical properties of MTA [12-14], in addition to the use of Silver/tin alloy by *Camilleri J* [15] as an alternative radiopacifier in calcium silicate cement for use as a root-end filling material without changing the hydration mechanism of the resultant material, however few studies evaluated the effect of additives on the antibacterial effect of MTA.

Recently, the nanoparticles have expanded applications in various fields, related to their unique physical and chemical characteristics, including high surface to volume ratio, high surface reactivity, and sizes in the range of 1–100 nm, where nano is defined as one billionth of a quantity, represented mathematically as 10^{-9} [16].

Studies have been conducted on silver nanoparticles (Ag-NPs) as an antimicrobial agent, or incorporating it in several antimicrobial applications, such as in bone prostheses coated or embedded with Ag-NPs, wound dressings, bandages, ointments and in surgical instruments, these may be related to its broad spectrum activity and lower tendency to induce microbial resistance compared to that of antibiotics [17].

Silver nanoparticles has been evaluated for its inhibitory effect on the oral bacterial growth of oral bacteria and accelerating effect on wound healing [18,19] thus the aim of this study was to assess the effect of addition silver nanoparticles on the antibacterial activity of MTA against *Enterococcus faecalis and Pseudomonas aeruginosa*, when used as root-end filling material.

MATERIALS AND METHODS

Test microorganisms and growth conditions:

Antibacterial activity of the tested materials was evaluated against the following facultative bacterial species: *Enterococcus faecalis and Pseudomonas aeruginosa*. Bacteria were grown aerobically from frozen stock cultures in Brain Heart Infusion broth (BHI broth, OXOID, England) at 37° C. With the two bacterial species, 18 to 20-hrs cultures 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.

Evaluation of the antibacterial activity:

The " Direct contact test" was used to assess the antibacterial activity of the tested materials; MTA-Angelus (Angelus, Londrina, PR, Brazil) mixed with sterile water and MTA-Angelus mixed with 25 part per million silver nanoparticles solution or 12 part per million nanoparticles solution, the test is based on determining the turbidity of bacterial growth in 96-well plates.

- Preparation of the samples:

The materials were mixed using a sterile stainless steel spatula and a clean, dry glass slab. For the MTA samples, powder was mixed with sterile water according the instructions provided by the manufacturer of MTA, where the material was dispensed and gradually incorporated with the liquid from the enclosed micro-ampoule; if needed, one or

two drops of sterile water were to be added to make the material into a thick creamy consistency with a final water-to-powder ratio of approximately 0.3 (1g: 3g) [20]. For the samples in which MTA powder was mixed with silver nanoparticles solution; 25 ppm (25AG) and 12 ppm (12 AG) were prepared by dilution of silver nanoparticles solution 0.1 mg/ml stock solution (nanotech, Nanotech Egypt for photo Electronics), then mixed with MTA powder according the instructions provided by the manufacturer of MTA.

Each of the tested materials was placed at the bottom of 96-well plates to a height of 1-2 mm and then condensed with a large plugger. The unfilled wells of the plate were used for the control group. All the procedures were carried out under aseptic conditions in a laminar flow cabinet.

The samples of each tested material were evaluated under three setting conditions as follows: fresh mix, where the samples were immediately exposed to the bacterial suspension, 3 days-old, where the samples were allowed to set at 37°C temperature and 100 % relative humidity in an incubator (Forma Series II water jacketed CO2 incubator, Thermo electron corporation, USA) for 3 days before testing, and 7 days-old, where the samples were allowed to set at 37°C temperature and 100 % relative humidity for 48-hrs then aged for 5 days in saline before testing.

- Spectrophotometric measurements:

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 ensuring direct contact between all the bacteria and the surface of the tested materials. BHI broth (245 µl) was then added to each of the wells and the plates were gently mixed for 2 min; 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 again mixed for 2 min [21].

The kinetics of bacterial growth in each plate was followed by continuous densitometric measurement every two hours for six hours, using a microplate spectrophotometer at a wavelength of 620 nm. The experiment was repeated three times under each condition for each of the two bacterial species: *Enterococcus faecalis* and *Pseudomonas aeruginosa* to ensure reproducibility.

Statistical analysis:

One-way analysis of variance (one way ANOVA) was used to compare the means of the optical density influenced by the type of the material (MTA, 25 AG, 12 AG and control) under each setting condition (Fresh mix, 3 days-old and 7 days-old) at each of the three reading points (2h, 4h and 6h). Tukey's post-hoc test was used for pair-wise comparisons of the experimental and control groups. Three-way ANOVA test was used to study the effect of the Material on the antibacterial activity. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 24 for Windows.

RESULTS

For the *Enterococus faecalis*, One-way ANOVA test showed a statistically significant difference among the groups ($p=0.001$, $p<0.05$) under different setting condition and all the reading points. In Fresh mix, the value of the mean optical density was highest for control group followed by MTA then 12 AG and 25 AG, where there was no significant difference between 12 AG and 25 AG group, while in 3 days-old and 7days-old mix, 25 AG showed the highest antibacterial effect followed by 12 AG, MTA and control (Table 1 and Figure 1). For *Pseudomonas aeruginosa*, One-way ANOVA test showed a statistically significant difference among the groups

(p=0.001, p<0.05) under different setting condition and all the reading points. In Fresh mix, a statistically significant difference existed between MTA and the control, between 25 AG and the control, as well as between 12 AG and the control (p<0.05), while in 3 days-old and 7 days-old mix, 25 AG showed the lowest mean optical density followed by 12 AG and MTA, then control (Table 2 and figure 2). According to the Three-way ANOVA test, there was a statistically significant effect of the Material on the antibacterial activity (p=0.001, p<0.05), where the 25 AG showed the highest antibacterial effect.

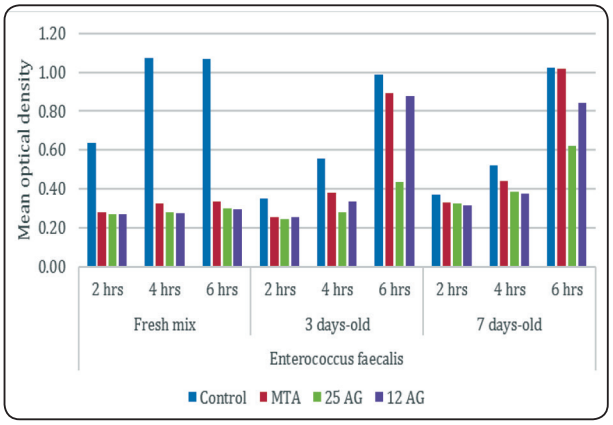

Fig. (1) Bar chart showing the mean optical density of Enterococcus faecalis with the tested materials and the control under the three setting condition (λ=620nm).

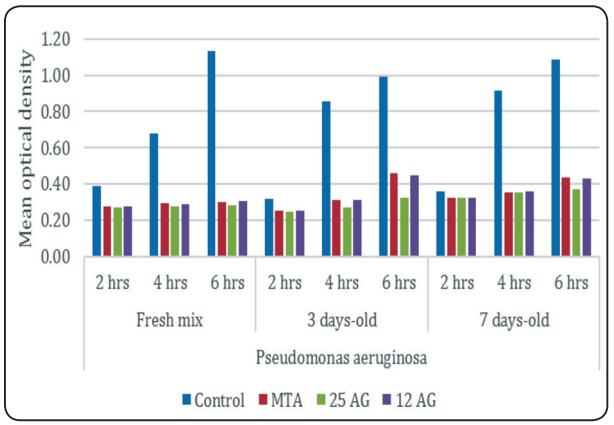

Fig. (2) Bar chart showing the mean optical density of *Pseudomonas aeruginosa* with the tested materials and the control under the three setting condition (λ=620nm).

TABLE (1) The means and standard deviation (SD) values of the Mean optical density of *Enterococcus faecalis* with MTA, 25 AG, 12 AG and control at the three setting conditions.

Setting conditions	**Reading**	**Control** Mean(SD)	**MTA** Mean(SD)	**25 AG** Mean(SD)	**12 AG** Mean(SD)	*p*-value
Fresh mix	**2 hrs**	0.64 ^{a} (0.03)	0.28^{b} (0.03)	0.27^{b} (0.01)	0.27^{b} (0.01)	0.001*
	4 hrs	1.07 ^{a} (0.03)	0.32^{b} (0.04)	0.28^{c} (0.01)	0.28^{c} (0.01)	
	6 hrs	1.07 ^{a} (0.02)	0.33^{b} (0.05)	0.30^{c} (0.02)	0.30^{c} (0.01)	
3 days-old	**2 hrs**	0.35^{a} (0.01)	0.26^{b} (0.01)	0.24^{c} (0.00)	0.26^{b} (0.01)	0.001*
	4 hrs	0.56^{a} (0.03)	0.38^{b} (0.05)	0.28^{c} (0.05)	0.34^{bc} (0.07)	
	6 hrs	0.99^{a} (0.04)	0.89^{a} (0.11)	0.43^{b} (0.26)	0.88^{a} (0.19)	
7 days-old	**2 hrs**	0.37^{a} (0.01)	0.33^{b} (0.01)	0.33^{b} (0.02)	0.32^{b} (0.02)	0.001*
	4 hrs	0.52^{a} (0.03)	0.44^{b} (0.07)	0.38^{c} (0.05)	0.37^{c} (0.03)	
	6 hrs	1.02^{a} (0.07)	1.02^{a} (0.08)	0.62^{b} (0.25)	0.84^{a} (0.19)	

: Significant at p ≤ 0.05, groups identified with different letters in the same row are statistically significantly different, same letters in the same row are not significantly different. SD= standard deviation.

TABLE (2) The means and standard deviation values of the Mean optical density of *Pseudomonas aeruginosa* with MTA, 25 AG, 12 AG and control at the three setting conditions.

Setting conditions	Reading	Control Mean(SD)	MTA Mean(SD)	25 AG Mean(SD)	12 AG Mean(SD)	*p*-value
Fresh mix	**2 hrs**	0.39[a] (0.01)	0.27[b] (0.01)	0.27[b] (0.01)	0.27[b] (0.01)	0.001*
	4 hrs	0.68[a] (0.04)	0.29[b] (0.01)	0.27[b] (0.01)	0.29[b] (0.01)	
	6 hrs	1.14[a] (0.04)	0.30[b] (0.01)	0.28[b] (0.01)	0.31[b] (0.01)	
3 days-old	**2 hrs**	0.32[a] (0.01)	0.25[b] (0.01)	0.25[b] (0.01)	0.25[b] (0.02)	0.001*
	4 hrs	0.86[a] (0.03)	0.31[b] (0.04)	0.27[b] (0.01)	0.31[b] (0.05)	
	6 hrs	0.99[a] (0.03)	0.46[b] (0.12)	0.33[c] (0.03)	0.45[b] (0.04)	
7 days-old	**2 hrs**	0.36[a] (0.01)	0.32[b] (0.01)	0.33[b] (0.01)	0.33[b] (0.02)	0.001*
	4 hrs	0.92[a] (0.02)	0.35[b] (0.01)	0.35[b] (0.02)	0.36[b] (0.01)	
	6 hrs	1.09[a] (0.02)	0.43[b] (0.07)	0.37[c] (0.03)	0.43[b] (0.02)	

: Significant at p ≤ 0.05, groups identified with different letters in the same row are statistically significantly different, same letters in the same row are not significantly different. SD= standard deviation.

DISCUSSION

An ideal endodontic material should adhere and adapt to the dentinal wall, prevent the leakage of the microorganisms and their byproducts, be insoluble in the tissue fluids, biocompatible; dimensionally-stable and easy to manipulate, not corrode or stain the periapical tissues and should have an antimicrobial effect [22].

In the present study, the materials were evaluated against the following facultative bacterial species: Enterococcus faecalis and Pseudomonas aeruginosa, Although the aerobic and facultative microorganisms are usually minor constituents of the primary infections, it was observed by *Sundqvist et al*. [23] that they have been found in cases of flare-ups and endodontic failures. It was reported that *Enterococcus faecalis, Pseudomonas aeruginosa and Staphylococcus aureus* are species resistant to several antimicrobial agents [24,11].

Enterococcus faecalis can grow as a monoinfection in treated canals in the absence of synergistic support from other bacteria, can colonize the dentinal wall under stressful conditions like nutrient deficiency, chemo-mechanical 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*. *a*nd *McHugh et al* [25, 26]; pH greater than 11.0 is needed to kill *Enterococcus faecalis*.

The antibacterial activity of the tested materials was evaluated by the direct contact test. As reported by *Shalhav et al*. [27], it is a reproducible and quantitative assay that allows testing of water- insoluble materials, continuous measurement of bacterial growth and could, also, be used for standardized aging studies. In the direct contact test, bacteria are brought in direct contact with the tested samples for a controlled period of time to allow the measuring of the effect of direct and close contact between microorganisms and the tested material on microbial 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 standardization of the inoculum density,

the growth medium, the agar viscosity and the storage condition of the agar plates. In addition, the agar diffusion test results were found to be influenced by the diffusibility of the materials across the medium; not only the antibacterial activity [28]. The agar diffusion test, also, was found not to be a reliable method for testing the antibacterial properties of calcium hydroxide-based materials; this substance has low solubility and may slowly diffuse in agar [21].

In the present study the results showed that MTA had an antibacterial activity against the tested microorganisms, which was in agreement with *Filho et al*. [29], *Eldeniz et al* .[21] and *Koruyucu et al* [30]. However, the results of the present study differed from those of *Miyagak et al*. (31) and *Yasuda et al*. [22] These could be attributed to the variations in the methods of the antibacterial activity evaluation as the direct contact test was used in the present study, while the others used the agar diffusion test or due to the variations in the materials used.

According to the manufacturer MTA-Angelus contains 80% Portland cement and 20% bismuth oxide with no calcium sulfate (gypsum). Portland cement is hygroscopic material; part of the water in the mix is consumed in the chemical reaction and another part is trapped in pores. Calcium hydroxide is the main soluble fraction produced by the hydrolysis of Portland cement. When the setting cement contacts with an aqueous environment, it absorbs water and the trapped water is released with calcium hydroxide [32]. *Siqueira, Lopes* [33] reported that most of the endodontic pathogens are unable to survive in the highly alkaline environment provided by calcium hydroxide, since its pH is about 12.5, and it was found that the water that had been in contact with MTA specimens had highly alkaline pH that ranged between 11.94 and 11.99 [32].

Results showed that MTA has extended antibacterial activity up to 7 days, which was in accordance with *Fridland and Rosado* [34], who reported that the high pH of MTA, ranging from 11.00 to 12.00, was maintained for 78 days.

MTA did not show any antibacterial effect in the 3days-old mixes and 7days-old mix with *Enterococus feacalis* at the 4h and 6h reading; this could be correlated to and explained on the basis that the initial rapid release of calcium hydroxide into the solution leaves an outer layer of calcium silicate hydrate, this is followed by a dormant period during which little hydration occurs due to the deposition of coating on the unhydrated cement grains, then eventually the coating ruptures because of the pressure of the products of hydration beneath, and hydration speeds again [35].

Recently, the study of the synthesis and effect of Ag-NPs has drawn the attention, because of its antimicrobial properties [36, 37]. In the present study, results showed that MTA mixed with silver nanoparticles solution has an extended antibacterial activity extended up to 7 days, which was in accordance *Abbaszadegan et al*. [38] who revealed that *Enterococus feacalis* did not survive after 4 and 24-hrs of contact with the most diluted form of Positively charged Ag-NPs, with *Zhuang et al*. [39] study, where 0.1% Ag-NPs had a strong bactericidal effect against *Enterococus feacalis* biofilm formed on dentine surface after 24-hrs of exposure and *Wu et al*. [40], who demonstrated that Ag-NPs were able to destroy *Enterococus feacalis* biofilm after an optimum time of interaction. These may be related to the sustained release of Ag+ by the progressive cement hydrolysis over time [41], which may create free radicals and induce oxid-dative stress, enhancing the bactericidal activity [42].

The present study showed that MTA mixed with silver nanoparticle solution had more antibacterial effect than MTA mixed with sterile water, these was in agreement with *Samiei et al* [11], *Bahador et al*. [43, 44] and *Jonaidi-Jafari* et al. [45], these could be attributed to the large surface area of the silver nanoparticles allowing better contact with microorganism, attachment and penetration [46], in addition to the interaction with phosphorus-containing com-

pounds like DNA and inhibiting its function [47, 48] and attacking the respiratory chain in the bacterial mitochondrial, leading to its death [49].

Results showed that 25AG showed better antibacterial effect than 12AG, these was in agreement with Bahador et al [44], Sondi I, Sondi B [50] and wu et al. [51] who reported that the antibacterial effect of silver nanoparticles depend on concentration and the time of interaction, regarding the cytotoxicity of silver nanoparticles, it has been reported by Miura and Shinohara [52] that 80 µg/ml nanosilver could be cytotoxic to Helal cells and Gomes-Filho et al [53], who observed mild tissue reaction with 23 ppm nanosilver, thus it could be advised to use 25ppm nanoparticles to improve the antibacterial effect of MTA.

REFERENCES

1. Harty FJ, Parkins BJ, Wengraf AM. The success rate of apicectomy. A retrospective study of 1016 cases. Br Dent J 1970; 129: 407-413.
2. Fogel HM, Peikoff MD. Microleakage of root-end filling materials. J Endod 2001; 27: 456-458.
3. Gartner AH, Dorn SO. Advances in endodontic surgery. Dent Clin North Am 1992; 36: 357-78.
4. Jou Y, Pertl C. Is there a best retrograde filling material? Dent Clin North Am 1997; 41:555-561.
5. Siqueira JF, Rôças IN, Abad E, Castro AJR, Gahyva SM, Favieri A. Ability of three root-end filling materials to prevent bacterial leakage. J Endod 2001; 27: 673-675.
6. Holland R, Otobonni-Filho JA, Souza V, Nery MJ, Bernabé PF, Dezan ED. Mineral trioxide aggregate repair of lateral root perforations. J Endod 2001; 27: 281-284.
7. Noetzel J, Ozer K, Reisshauer BH, Anil A, Rössler R, Neumann K, et al. Tissue responses to an experimental calcium phosphate cement and mineral trioxide aggregate as materials for furcation perforation repair: a histological study in dogs. Clin Oral Invest 2006; 10: 77-83.
8. Asgary S, Eghbal MJ, Parirokh M, Ghanavati F, Rahimi HA. Comparative study of histologic response to different pulp capping materials and a novel endodontic cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106: 609-614.
9. Seong TH, Kwang SB, Seung HB, Kee YK, Woo CL. Microleakage of Accelerated Mineral Trioxide Aggregate and Portland Cement in an In Vitro Apexification Model. J Endod 2008; 34: 56-58.
10. Bortoluzzi EA, Broon NJ, Bramante CM, Garcia RB, Moraes IG, Bernardineli N. Sealing ability of MTA and radiopaque Portland cement with or without calcium chloride for root end filling. J Endod 2006; 32: 897-900.
11. Samieia M, Aghazadehb M, Lotfia M, Shakoeia S, AghazadehcZ, Mahdi S. Antimicrobial Efficacy of Mineral Trioxide Aggregate with and without Silver Nanoparticles. Iran Endod J 2013; 8:166-170.
12. Ber B, John FH, Gregory PS. Chemical modification of ProRoot MTA to improve handling characteristics and decrease setting time. J Endod 2007; 33: 1231-1234.
13. Wiltbank KB, Schwartz SA, Schindler WG: Effect of selected accelerants on the physical properties of mineral trioxide aggregate and Portland cement. J Endod 2007; 33: 1235-1238.
14. Parirokh M, Torabinejad M. Mineral trioxide aggregate: comprehensive literature reviews-part I: chemical, physical and antibacterial properties. J Endod 2010; 36: 16-27.
15. Camilleri J. Hydration characteristics of calcium silicate cements with alternative radiopacifiers used as root-end filling materials. J Endod 2010; 36: 502-508.
16. Naidu KSB, Govender P, Adam JK. Biomedical applications and toxicity of nanosilver: a review. Med Techno SA 2015; 29: 13-19.
17. Nour el Din S, El-Tayeb T, Abou-aisha K, El-azizi M. In vitro and in vivo antimicrobial activity of combined therapy of silver nanoparticles and visible blue light against Pseudomonas aeruginosa. Int J Nanomedicine 2016:11: 1749-1758.
18. Zhao L, Wang H, Huo K, Cui L, Zhang W, Ni H, et al. Antibacterial nanostructured titania coating incorporated with silver nanoparticles. Biomaterials 2011; 32:5706-5716.
19. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: Review of the literature. Burns 2007; 33:139-148.
20. Dammaschke T, Gerth HUV, Zuchner H, Schafer E. Chemical and physical surface and bulk characterization of white ProRoot MTA and two Portland cement. Dent Mater 2005; 21: 731-738.

21. Eldeniz AU, Hadimli HH, Ataoglu A, Orstavik. Antibacterial effect of selected root end filling materials. J Endod 2006; 32: 345-9.

22. Yasuda Y, Kamaguchi A, Saito T. In vitro evaluation of the antimicrobial activity of a new resin-based endodontic sealer against endodontic pathogens. J Oral Scie 2008; 50:309-313.

23. Sundqvist G, Figdor D, Endo D, Persson S, Sjogren U. Microbiological analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85: 86-93.

24. Eldeniz AU, Erdemir A, Hadimli HH, Belli S, Erganis O, Konya A, Kirikkale. Assessment of antibacterial activity of EndoRez. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102: 119-126.

25. Love RM. Enterococcus faecalis- a mechanism for its role in endodontic failure. Int Endod J 2001; 34: 399-405.

26. McHugh CP, Zhang P, Michalek S, Eleaser PD. pH required to kill Enterococcus faecalis in vitro. J Endod 2004; 30: 218-219.

27. Shalhav M, Fuss Z, Weiss EI. In vitro antibacterial activity of a glass ionomer endodontic sealer. J Endod 1997; 23:519-522.

28. Siqueira JF and De Uzeda M. Intracanal medicament: evaluation of the antibacterial effect of chlorohexidine, metronidazole and calcium hydroxide associated with three vehicles. J Endod1997; 23: 167-169.

29. Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. In vitro antimicrobial activity of endodontic sealers, MTA-based cement and Portland cement. J Oral Scie 2007; 49:4-45.

30. Koruyucu M, Topcuoglu N, Tuna B, Ozel S, Gencay K, Kulekci G, et al. An assessment of antibacterial activity of three pulp capping materials on Enterococcus faecalis by a direct contact test: An in vitro study. Eur J Dent 2015; 9: 240-245.

31. Miyagak DC, Carvalho EM, Robazza CR, Chavasco JK, Levorato GL. In vitro evaluation of antimicrobial activity of endodontic sealers. Braz Oral Rese 2006; 20: 303-306.

32. Fridland M, Rosado R. Mineral trioxide aggregate (MTA) solubility and porosity with different water to powder ratios. J Endod 2003; 29: 814-17.

33. Siqueira JF, Lopes H D. Mechanism of antibacterial activity of calcium hydroxide: a critical review. Int Endod J 1999; 32: 361-369.

34. Fridland M, Rosado R. MTA solubility: A long term study. J Endod 2005; 31: 376-379.

35. Neville AM. Properties of concrete. In: Neville AM, editor. 3rd edition. England, London; Marshifield Mass:Pitman Pub Inc; 1981.p.779.

36. Rai, A. Yadav, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances 2009; 27: 76-83.

37. Eckhardt S, Brunetto PS, Gagnon J, Priebe M, Giese B, Fromm KM, Nanobio silver: its interactions with peptides and bacteria, and its uses in medicine, Chemical Reviews 2013; 113:. 4708–4754.

38. Abbaszadegan A, Nabavizadeh M, Gholami A, Aleyasin ZS, Dorostkar S, Saliminasab M, et al. Positively charged imidazolium-based ionic liquid-protected silver nanoparticles: a promising disinfectant in root canal treatment. Int Endod J 2014; 1-11.

39. Zhuang P, Gao Y, Ling J, Hu X, Wang Z. Bactericidal effect of nano-silver against E. faecalis biofilm on dentin. Chinese Journal of Stomatological 2011; 5, 463-469.

40. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod 2014; 40, 285–290.

41. Alt V, Bechert T, Steinrücke P, Wagener M, Seidel P, Dingeldein E An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement Biomaterials 2004, 25, 4383-4391.

42. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, et al. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005; 16: 2346-2353.

43. Bahador A, Khaledi A, Ghorbanzadeh R. Evaluation of antibacterial properties of nano silver Iranian MTA against Fusobacterium nucleatum. Eur J Exper Biolo 2013; 3:88- 94.

44. Bahador A, Pourakbari B, Bolhari B, Hashemi FB. In Vitro Evaluation of the Antimicrobial Activity of Nanosilver-Mineral Trioxide Aggregate against Frequent Anaerobic Oral Pathogens by A Membrane-enclosed Immersion Test. Biomed J 2015; 38: 77-83.

45. Jonaidi-Jafari M, Izadi M, Javidi P. The effects of silver nanoparticles on antimicrobial activity of ProRoot mineral

trioxide aggregate (MTA) and calcium enriched mixture (CEM). J Clin Exp Dent. 2016; 8: e22-e26.

46. Bryaskova R, Pencheva D, Nikolov S, Kantardjiev T. Synthesis and comparative study on the antimicrobial activity of hybrid materials based on silver nanoparticles (AgNps) stabilized by polyvinylpyr-rolidone (PVP). J chem Biol. 2011; 4: 185-191.

47. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, et al. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16: 2346-2353.

48. Matsumura Y, Yoshikata K, Kunisaki Si, Tsuchido T. Mode of Bactericidal Action of Silver Zeolite and Its Comparison with That of Silver Nitrate. Appl Environ Microbiol. 2003; 69:4278-4281.

49. Sondi I, Goia DV, Matijević E. Preparation of highly concentrated stable dispersions of uniform silver nanoparticles. J colloid Interface sci. 2003; 260:75-81.

50. Sondi I, Sondi BS. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J colloid Interface sci 2004; 275: 177-182.

51. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the Antibacterial Efficacy of Silver Nanoparticles against Enterococcus faecalis Biofilm. J Endod 2014; 40: 285-290.

52. Miura N, Shinohara Y. Cytotoxic effect and apoptosis induction by silver nanoparticles in Hela cells. Biochem Biophys. Res Commun. 2009; 390: 733-737.

53. Gomes-Filho JE, Oliveira Silva F, Watanabe S, Angelo Cintra LT, Tendoro KV, Dalto LG, et al. Tissue Reaction to Silver Nanoparticles Dispersion as an Alternative Irrigating Solution. J Endod 2010; 36:1698-1702.