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COMPARATIVE STUDY OF THE ANTIBACTERIAL EFFECT OF MTA, NANO-MTA, PORTLAND CEMENT, AND NANO-PORTLAND CEMENT

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ABSTRACT

The root end filling materials antibacterial properties is a must nowadays as there is none of the available root canal filling materials can provide a hermetic seal.The aim of the current study isto compare the antibacterial effect of Gray Portland cement and white MTA angelus in the conventional and nano-particle forms. Agar diffusion test was used to examine the tested materials, where Enterococcus faecalis ATCC29212 (American Type Culture Collection 29212) cultured in agar BHI (brain heart infusion) in anaerobic condition at 37°Cfor 72 hours.Then, the agar was added to 12 sterile petri plates and the prepared bacterial suspension was inoculated. Punch holes of 6 mm diameter were formed on each agar plate and each 3 plates were filled with the materials tested (Portland cement, Nano-Portland cement, MTA angelus, and Nano-MTA angelus). The results showed that conventional Portland cement has the highest antibacterial property, followed by Nano-Portland cement, MTA angelus, Nano-MTA angelus respectively with significant difference according to Kruskal Wallis ANOVA test followed by Mann-Whitney U test.

INTRODUCTION

Microorganisms are the main cause of endodontic treatments failures. It is imperative to eliminate microorganisms from theroot canal system. However, some bacteria may remain in the root canal system.(1)

Successful endodontic treatment depends on capability of bacterial elimination and sealof the root canalsystem (2). Since none of the available root canal filling materials can provide a hermetic seal, they must have antibacterial properties to prevent infection. Many root canal filling materials have been introduced with such properties but none of them are ideal (3).

A wide variety of materials was used for retrograde fillings and perforation repair throughout the dental history.(4,5) An ideal root-end filling material should produce a complete apical seal, be nontoxic, well tolerated by the periradicular

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tissues, dimensionally stable, easy to manipulate, and radiopaque in radiograph as well as not to be easily resorbed .In addition, it should be bactericidal or bacteriostatic. Numerous materials have been recommended as root-end filling materials, but none has so far been found to be totally ideal.

Most endodontic failures are caused by inadequate cleansing of the root canal and egress of bacteria and other antigens into the periradicular tissues. Inaddition to sealing ability and biocompatibility, root-end filling materials should ideally have some antibacterial activity to prevent bacterial growth.(6)

It is evident that an infected root canal system is a unique niche for selectivespecies of microorganisms, so success of any endodontic material also depends on its antimicrobial activity. Enterococcus faecalis is considered "The star survivor in the root canal" and is the most frequently recovered microorganism from periodontitis.(7)

Enterococcus faecalis is the most commonly isolated species from endodontic retreatment cases as it penetrates the dentinal tubules of root. It colonize root canal and survive without the support of other bacteria. The prevalence of different Enterococcal species appears to vary according to host and is also influenced by age, diet and other factors that may be related to changes in physiologic conditions such as demographic factors, underlying diseases and antimicrobial therapy.(8)

Scientific researches studing the comparisonbetween mineral trioxide aggregate (MTA) with Portland cement (PC) was recently evolved, especially in the evaluation of PC as a low-cost alternative to MTA. Several studies have demonstrated some similarities between both materials in terms of their biocompatibility, composition, mechanism of action, and cytotoxicity.(9-12)

MTA iscomposed of 75% Portland cement, 20% bismuth oxide and 5% gypsum. The MTA was first introduced as a root end filling material but later it was later used in pulp cappingand pulpotomy treatments, for the induction ofapical barrier formation in open apex teeth, repair of perforation and root canal filling.(13,14)

PC is used in dental applications as it is a main component of MTA, which is already widely used in endodontic field including root end filling, root repair, and pulp capping due to its biological and physicochemical properties(15,16). Mineralized tissue formation is stimulated by PC (17). PC-based materials have limited or controversial antimicrobial effects against some microorganisms.(18,19)

Retrograde filling materials cannot provide perfect sealing and thus, there remain microscopic spaces between the retrograde cavity and the filling material.(20) Microorganisms and their products may penetrate into root canal system through these spaces. Therefore, antimicrobial activities of the materials used as retrograde filling are necessary to be advantageous.(21)

Evidence of antibacterial properties of MTA was also existing but these properties have not been well confirmed.(22)

Many studies over the years have been conducted for the assessment of the antibacterial efficacy of MTA against *E. faecalis* which concluded that MTA has a limited inhibitory action against this resistant microorganism.(19,23)

Nanotechnology is a new evolution in all aspects of health including the dentistry. It is the science of producing functional materials and structures in range of 0.1 nm to 100 nm.(24)

The root canals disinfection with nanoparticles has gained popularity in the recent years as most efficient as the ordinary disinfectants. This is mainly due to the broad spectrum antibacterial activity.(25,26)

Handling and setting properties of the tricalcium silicate powders are enhanced by decreasing the particle size of the powder.Smaller particles may penetrate tubules and also hydrate faster than larger

particles because of their higher surface-to-volume ratio. If the tricalcium silicate material dissolves during setting and precipitates to penetrate the tubules, sealing is enhanced. Dentinal tubules range in size from 2-5 μm.[27]

Agar diffusion test is a standard method for assessment of antibacterial properties of materials in vitro. It is extensively used for accurate and direct comparison of antibacterial effects of materials.[1,22]

MATERIALS AND METHODS

Tested materials

1) White-MTA Angelus (Angelus, Londrina, PR, Brasilia)
2) White-MTA Angelus (Angelus, Londrina, PR, Brasilia) nano form.
3) Gray Portland cement with Barium sulfate in ratio 3:1by weight.
4) Gray Portland cement with Barium sulfate in ratio 3:1 by weight manufactured in nano form by top-down technique.

Preparation of tested materials:

In this study study, the antibacterial activity of MTA Angelus, MTA Angelus nano-particles, Portlandcementand Portland cementnano-particleswere determined by Agar Diffusion Test (ADT).[28]

Enterococcus Faecalis ATCC29212 (American Type Culture Collection 29212) was cultured in agar BHI (brain heart infusion) in anaerobic condition at 37ºC for 72 hours then 3 to 4 colonies were isolated and inoculated in 3 mL of a BHI broth under the same conditions previously mentioned. Dilution of the culture was done in sterile saline to reach 0.5 McFarland.

The antimicrobial activity was achieved on blood agar plates (30g dehydrated culture media"Thermoscientific, UK"/1L distilled water in 50 mL sheep blood). Agar was added to 12 sterile petri plates and the prepared bacterial suspension was inoculated. Punch holes of 6 mm diameter were formed on each agar plate and then each 3 plates were filled with the materials tested as follow

1. **Group 1:** The punch hole of the 3 plates was filled by MTA Angelus.
2. **Group 2:** The punch hole of the 3 plates was filled by MTA Angelus nano-particles.
3. **Group 3:** The punch hole of the 3 plates was filled by Portland cement.
4. **Group 4:** The punch hole of the 3 plates was filled by Portland cement nano-particles.

The agar plates were incubated at 37ºC, and after the first 24 hours the diameters of the inhibition growth zones were measured with a vernier caliper.

The antimicrobial activity was rated based on the diameters of the inhibition growth zones with meansand standard deviationswere calculated for each group and statistically analyzedto determine the presence or absence ofsignificant differences.

RESULTS

Data were expressed as mean ± SD. Comparison between values of different parameters in the four studied groups were performed using Kruskal Wallis ANOVA test followed by Mann-Whitney U test if significant results were recorded. Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis. P value ≤ 0.05 was considered significant.

The largest diameters of inhibition zones were recorded by Portland cement followed by nano-Portland cement then MTA, while the lowest inhibition zones were recorded by nano-MTA.

The results showed high significant difference between Portland cement and the other groups except nano-Portland cement where there is low significant difference between them.

Nano-MTA showed the lowest antibacterial activity among all the groups.

TABLE (1): Comparison between mean values of the diameters of the inhibition growth zones in the four studied groups.

	MTA (n= 4)	Nano-MTA (n= 4)	Portland (n= 4)	Nano-portland (n= 4)	P value
Mean ± SD	17.75 ± 0.65	0.17 ± 0.07	22.32 ± 0.47	20.82 ± 0.71	0.001*
P value vs MTA	----	0.021*	0.020*	0.021*	
P value vs nano-MTA	----	----	0.021*	0.029*	
P value vs portland	----	----	----	0.020*	

Data are expressed as mean ± SD. ****p≤ 0.05= Significant***

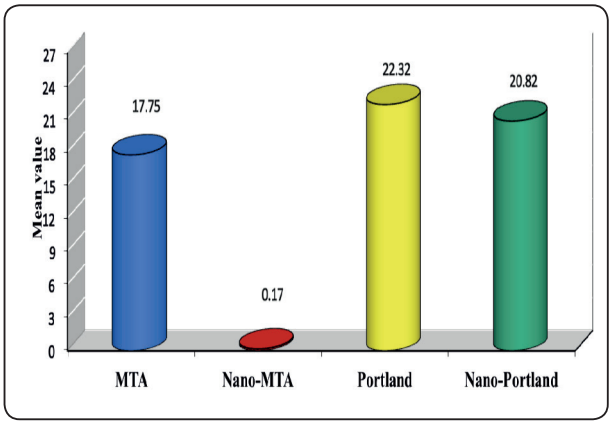

Fig. (1) : Mean values of the diameters of the inhibition growth zones in the four studied groups.

DISCUSSION

MTA is considered the best for root perforations repair, pulp capping and retrograde root canal filling.[13] So antibacterial activity is needed to avoid recontamination of the area covered by MTA to avoid the failure of pulp capping, repaired perforation, or retrograde filled root.

MTA has favorable properties such as providing an excellent seal, biocompatibility, low toxicity, low solubility, hard tissue formation, high pH and radiopacity. However, it also has several drawbacks such as long setting time [about four hours], tooth discoloration, difficult handling, limited antimicrobial activity and high cost.

Some studies investigated the mechanism of setting reaction of PC which may not produce enough free radicals and also PC has limited antimicrobial effect to some microorganisms.[18,29]

Other studies stated that the variation of the origin of MTA will cause variation in its antibacterial activity.[30]

The antibacterial properties of MTA is controversial as previous studies have reported different results. For example, antimicrobial activity of MTA has been reported to bein significant in some studies [18,19] and sufficiently high against E.faecalis in some other studies[2,31]. However, it should be noted that the antibacterial properties of MTA still depend on its concentration and the preparation method.[30]

The high pH and release of the materials that can be well diffused in the medium were correlated to the antimicrobial activity of MTA.[32] Torabinejad et al, in 1995 reported that the primary pH of MTA was 10.2, which increased to 12.5 after three hours. [21] A rise in the pH to 12 further inhibited the growth of microorganisms.[33]

Based on the results of current study, Portland cement showed the highest antibacterial effect over nano-Portland cement, MTA-angelus, and nano-MTA- angelus against E. faecalis.

It was reported by previous studies that Portland cement has similar biological and mechanical behavior as MTA, which was shown in the current study as both have antibacterial activity.

The reduction in antibacterial effect in the nano-form of both materials might be due to the

change occurs in the setting time of the materials in nano- form where setting time reduces due to the acceleration of hydration process[34], which in turn will decrease the time for diffusion of materials in the medium that correlated to the antimicrobial effect of Portland cement and MTA. Also the time taken during the reaction to increase the pH will be decreased which may affect the antimicrobial activity due to the highly alkaline pH 12.

It was stated in a previous study by Bedier et al. that the antibacterial activity of both Portland cement and MTA were similar against Enterococcus faecalis, Pseudomonas aeruginosa, and Staphylococcus aureus, while MTA had more antibacterial activity than PC against S. aureus.As they examined the materials using direct contact test.[35]

Also Oliviera et al compared the chemical composition of Portland cement with Pro-root MTA and MTA Angelus using scanning electron microscopy and energy dispersive spectroscopy concluding that the compositions of tested materials were similar but MTA contains bisthmus cements to provide radiopacity.[36]

As the results came in conjunction with the previous studies which showed the presence of antibacterial properties of MTA and Portland cement but this property decreased in nano-form that is thought to be due to decreased setting time of the nano-formed materials that decreased the pH and released radicals during the setting reaction of the present tested nano-form materials.

Barium sulfate was added in this study to increase the radiopacity of Portland cement to simulate the radiopacity of MTA, but also barium sulfate has antibacterial properties as examined by Verma et al.[37] they proved that $BaSO_4$ DAP at 1 mg/mL provided significantly superior residual antibacterial effects. So the reason for the superior results of Portland cement antibacterial properties could be referred to the addition of barium sulfate during the preparation of tested Portland cement and nano-Portland cement as it was prepared from the same mixture in nano-form.

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