Original

Antibacterial effects of five different root canal sealing materials

Saeed Asgary[1] and Farshid Akbari Kamrani[2]

[1]Iranian Center for Endodontic Research, Dental Research Center, Dental School,
Shahid Beheshti University MC, Tehran, Iran
[2]Dental Research Center, Shahid Beheshti University MC, Tehran, Iran

Abstract: The aim of this *in vitro* study was to assess the antibacterial effects of gray and white mineral trioxide aggregate (GMTA and WMTA), calcium hydroxide (CH), Portland cement (PC) and a new endodontic cement (NEC) on various species of microorganisms, using agar diffusion test. A base layer of Petri plates was made using Muller-Hinton agar. Five cavities were made in agar and filled with fresh mixed materials after 24 h. *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and a mixture of these bacteria were seeded by pour plate. The plates were preincubated for 2 h at room temperature followed by incubation at 37°C. The inhibition zone diameters were measured at 24, 48 and 72 h. The highest mean diameters of growth inhibition zones were observed around NEC and CH. According to one-way ANOVA, there was a significant difference among test groups (*P* < 0.001), while post-hoc test revealed no significant difference between the mean zone diameters of NEC and CH, and also between MTAs and PC. However, there was a significant difference between CH and NEC in comparison with MTAs and PC groups (*P* < 0.001). It appears that NEC may act as a potent antibacterial agent similar to CH. (J. Oral Sci. 50, 469-474, 2008)

Keywords: antibacterial agents; calcium hydroxide; dental cement; endodontics; mineral trioxide aggregate; new material.

Correspondence to Dr. Saeed Asgary, Iranian Center for Endodontic Research, Dental School, Shahid Beheshti University MC, PO Box 1983963113, Evin, Tehran, Iran
Tel: +98-21-22413897
Fax: +98-21-22427753
E-mail: saasgary@yahoo.com

Introduction

Bacteria are mainly responsible for the development of pulp/periapical diseases (1). Therefore, elimination of bacteria during root canal treatment by instrumentation, irrigation and intracanal medication has always been an important part of successful endodontic treatment (2). Although bacteria superficially adhering to root canal dentin might be more easily killed than those protected in the depths of dentinal tubules, bacteria inside the dentinal tubules might also be affected by antibacterial components leaching from the irrigation solution, intracanal medication, and endodontic filling and sealing materials (3). However, even after these procedures, bacteria are still found inside the dentinal tubules, with the potential for disease to persist or emerge (4).

Endodontic treatment outcome will depend on an effective seal to prevent further recontamination and also successful reduction of the associated microorganisms. Since many existing materials may not provide a perfect and hermetic seal, it was expected that these materials may prevent bacterial growth (5). Therefore, antibacterial testing of biomaterials should consider this effect. The agar-diffusion test (ADT) is the most commonly used technique for evaluating this property of dental materials (6).

Numerous studies have been performed to assess the antibacterial activity of different materials used in dental treatment. Calcium hydroxide (CH) was first introduced to dentistry in 1920 (7). Some biological properties have been attributed to CH such as induction of hard tissue formation (8), inhibition of root resorption (9), antibacterial action (10), and tissue dissolution (11). Because of these biological properties, CH is recommended for many purposes in endodontics.

Mineral trioxide aggregate (MTA), which was introduced

in 1993 (12), has been examined since 1995 as a potential antibacterial material (5). MTA is a powder consisting of fine hydrophilic particles that, in the presence of water, becomes a colloidal gel that solidifies to form hard cement within approximately 4 h (13). ProRoot MTA is marketed in gray-colored (GMTA) and white-colored (WMTA) preparations. GMTA may cause tooth discoloration particularly when it is used to cap or seal a perforation, where aesthetics is the priority. WMTA was introduced in order to address this issue (14). Major compositional differences in the concentrations of periclase (MgO) and especially FeO between GMTA and WMTA have been reported. These elements were found to be considerably lower in WMTA (15-16). According to recent studies, MTA is a biocompatible dental material and it was suggested that these biological properties may be due to its excellent sealing ability (17), high alkalinity (18) induction of hard tissue formation (19), and antibacterial effects (5). Because of its physical and chemical properties, the use of MTA as a biomaterial has been recommended for a wide variety of endodontic treatments (20).

It is reported that Gray MTA and gray Portland cement (PC) appear to be almost identical macroscopically, microscopically and when using X-ray diffraction analysis (21). In another study, it has been shown that white PC contains the same chemical elements as WMTA, except bismuth (22). It is concluded that comparable tissue responses to PC and MTA are due to similarities in their chemical composition (23). Although MTA has excellent biocompatibility, it has a delayed setting time (13) and poor handling characteristics (24), as well as being an expensive material.

Recently, the first author developed a new endodontic cement (NEC) consisting of different calcium compounds (i.e., calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, and calcium chloride) that is compliant with the ISO 6876 (the International Organization for standardization) standard for dental root canal sealing materials (25). The clinical uses of NEC are similar to MTA.

NEC is biocompatible, can stimulate hard tissue healing (26), is easy to handle, sets in an aqueous environment, has appropriate setting time and good handling characteristics (25), and forms an effective seal when used as a root-end filling material (27). The results of recent research revealed that NEC comprises water-soluble calcium and phosphate, and forms hydroxyapatite after setting (28).

The aim of the present *in vitro* study was to compare the antimicrobial activities of CH, GMTA, WMTA, PC and NEC on four microorganisms commonly associated with endodontic infections and a mixture of these using the agar-diffusion test.

Materials and Methods

In this study, we had five experimental groups as follows: group 1, calcium hydroxide (Sealapex, Kerr, Orange, CA, USA); group 2, ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA); group 3, ProRoot MTA tooth-coloured formula, (Dentsply Tulsa Dental); group 4, white Portland cement (Abyek white cement, Abyek, Qazvin, Iran); and group 5, new endodontic cement (NEC).

Agar diffusion test

The study was conducted on double-layered plates, in which the base layer was made of 10 mL of sterilized Muller-Hinton agar (MH) poured into 2×10 cm sterilized Petri plates. Five uniform cavities (4-mm diameter, one for each test material) were punched at equidistant points in agar by means of a sterile copper coil after 24 h. The cavities were filled by materials immediately after being mixed according to the manufacturer's instructions.

Microorganisms

Antibacterial activities of the selected materials were evaluated against the *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and a mixture of these bacteria using an agar diffusion method. The strains were obtained from the Department of Microbiology, Faculty of Medicine, Shahid Beheshti University MC, Tehran, Iran. After activation from stock culture, microorganisms were maintained in MH broth until use. Overnight cultures of the microorganisms were done. All the microbial strains were grown at 37°C for 24 h in MH broth and then seeded into 15 mL of the MH agar, to produce a turbidity of 0.5 on the McFarland scale, which corresponds to a concentration of 10^8 colony forming units ml^{-1}. For the mixture group, the second layer contained equal concentrations of each microorganism. The seeded agar was added over the plates immediately after the insertion of freshly mixed test materials. The plates were kept at room temperature for 2 h for pre-diffusion of the materials and then incubated at 37°C for 72 h.

A total of 42 plates were used: plates were divided randomly into five test groups with eight plates each, so microorganisms were tested eight times. Positive and negative controls were prepared, maintaining the plates with and without inoculums, for the same period and under identical incubation conditions. All assays were carried out under aseptic conditions.

Data recording

The diameter of bacterial growth inhibition zones was measured with a millimeter ruler with an accuracy of 0.5 mm in two perpendicular locations for each sample by an independent observer.

Statistical analysis

Statistical analysis was performed using a one-way ANOVA for the mean zones of growth inhibition among test materials. The post-hoc test was run for multiple comparisons. Statistically significant differences among the groups were set at $P < 0.05$.

Results

The positive control showed bacterial growth, while the negative control showed no bacterial growth. All bacterial strains were inhibited by all test materials. The antimicrobial activities of test materials determined by the means and standard deviation of inhibition growth zones in millimeters on all test microorganisms after 24, 48, and 72 h are shown in Table 1. The results of 24-h incubation revealed that the antimicrobial effect of CH and NEC on all test microorganisms was superior to that of the MTAs and PC. Decreasing order of inhibition zones produced by NEC, CH, WMTA, GMTA, and PC on all microorganisms ranged from 3.37 to 7.06, 3.81 to 6.81, 2.25 to 5.18, 1.31 to 4.93, and 0.56 to 4.68 mm at 24 h,

respectively. The results for 48 and 72 h were similar with that for 24 h in all experimental groups.

The highest mean diameters of inhibition zones of bacterial growth were founded in NEC and CH groups, while the observed zones related to MTAs and PC groups were smaller. There was no statistically significant difference between the antibacterial activity of CH and NEC, and also between MTAs and PC. However, CH and NEC showed significantly better antibacterial effect than MTAs and PC ($P < 0.001$). There were no statistically significant differences between results of the mixture group and other groups. Additionally, there were no significant differences between results from 24, 48 and 72 h for each group; however, it decreased in order of 24, 48 and 72 h as expected. The highest inhibition zone in all groups was against *Enterococcus Faecalis*, followed by mixture of bacteria, *Staphylococcus Aureus*, *Escherichia Coli* and *Pseudomonas Aeruginosa* in a decreasing order.

Discussion

In this study, we used ADT, the most widely used *in vitro* method for the evaluation of antibacterial activity (29), which identifies the materials more likely to have an antimicrobial effect within the root canal system *via* direct comparisons between them (30). ADT results are highly influenced by the diffusion ability of the material across the medium (31). However, the selection of the agar

Table 1 The antibacterial activity of test materials toward four bacterial spp. and their mixture

Microorganisms	Time (hour)	Materials				
		NEC	CH	WMTA	GMTA	PC
Escherichia Coli	24	5.37(0.64)*	5.12(0.35)	2.93(1.20)	2.37(1.02)	0.56(1.05)
	48	5.37(0.64)	4.75(0.46)	2.75(1.16)	1.93(0.97)	0.50(0.92)
	72	4.93(0.72)	4.56(0.49)	1.68(1.06)	0.31(0.88)	0.0
Staphylococcus Aureus	24	6.37(0.79)	6.50(0.46)	4.37(0.44)	3.56(0.41)	3.56(0.67)
	48	5.93(0.67)	6.12(0.35)	4.12(0.44)	3.31(0.53)	3.31(0.45)
	72	5.87(0.79)	6.06(0.32)	3.93(0.49)	3.18(0.45)	3.18(0.37)
Pseudomonas Aeruginosa	24	3.37(0.35)	3.81(0.37)	2.25(0.46)	1.31(0.75)	1.87(0.83)
	48	3.37(0.35)	3.81 (0.37)	1.87(0.99)	0.93(1.08)	1.87(0.83)
	72	3.25(0.25)	3.18 (0.53)	1.87(1.03)	0.81(1.13)	1.67(1.32)
Enterococcus Faecalis	24	7.06(0.17)	6.81(0.25)	5.18(0.45)	4.93(0.17)	4.68(0.25)
	48	7.06(0.17)	6.81(0.25)	5.18(0.45)	4.93(0.32)	4.68(0.25)
	72	7.00(0.25)	6.68 (0.23)	5.06(0.32)	4.93(0.32)	4.56(0.17)
Mixed	24	6.93(0.32)	6.81(0.44)	4.41(0.32)	4.06(0.17)	4.62(0.35)
	48	6.93(0.32)	6.81(0.44)	4.41(0.32)	3.68(0.37)	4.50(0.37)
	72	6.87(0.35)	6.69(0.34)	4.25(0.26)	3.43(0.32)	4.37(0.23)

The growth inhibition zones presented in millimeters.
*Mean (Standard Deviation), NEC: new endodontic cement, CH: calcium hydroxide, WMTA: white mineral trioxide aggregate, GMTA: gray mineral trioxide aggregate, PC: Portland cement

medium and microorganisms, control and standardization of inoculation density, incubation and reading point of the zones of inhibition are factors that affect the results of diffusion tests in an agar medium. Many different media, different methods of inoculum preparation or both have been used (32).

True endodontic pathogens or those associated with therapy-resistant cases (2) were selected as test bacteria for this experiment. Although aerobic and facultative bacteria are usually minor constituents of primary infections, they have been found with higher frequency in cases of treatment failure (33). These bacteria can enter the root canal system before, during or after treatment and cause secondary infections (34). An attempt was made to select representative Gram-negative/positive and cocci/bacilli bacteria commonly isolated from endodontic infection. To simulate the oral and teeth conditions, we included a mixture group which corresponded to the mixed nature of pathogens to determine whether they affect the result of inhibition by synergistic effects.

In this study, freshly mixed cements were immediately transferred into agar plates. Because of various transitory or permanent products, the material should be tested immediately after mixing and also after it is assumed to reach its final chemical structure. CH and MTA are inserted into the tooth in a freshly mixed, incompletely set stage, and thus it is likely that during a period after clinical application of the material, local responses are provoked by components with no or partial reaction. After setting, the release of active ingredients from the materials is still possible. The difference in antibacterial patterns of various materials may also be related to the degree of setting (6).

CH shown to be appropriate for elimination of bacteria depends on ionization that releases hydroxyl ions, causing an increase in pH. A pH of more than 9 may reversibly or irreversibly inactivate cell membrane enzymes of the microorganism, resulting in a loss of biological activity (35). The culture medium can influence the solubility, ion release and alkalinity of CH, which are essential conditions for the antimicrobial effect (36). However, our results showed effective antibacterial activity of CH which caused greater growth inhibited zones of tested bacteria than MTAs and were in agreement with those of Amorim et al. (37). They had reported that calcium hydroxide paste formed inhibition zones around *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis* and *C. albicans* strains.

The antimicrobial activity of MTA was reported by Torabinejad et al. (5), who detected its efficiency against some facultative bacteria; however, no activity was found against *E. faecalis*, *S. aureus*, *B. subtilis* and *E. coli* or against anaerobic bacteria. Estrela et al. (35) demonstrated that MTA or PC did not reveal any antimicrobial activity against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis*, or *C. albicans*. In this study, the same poor result obtained with MTAs also applies to PC, with no significant difference. The fact that the main components of MTAs are also found in PCs (21, 22) can justify the similarity of their antimicrobial activity. Our results are also in partial agreement with those of Stowe et al. (38) who assessed the antimicrobial properties of MTA and found that it inhibited the growth of both *E. faecalis* and *S. sanguis*.

Our results showed the effective antibacterial activity of NEC, which is comparable with CH and significantly better than MTAs and PC groups. This could imply that NEC contains more potent antibacterial inhibitors than MTAs and PC. Alkaline earth metal oxides and hydroxides (e.g. calcium oxide and calcium hydroxide), calcium phosphate and calcium silicate are the important constituents of NEC. During and after mixing with its liquid, CH is produced through hydration reactions, mainly because of the reactions involving calcium silicates, calcium phosphate, and calcium oxide in addition to presence of CH alone. When NEC is transferred to agar plates and contacts the medium, CH dissociated into calcium and hydroxyl ions, increasing the pH and calcium concentrations. These mechanisms may partially explain the superior antibacterial activity of this material. An alternative explanation is that the antibacterial components of NEC have better diffusion properties.

Under the conditions of this *in vitro* study, it was concluded that the favorable results of NEC and CH in comparison with WMTA, GMTA, and PC, indicate potentiality of NEC as an antibacterial agent. However, it is necessary to investigate other properties of this new material.

Acknowledgment

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