ASC
Acta Stomatologica Cappadocia

Volume 2 / Issue 1 / June 2022

Research Article

Comparison of Antibacterial Efficacy of Silver Particle Added Portland Cement and Mineral Trioxide Aggregate

Güldağ Herdem, DDS, PhD[1]*, Mustafa Aydınbelge, DDS, PhD[2]

[1]*Cappadocia University, Department of Pediatric Dentistry, Urgup, Turkey*
[2]*Erciyes University, Department of Pediatric Dentistry, Kayseri, Turkey.*
**Corresponding author: guldag.herdem@kapadokya.edu.tr*

Herdem, Güldağ. & Aydınbelge, Mustafa. "Comparison of Antibacterial Efficacy of Silver Particle Added Portland Cement and Mineral Trioxide Aggregate". *Acta Stomatologica Cappadocia.* 2;1 (June 2022): 37-45.
DOI: https://doi.org/10.54995/ASC.2.1.3

Received: 18.05.2022; Accepted: 21.06.2022

Comparison of Antibacterial Efficacy of Silver Particle Added Portland Cement and Mineral Trioxide Aggregate

Güldağ Herdem, Mustafa Aydınbelge

Abstract

Statement of the problem: Despite the studies on the antibacterial effect of mineral trioxide aggregate (MTA), there are few studies comparing the antibacterial activity of MTA with silver nanoparticle added Portland Cement (PC).

Objective: The study aimed to compare the antibacterial activity of MTA with silver nanoparticle added PC.

Materials & Methods: MTA, mixed with distilled water, poured into silicone molds containing disc-shaped spaces with a diameter of 5 mm and a height of 2 mm (n = 7). Silver nanoparticles of 100 nm size were added to PC at a concentration of 1% by volume, and 7 disc samples were prepared by mixing with distilled water (PAG-DW group). Finally, PC containing 100 nm silver particles at 1% concentration was mixed with 0.12% chlorhexidine gluconate (CHG) and seven specimens of PAG-CHG were obtained by pouring this mixture into the same silicone molds. Then, all samples in these three groups were placed in the diffusion agar test (DAT) created with six different microorganisms. Inhibition zone diameters formed by the discs were measured to determine the antibacterial activity.

Results: The samples of PAG-DW and PAG-CHG formed statistically significantly larger inhibition zones in DAT when compared to samples of MTA (P=0.001).

Conclusion: The antimicrobial activity of PC with added silver nanoparticles was significantly higher than that of MTA. Mixing PC with CHG also increased its the antibacterial activity.

Keywords: Antibacterial Activity, Portland Cement, Chlorhexidine Gluconate, Silver Nanoparticles, Mineral Trioxide Aggregate, Diffusion Agar Test, Inhibition Zone

Introduction

Mineral trioxide aggregate (MTA) is a root canal filling material that has been in use for over 30 years.[1] Despite the disadvantages of the material such as high cost, moisture sensitivity, application difficulties and microleakage, it has extremely important advantages such as excellent covering, high alkalinity and stimulation of hard tissue formation.[1-3] Although Zhou et al1 reported that the material showed strong antibacterial properties, there are also studies reporting that it has no antibacterial activity against E. faecalis, S. aureus, B. subtilis and E. coli, which were shown to be primary sources of infection in root canals. [4,5]

Today, the antibacterial activity of different biomaterials can be increased using different methods. One of the methods applied to increase the antibacterial activity and spectrum of materials used in endodontic treatments is the addition of silver nanoparticles into the structure.[4] It has been shown that with the added silver nanoparticles, MTA gains antibacterial activity against E. feacalis, P. aeruginosa and C. albicans.4 The smaller the size of silver nanoparticles, the greater the antibacterial activity.[4] Silver is thought to exert an antibacterial effect by destroying the cell wall or disrupting RNA structure.[4]

One of the antibacterial agents frequently used in dentistry is chlorexidine gluconate (CHG). In particular, many studies have been published on the antibacterial activity of CHG, which is effective on E. feacalis, when mixed with MTA.[6-8] Holt et al[7] reported increased antibacterial activity of MTA mixed with 2% CHG. However, researchers reported that the resistance to pressure of MTA prepared with CHG is less than that of MTA prepared with distilled water.[7] Bidar et al[8] also reported that the antibacterial activity of CHG mixed with MTA increased. However, according to Bidar et al[8], there was no statistically significant difference in antibacterial activity of CHG added to MTA at different concentrations.

Most of MTA's content is PC, which incorporates ferrite, aluminate, alite and belite, obtained by heating limestone and crushing it into gypsum after adding clay.[9] PC makes MTA very useful in challenging endodontic procedures.[9] The effect of antibacterial agent additions to MTA on bacteriostatic or bactericide activity is well documented.[10] However, there is a lack of information in the literature about the antimicrobial effectiveness of PC with and without additional antibacterial agents.

The present study aimed to compare PAG-DW, PAG-CHG and MTA with regard to their antibacterial activities. The null hypothesis was that the antibacterial activities of PAG-DW and PAG-CHG are not significantly different than that of MTA.

Materials & Methods

The study was approved by the Ethics Committee of Erciyes University Faculty of Dentistry and was conducted in accordance with the World Medical Association Declaration of Helsinki.

The study was carried out in three stages, namely preparation of the specimens, investigation of antibacterial activity using Diffusion Agar Test (DAT), and statistical analysis.

-Preparation of the specimens

A total of three different specimen groups were formed. In the first group, the powder and the liquid of MTA (Angelus, Londrina, PR, Brazil) were mixed by following the manufacturer's instructions. The resulting mixture was poured into silicone molds (with a diameter of 5 mm, and a height of 2 mm). Seven disc shaped specimens were produced. This group was named as MTA because no antibacterial was added to the content.

Portland cement, to which silver particles were added at a concentration of 1% by volume and 100 nm in length, was mixed with distilled water and the mixture was poured into same silicone molds mentioned above. Thus, seven more disc-shaped specimens were obtained in this group, which was named as PAG-DW.

In another group (PAG-CHG), Portland cement, to which silver nanoparticles were added at 1% by volume, was mixed with 0.12% CHG. The resulting mixture was poured into molds, and seven discs were produced.

-Investigation of antibacterial activity using DAT

The antibacterial activity of the specimens from MTA, PAG-DW and PAG-CHG was compared on standard strains of E. faecalis (ATCC 29212), E. coli (ATCC 25922), S. mutans (ATCC 25175), P. aeruginosa (ATCC 27853) ve S. aureus (ATCC 25923) C. albicans using DAT. Mueller Hinton Agar (Merck, Darmstadt, Germany) medium was used for E. coli, P. aeruginosa and E. faecalis. Five percent Sheep Blood Müller Hinton Agar (Merck, Darmstadt, Germany) was used for S. mutans, and Roswell Park Memorial Institute 1640 (RPMI 1640) (Biological Industries, Kibbutz, Israel) medium was used for C. albicans.

After the microorganisms were removed from the stock, fresh passages were made and dissolved in sterile 0.9% saline so as to provide Mcfarland 0.5 standard turbidity. In the medium in the Petri dish, wells of 5 mm in diameter were prepared to place the specimens to be tested. The prepared microorganism solutions were spread homogeneously on the media with the help of a sterile swab. A total of 21 disc samples of MTA, PAG-DW and PAG-CHG were placed in these wells. Culture plates were evaluated after they were kept in an oven at 37ºC for 24 hours in a normal atmospheric environment. The diameters of the inhibition zones formed were measured three times by the same observer with a millimetric ruler and the average values were recorded.

-Statistical analysis

All data were tabulated and analyzed using Statistical Package for Social Science 22 software for Windows (SPSS®, SPSS Inc., Chicago IL, USA). First, descriptive statistical analysis of the measured values was made and the distribution of variables was evaluated with Kolmogorov-Smirnov Test. The Kruskal-Wallis and Mann-Whitney U tests were for the analysis of quantitative data, and the Wilcoxon test was used for the analysis of repetitive measurements. For all analyses, the level of significance was set as $\alpha = 0.05$.

Results

The descriptive values of inhibition zone measurements obtained after 24 hours are presented in Table 1.

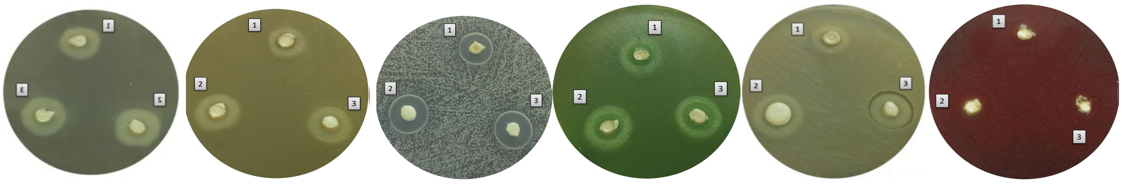
Table 1. Descriptive values of inhibition zone measurements after 24 hours in the study groups and Kruskal-Wallis test results.

	Inhibition Zone Sizes							
	Mean ± Standard Deviation (mm)			Median	Minimum-Maximum (mm)			P
PAG-CHG	**13,0**	±	1,7	13,0	10	-	15	0.001*
PAG-DW	10,2	±	3,1	10,0	6	-	14	
MTA	8,7	±	2,8	8,5	4	-	12	

* Kruskal-Wallis test

A statistically significant difference was found in the sizes of the inhibition zones, namely in the antibacterial activities, formed by the samples belonging to the MTA, PAG-DW and PAG-CHG groups. It was determined that the specimens of PAG-CHG showed higher antibacterial activity than the samples of PAG-DW and MTA (Figure 1).

Figure 1. Examples of cements belonging to bacteria 1-MTA, 2- PAG-DW, 3- PAG-CHG



E. Fecalis E. Coli C. Albicans. P. Aeruginosa. S.aureus. S. Mutans

The descriptive results of the inhibition zone sizes formed by the samples belonging to the groups on different microorganisms are given in Table 2.

Table 2. Analysis of inhibition zone sizes in different microorganism strains.

Microorganism	Mean ± Standard Deviation (mm)			Median	Minimum-Maximum (mm)			P
S. aerous	11,7	±	3,3	11,0	8	-	15	0.051*
P. aeruginosa	9,4	±	0,5	9,0	9	-	10	
S. mutans	12,9	±	0,9	13,0	11	-	14	
E. coli	10,4	±	3, 4	8,0	7	-	14	
E. faecalis	8,3	±	4, 8	6,0	4	-	14	
C. albicans	12,9	±	0,4	13,0	12	-	13	

* Kruskal-Wallis test

The antibacterial activity of MTA, PAG-DW and PAG-CHG on different bacteria, and even fungi did not differ significantly ($P > 0.05$).

Discussion

According to the results of the present study the null hypothesis was rejected. In PC-DW and PC-CHG groups, where 1% silver particles by volume were added and mixed with distilled water or 0.12% CHG, significantly larger inhibition zones were formed when compared with the zones obtained in MTA.

The findings are in line with the previous studies, where the antibacterial activity of silver nanoparticles were evaluated. Gomes-Filho et al[11] evaluated the tissue response after the implantation of polyethylene tubes filled with silver-impregnated sponge. They determined that as the size of the silver particles decreased, the antibacterial activity of the material to which it was added increased. The authors reported that the most effective size and concentration were to be 150 nm, and 1% (by weight), respectively.[11]

The present study utilized silver nanoparticles as in the study of Gomes-Filho et al.[11] However, unlike that study where silver particles were added at 1% by weight, this study preferred to add 1% by volume. The reason for this is that at a concentration of 1% by weight, there might not be enough particles to provide sufficient effectiveness compared to a concentration of 1% by volume. In addition, in the study by Gomes-Filho et al[11], 150 nm silver particles were used. However, it is known that the effectiveness of the silver particles increases as the size of them decreases.[4] Hence, the present study was conducted with silver nanoparticles of 100 nm size.

Studies using fluorence spectrometry showed that the molecular structures of PC and MTA were largely similar.[2,12] It is not surprising that these two materials with similar molecular structures, show higher antibacterial activity when mixed with CHG than with distilled water. It is known that CHG has high antibacterial activity against gram +, gram - bacteria, bacterial spores and different microorganisms.[13] However, CHG is a solution that is not only antibacterial but also cytotoxic. In other words, it is necessary to determine its minimal concentration that will show the maximum antibacterial effect in the dental material to which it is added. There has been extensive studies in order to understand the concentration of CHG at which it will produce the highest bacterial activity but the lowest cytotoxic effect when it is mixed with MTA. It has been reported that the antibacterial activity of MTA mixed with 2% CHG increased, but its resistance to mechanic stresses decreased.[7] It has also been reported that the antibacterial efficacy of MTA mixed with CHG at concentrations of 0.12%, 0.2% and 2% did not show a significant difference.[8] Considering that HCG can decrease the strength of the material and increase its cytotoxic effect at high concentrations, silver nanoparticle added PC was mixed with 0.12% CHG in the current study. According to the findings, even with these low concentrations of CHG, the antibacterial activity of PC significantly increased. However, the effect of mixing PC with distilled water or CHG on the mechanical properties of the material was not investigated in the present study.

The present study has three limitations. First is the lack of data on whether the mechanical strength of PC is also positively or negatively affected. As mentioned above, it has been observed to have increased antibacterial activity when mixed with CHG. In our opinion, however, the most important of the limitations is that the DAT method used to determine the antibacterial activity. It is not a selective method and it is almost impossible to distinguish whether the activity of a material is bacteriostatic or bactericidal. With DAT, the antibacterial or antifungal activity of any material is determined by the inhibition zone size formed as a result of the diffusion of the material to the medium. The results obtained in DAT are subject to the influence of variables such as the size of the test material or the incubation time.[14] Therefore, the studies that are conducted with more advanced methods to eliminate the effects of these variables would be more enlightening. In the present study, the antibacterial activity of the specimens was investigated on a limited number of microorganism species. However, many microorganisms have the potential to cause acute or chronic inflammation in soft or hard oral tissues. This might be another important study limitation.

Conclusion

Within the limitations of the present study, the antimicrobial activity of PC with added silver nanoparticles is significantly higher than that of MTA. Mixing PC with CHG increased the antibacterial activity of the material. It seems necessary to investigate the mechanical properties of PC when silver nanoparticles and the material was mixed with CHG.

References

1.Zhou HM, Shen Y, Wang ZJ, Li L, et al. In vitro cytotoxicity evaluation of a novel root repair material. J Endod 2013;39:478-83.

2.Camilleri J, Pitt Ford TR. Mineral trioxide aggregate: a review of the constituents and biological properties of the material. Int Endod J 2006;39:747-54.

3.Torabinejad M, Watson TF, Ford TRP. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 1993;19:591-5.

4.Samiei M, Aghazadeh M, Lotfi M, Shakoei S, et al. Antimicrobial efficacy of mineral trioxide aggregate with and without silver nanoparticles. Iran Endod J 2013;8:166-70.

5.Asgary S, Akbari Kamrani F, Taheri S. Evaluation of antimicrobial effect of MTA, calcium hydroxide, and CEM cement. Iran Endod J 2007;2:105-9.

6.Ehsani M, Amin Marashi M, Zabihi E, Issazadeh M, et al. A comparison between antibacterial activity of propolis and aloe vera on Enterococcus faecalis (an in vitro study). Int J Mol Cell Med 2013;2:110-6.

7.Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, et al. 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.

8.Bidar M, Naderinasab M, Talati A, Ghazvini K, et al. The effects of different concentrations of chlorhexidine gluconate on the antimicrobial properties of mineral trioxide aggregate and calcium enrich mixture. Dent Res J (Isfahan) 2012;9:466-71.

9. Shahriar S, Elaheh F, Hamidreza Y, Solmaz Maleki D, et al. Portland Cement: An overview as a root repair material. BioMed Research International 2022;6;2022:3314912.

10.Samiei M, Aghazadeh M, Lotfi M, Shakoei S, et al. Antimicrobial efficacy of mineral trioxide aggregate with and without silver nanoparticles. Iran Endod J 2013;8:166-70.

11.Gomes-Filho JE, Silva FO, Watanabe S, Cintra LTA, et al. Tissue reaction to silver nanoparticles dispersion as an alternative irrigating solution. J Endod 2010;36:1698-702.

12.Mitchell PJC, Ford TRP, Torabinejad M, McDonald F. Osteoblast biocompatibility of mineral trioxide aggregate. Biomaterials 1999;20:167-73.

13.Lessa FCR, Aranha AMF, Nogueira I, Giro EMA, et al. Toxicity of chlorhexidine on odontoblast-like cells. J App Oral Sci 2010;18:50-8.

14.Samiei M, Aghazadeh M, Lotfi M, Shakoei S, et al. Antimicrobial efficacy of mineral trioxide aggregate with and without silver nanoparticles. Iran Endod J 2013;8:166-70.