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Antimicrobial Activity of Calcium-Enriched Mixture Cement and Biodentine on *Enterococcus faecalis*: An *in Vitro* Study

Mahdieh Nourzadeh [a*], Arezu Amini [b], Farzaneh Fakoor [c], Saeed Asgary [a]

a Iranian Center for Endodontic Research, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran; b Researcher, Dental School, Zanjan University of Medical Sciences, Zanjan, Iran; c Department of Microbiology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

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**Corresponding author*: Mahdieh Nourzadeh, Iranian Center for Endodontic Research, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Tel: +98-21-22413897
E-mail: m_nourzadeh1986@yahoo.com

ABSTRACT

Introduction: The main cause of endodontic failure is residual bacteria in the root canal system. *Enterococcus faecalis* (*E. faecalis*) is the predominant species isolated from infected root canals. This study aims to compare the antibacterial activity of calcium-enriched mixture (CEM) cement and Biodentine as root canal filling materials on *E. faecalis*. **Methods and Materials:** Seventy extracted human single-rooted teeth were prepared and infected with *E. faecalis* for 24 h. Specimens were randomly divided into control or experimental groups; the later were filled with either CEM cement or Biodentine. Dentinal samples were collected after 7 and 30 days and transferred to test tubes. After incubation, the number of colony forming units (CFUs) were counted and analyzed using the Kruskal-Wallis test, followed by the Mann Whitney U test. The level of significance was set at 0.05. **Results:** The reduction in mean CFU level of *E. faecalis* was significantly more in the presence of CEM cement at both time intervals ($P<0.001$). Compared to the positive control, Biodentine significantly reduced the mean CFU level only after 30 days ($P<0.01$). **Conclusion:** Although both biomaterials exerted antibacterial activity against *E. faecalis*, the CEM cement had more antibacterial activity than Biodentine.

Keywords: Antibacterial Agent; Biodentine; Calcium-Enriched Mixture Cement; CEM Cement; Endodontics

Introduction

Bacteria are the main etiology of endodontic diseases. Resistant bacteria in the root canal system after primary root canal therapy (RCT), particularly in dentinal tubules, are known as the main causes of endodontic treatment failures [1]. *Enterococcus faecalis (E. faecalis)* (a gram-positive, facultative, anaerobic cocci) composes a small proportion of the flora in untreated canals, whereas it is considered as the major etiology of periradicular lesions after RCT. It is frequently present in root canal failure cases (22-77%) [2]. *E. faecalis* can survive starvation because of its physicochemical characteristics, including the formation of biofilm, innate antibacterial resistance, and capacity to invade the dentinal tubules where they are protected from endodontic medicaments [3].

When healing does not happen after non-surgical endodontic therapy and the retreatment is impossible or failed, root-end surgery is chosen to preserve the tooth. The procedure includes exposure of the apex, root-tip resection, root-end cavity preparation, and placement of root-end filling/sealing material in the cavity [4, 5]. An ideal root-end filling material should be biocompatible, dimensionally stable, and insoluble in tissue fluids. It should also have adequate sealing ability, stimulate tissue regeneration, and exert antimicrobial properties [6].

As a root-end filling material, calcium-enriched mixture (CEM) cement has similar clinical applications to mineral trioxide aggregate (MTA), but a different chemical composition. It stimulates hard tissue healing and forms an effective seal similar to MTA [7, 8], but with proper setting time, better handling characteristics, lower tooth discoloration

and at a lower cost than MTA [9-11]. Furthermore, it has been proven that CEM cement is a potent inhibitor of bacterial growth [12-14].

Biodentine is a new calcium-silicate based biomaterial. The powder mainly comprises dicalcium/tricalcium silicate and calcium carbonate. The liquid contains calcium chloride with a mixture of polycarboxylate. The antibacterial activity of Biodentine has been demonstrated in several studies [15, 16]. Using the agar diffusion test (ADT), the inhibitory effects of Biodentine against *E. faecalis* was more than ProRoot MTA [17].

A recent bacterial leakage study using *E. faecalis* showed that the sealing ability of CEM cement and Biodentine was comparable in repair of furcation perforation in primary molars [18]. However, as no study has compared the antimicrobial activity of CEM cement and Biodentine to date, the aim of this *in vitro* study was to compare the anti *E. faecalis* effect of two endodontic biomaterials in dentin.

Materials and Methods

Seventy extracted caries-free human single-rooted teeth were selected for this *in vitro* study. The teeth had single root canals with no signs of cracks, grooves, resorption or calcification. The external surfaces of the teeth were cleaned using periodontal instruments. Then they were stored in 2.5% NaOCl. Teeth were cut from the cementoenamel junction (CEJ) and apical end to create standard segments of 7-mm in length. Under copious irrigation, the root canals were prepared using K-files (Dentsply, Maillefer, Ballaigues, Switzerland) up to #30 and the RaCe rotary system (FKG Dentaire, La Chaux-de-Fonds, Switzerland). In order to remove smear layer, the samples were treated in an ultrasonic bath in 17% ethylenediaminetetraacetic acid (EDTA) for 10 min followed by 1% NaOCl for 10 min. The samples were stored in distilled water for 1 h to remove chemical agents. External surfaces and root apices of samples were covered with nail polish and resin (respectively) to prevent bacterial leakage [19].

The specimens were placed into glass tubes of brain heart infusion (BHI) broth medium (Merck, Germany), autoclaved at 120°C for 15 min, and kept in an incubator at 37°C for 48 h. Obtained *E. faecalis* (ATCC 29212; Iranian Research Organization for Science and Technology (IROST), Tehran, Iran) grown overnight in BHI to reach a turbidity of 0.5 McFarland standard (1.5×10^8 CFU/mL). The bacteria suspension was inserted into the root canals with a sterile 29-gauge syringe and incubated at 37°C for 24 h. The purity of infection was checked by gram staining and colony morphology on BHI blood agar and *Streptococcus faecalis* (SF) broth and bile-esculin tests after 24 h. If any contaminants were observed, the samples were excluded.

After 24 h, the excess broth was removed from the root canals with sterile paper points and the external surfaces of the teeth were dried with sterile gauze. The nail polish and cementum layers were removed from the specimens using a sterile round diamond bur (Komet, Lemgo, Switzerland) at high speed. The samples were randomly divided into four groups as follows: group 1; CEM cement (n=25), group 2; Biodentine (n=25), group 3; positive control (n=10), and group 4; negative control (n=10).

CEM cement (BioniqueDent, Tehran, Iran) and Biodentine (Septodont, Saint Maur des Fosses, France) were prepared according to the instructions of their manufacturers and used to fill the root canals. The samples were placed in an incubator set at 37°C, 95% humidity, and 5% CO_2. Dentinal samples were taken from buccal and lingual sides of the teeth after 7- and 30-day time intervals, respectively. A sterile round bur at high speed was used to drill a 1-mm hole into the middle part of the buccal/lingual side of the root. The shavings fell into separate test tubes containing BHI and CFUs were then counted. All assays were done three times. The purity of the infection was checked as described above.

Statistical analysis

After $\log_{10}$ transformation of CFU+1, data was analyzed using the Kruskal-Wallis test followed by the Mann-Whitney U test. A level of P<0.05 was considered significant in all analyses.

Table 1. Antibacterial action of the materials against *E. faecalis* in relation to the incubation time

Materials	Time	Mean (SD)	Median	Max	Min
CEM cement	Day 7***	0.958 (0.233)	0	3.109	0
	Day 30***	0.53 (0.209)	0	3.006	0
Biodentine	Day 7	3.205 (0.202)	3.562	4.197	0
	Day 30**	2.35 (0.271)	2.788	4.409	0
Control	Day 7	3.425 (0.174)	3.217	4.284	2.603
	Day 30	3.79 (0.113)	3.812	4.176	3.301

**** P<0.001 versus Control and Biodentine; ** P<0.01 versus Control*

Results

Compared to the positive control and Biodentine, CEM cement significantly decreased bacteria at both time intervals (P<0.001). Compared to the positive control, Biodentine decreased the number of bacteria at both time intervals, but a statistical significance was seen only after 30 days (P<0.01). The mean CFU levels in both groups were reduced with time, but the reduction was significant only in Biodentine group (P<0.01) (Table1).

Discussion

The bacterial reduction in dentinal tubules after root canal fillings with two endodontic biomaterials -CEM cement and Biodentine- was evaluated in this *in vitro* study; both biomaterials reduced *E. faecalis,* the presence of which generally tends to increase with time.

Researchers have used various methods to evaluate the antibacterial effects of endodontic materials. ADT is a common method for assessment of antibacterial effect of endodontic biomaterials *i.e.* MTA, CEM cement, and Biodentine against microorganisms and specifically *E. faecalis* [17, 20-23]. The obtained results were heterogeneous. The results of ADT is based on the diffusion from the biomaterials into the agarose medium which leads to creation of inhibition zones of bacterial growth. Because of the limitations of ADT which is reviewed by Tobias [24] and are responsible for heterogeneous results, the dentin block model was used in the present study. In this method, the bacteria are embedded within dentinal tubules so the agents do not have direct contact with them; the method is established to assess the antimicrobial activity of materials diffusing into dentinal tubules.

E. faecalis -a part of normal human microflora- is the most prevalent bacteria in teeth with apical periodontitis and the most resistant one to intracanal medicaments due to its resistance against high alkalinity and its ability to invade into dentinal tubules [25, 26]. For these reasons, *E. faecalis* was chosen as the test organism in this study.

The most noticeable antibacterial activity was demonstrated by CEM cement after seven and 30 days, without any significant difference between the 2 time intervals. The great antibacterial activity of CEM cement is related to its bacterial inhibitors that are more potent than those of other calcium-silicate materials. Alkaline metal oxides and hydroxides (calcium oxide and calcium hydroxide) are important elements of CEM cement. In addition to its inherent presence in the material, calcium hydroxide is formed through the hydration (during and after mixing) when it dissociates into calcium and hydroxyl ions and results in rises in pH and calcium concentration. The other reason for the favorable antibacterial activity of this cement is the superior diffusion properties of its components in comparison with other calcium silicate-based biomaterials [14, 20, 27].

Even though Biodentine reduced bacteria at both time intervals, roots filled with Biodentine had significantly fewer bacteria than the control group only after 30 days. The antimicrobial properties of Biodentine are associated with calcium release and alkalinity. Colloidal gel, formed during hydration of the cement, leads to the release of calcium hydroxide, which in turn inhibits bacteria. In addition, the pH of cement rises up to 12.5 during setting, which inhibits bacterial growth and disinfects adjacent areas [28].

There is a limited number of studies evaluating the activity of calcium silicate cements in presence of dentin. Although dentin's buffering action and its inhibitory influence on the antibacterial effect of endodontic materials have been established [29], there is also evidence suggesting the enhanced antibacterial effect of materials in the presence of dentin. Zhang *et al.* [25] reported that the addition of dentin powder to the suspension of either BioAggregate or MTA powder enhanced their activity in the elimination of bacteria. Razmi *et al.* [14] evaluated the activity of CEM cement and MTA against *E. faecalis* in the presence of dentin. They found that the addition of dentin powder to the suspension of CEM or MTA improved the elimination of bacteria. Prestegaard *et al.* [30] evaluated the antibacterial effects of MTA, IRM, and calcium hydroxide against *E. faecalis* in dentinal blocks. Following an incubation period of three weeks, the antibacterial effect was assessed after 1- and 7-days [30]. All materials significantly reduced bacteria in comparison with the control group. It is worth noting that a material placed in greater amounts into the root canal acts as a mass and provides more antibacterial active components; it may also be less affected by the buffering effect compared to one placed as a thin film.

In the current study, the number of recovered bacteria decreased from day seven to day 30 of treatment in both experimental groups; the decrease was not significant in the CEM cement group. This finding is inconsistent with that of

Prestegaard *et al*. [30] who showed an increase in the amount of bacteria from day one to day seven in experimental groups which was not significant in MTA and calcium hydroxide groups. It is interesting to note that the antibacterial activity is superior at freshly-mixed-phase (during day one), but reduces with time. It seems that the initial toxicity of the materials during setting inhibits the bacteria. When the material sets (during seven-days of treatment), the antibacterial activity reduces, and bacteria gradually adapt to the environment and regrow [31, 32].

Another interesting finding in the current study is that, despite the reduction of bacteria caused by CEM cement and Biodentine, the dentin was not bacteria-free, which is in agreement with others; the inhibitory effect caused by the dentin buffering action, insufficient penetration depth of agents, and the presence of microbial biofilms may explain the incomplete killing of bacteria [30, 33].

Finally, it should be considered that outcomes from one study on one specific bacteria cannot be extrapolated, because phenotypic differences between bacteria are possible. Thus, other *in vitro* and clinical studies on other strains of bacteria should be done.

Conclusion

Within the limitations of the present *in vitro* study, it was revealed that CEM cement had a greater inhibitory effect on *E. faecalis* than Biodentine.

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Conflict of Interest: 'None declared'.

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