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Antibacterial Properties of New Cement Based Capping Material Prepared from Egg Shell and Biopolymer (Chitosan)

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Abstract

Backgrounds : This study evaluated the antimicrobial properties of a newly prepared, cement-based capping material and biopolymer (chitosan) with Mineral Trioxide Aggrigate MTA and Biodentine. **Method:** The antibacterial effects of a set of specimens against *Streptococcus mutans, Rothia dentocariosa,* and *Enterococcus faecalis* were evaluated by agar diffusion tests. Thirty disc-shaped specimens (10 of each type of materials; 6 mm in diameter × 2 mm in thickness) were prepared. One specimen for each material was placed on each agar plate, and the plates incubated for 24 h. After incubation, the diameter of the inhibition zone was calculated at three different points and averaged. **Results**: Statistically significant differences were found among new calcium-based capping material, MTA, and Biodentine. An ANOVA test was used to evaluate the effect of materials against each type of bacteria. This revealed that the inhibition zones produced by the new cement based capping material mixture were statistically significantly larger than those produced by the other materials. **Conclusions:** Within the limitations of the experimental methods employed in the present study, the cement-based capping material prepared from egg shells and the biopolymer chitosan has better antimicrobial properties than Biodentine and MTA.

Key words: *Egg shell and biopolymer (chitosan), antibacterial , Eggshell*

Backgrounds

Streptococci, lactobacilli, and Actinomyces species are the main cariogenic microflora presented on the surface of fissures, smooth-surface coronal caries, or root-surface caries. Members of the mutans group of streptococci spacially, *Strep. mutans* and *Strep. sobrinus* are considered to be the primary etiological causes in the induction of coronal and root caries [1].

One of the bacteria most frequently involved bacteria in dental caries is Streptococcus mutans; it efficiently degrades fermentable carbohydrates to acids, which can demineralize tooth tissue (Van Houte et al; 1991; Brukiene et al; 2005; Konradsson et al; 2006)[2]. Superficial infected dentin contain greater numbers of bacteria compared with deeper dentin. The application of strict anaerobic sampling and cultivation methods always reveals greater bacterial retrieval, including that the environment of carious dentin promotes the survival of obligately anaerobic bacteria. Thus, species of Propionibacterium, Eubacterium, and Bifidobacterium are predominate microflora of deep carious dentin, including Actinomyces, Lactobacillus, and some streptococci, but rarely *S. Mutans* [3]. The antibacterial effect is an important property because killing bacteria is a direct strategy for eliminating the cause of dental caries [4, 5]. Dental cements play an important role in assuring a healthy, infection-free oral cavity because they facilitate the sealing of damaged areas. However, it is still likely for infections to appear within these cements, and as such, antimicrobial activity must be one of the essential properties of dental cements [6, 7]. Dental cements are greatly used in odontological treatment. However, due to the dangerous nature of some of the materials used and the reduced biological efficiency, newer and safer substitutions are needed, particularly those possessing higher antimicrobial activity than their traditional counterparts [8–10].

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Antibacterial activity and sterilization of carious dentin could be supplied by cements containing calcium hydroxide [11]. Among the pioneer contaminants of dental cements, there are several types in the oral cavity, such as Enterococcus, Lactobacillus, and Streptococcus species. The inability to prevent these species may lead to tissue invasion with consequential pulpal necrosis and tooth loss. Thus, the search for new compounds that may ameliorate biocompatibility and functionality via enhanced antibacterial properties of cements has gained importance [12]. Antimicrobial action and the ability to enhance the formation of mineralized tissue are both dependent on an alkaline pH [13].

The aim of this study was to compare and evaluate the antimicrobial action changes caused by new capping cement material (prepared from egg shells and biocompatible chitosan) with MTA and Biodentine materials.

Method

In this study, we compared three experimental groups:

• New cement based capping material prepared from egg shells and the biopolymer chitosan (experimental material). The powder to liquid ratio was 1 spoon of powder to 8 drops of liquid; mixing time: 45 seconds, working time: 2 min, setting time: 5.45 min.

• Mineral Trioxide Aggrigate (Rootdent, Tehnodent, Russia) (control group).

• Biodentine material (septodont, France) (control group).

The microbiological study was carried out in the Bio® Laboratory of Microbiology in Erbil, Iraq.

The antibacterial effects of specimens against *S. mutans*, *Enterococcus faecalis,* and *Rothia dentocariosa* were evaluated using agar diffusion tests as described by Bauer et al. [14]. After isolation and identification, a single colony from each type of bacteria was transferred into 5 mL of sterile Brain Heart Infusion BHI broth (Lab, UK) and incubated at 37°C for 24 h. In order to prepare the experimental suspensions, a McFarland 0.5 turbidity tube was prepared and used to make suspensions of the strains in a brain-heart infusion at approximately 1.5×10^8 organisms/mL, which were flood-inoculated onto the surface of Muller-Hinton agar (Lab, UK) plates [14].

Thirty Petri plates with 20 mL of Muller-Hinton agar were inoculated with the microbial suspensions using sterile swabs that were spread onto the medium. Ninety disc-shaped specimens (30 of each type of materials; 6 mm in diameter × 2 mm in thickness) were prepared and divided into three groups:

• Group A: 30 experimental specimens composed of calcium-based cement subdivided into:

A1: 10 specimens inoculated with *E. fecalis*

A2: 10 specimens inoculated with *S. mutans*

A3: 10 specimens inoculated with *R. dentocariosa*

• Group B: 30 specimens composed of Biodentitn subdivided into:

B1: 10 specimens inoculated with *E. fecalis*

B2: 10 specimens inoculated with *S. mutans*

B3: 10 specimens inoculated with *R. dentocariosa*

• Group C: 30 specimens composed of MTA subdivided into:

C1: 10 specimens inoculated with *E. fecalis*

C2: 10 specimens inoculated with *S. mutans*

C3: 10 specimens inoculated with *R. dentocariosa*

Three discs (one for each material) were placed on each agar plate using sterile forceps and incubated in an aerobic candle jar at 37°C for 24 h. After incubation, the diameter of the inhibition zone was calculated at three different points and averaged. The sizes of the inhibition zones for each material were measured based upon the diameters of the halo of inhibition and the disc's diameter as follows:

Size of inhibition zone = diameter of halo – diameter of the disc (Figures 1–3).

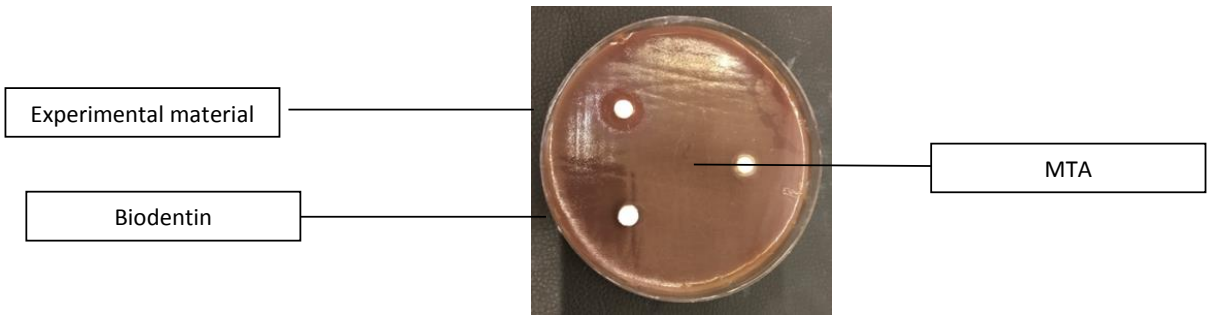

Figure 1: The zones of bacterial growth inhibition against (Streptococcus mutans).

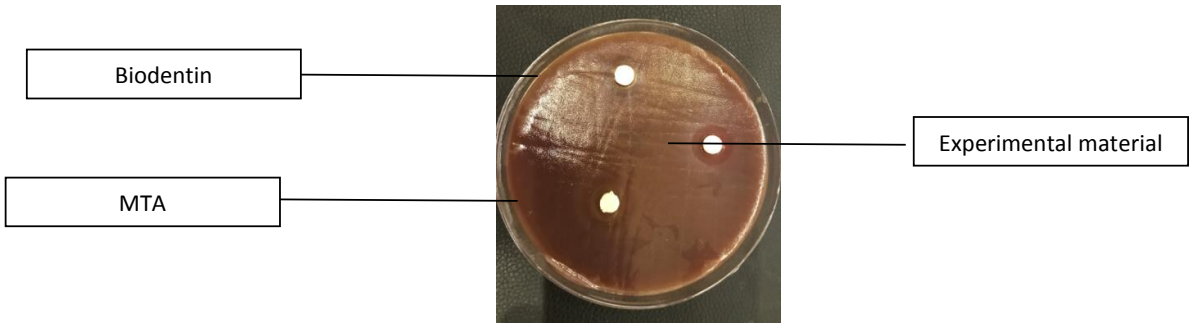

Figure 2: The zones of bacterial growth inhibition against (Enterococcus fecalis).

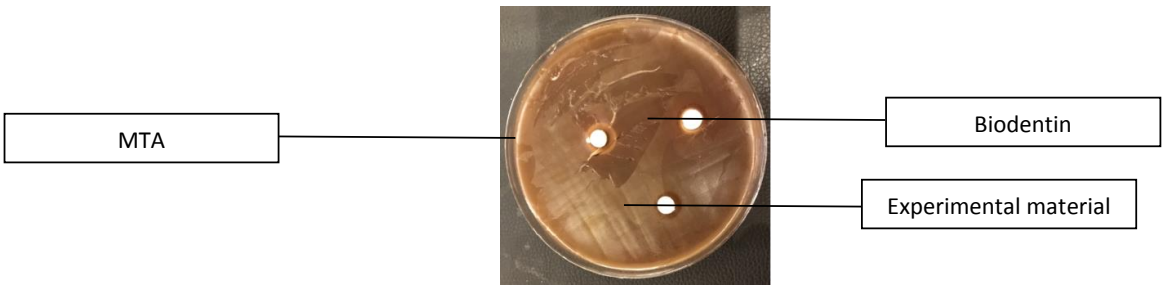

Figure 3: The zones of bacterial growth inhibition against (Rothia dentocarisa).

Results

The antibacterial activities of the tested materials determined by the means and standard deviations of growth inhibition zones for various microorganisms after 24 h are shown in Table 1. All materials showed antibacterial activity. The results of the 24-h incubation revealed that the experimental materials were the most effective against all tested bacterial strains, while the growth zones for the MTA and Biodentin groups were smaller.

Table 1. The diameters (in mm) of the zones of bacterial growth inhibition against the bacterial strains.

Materials	E. fecalis		S. mutans		R. dentocariosa	
	Mean	SD	Mean	SD	Mean	SD
Experimental cement	13.500	0.3590	11.700	1.1353	8.000	1.4907
Biodentin	9.200	1.6865	9.700	1.1353	6.300	0.9487
MTA	6.560	0.4142	8.000	1.4907	5.000	0.7454

The ANOVA test showed that there were statistically significant differences ($p < 0.0001$) among the sizes of the inhibition zones produced by the tested materials for all bacterial strains (Tables 2–4).

Table 2. ANOVA shows the effect of testing materials against *E. fecalis*.

Group	Sum of Squares	Df	Mean Square	F	Sig.
Between groups	45.267	2	22.633	18.462	0.000
Within groups	33.100	27	1.226		
Total	78.367	29			

Table 3. ANOVA test shows the effect of testing materials against S. mutans.

Group	Sum of Squares	Df	Mean Square	F	Sig.
Between groups	68.600	2	34.300	21.437	0.000
Within groups	43.200	27	1.600		
Total	111.800	29			

Table 4. ANOVA shows the effect of testing materials against R. dentocariosa.

Group	Sum of Squares	Df	Mean Square	F	Sig.
Between groups	245.411	2	122.705	117.052`	0.000
Within groups	28.304	27	1.048		
Total	273.715	29			

Discussion

The development and progression of pulpal and periapical diseases, as well as endodontic treatment failure were essentially attributed to Microorganisms. Treatment consequences will depend on the successful elimination of the associated microorganisms and infected tissues [15].

The agar diffusion test is routinely used to explore the antimicrobial properties of dental materials. This method involves placing the tested material on an agar plate inoculated with oral bacteria. Using this method, an inhibition zone around the material is produced. To produce a zone of inhibition, the material needs to ooze a soluble antimicrobial agent. If the amount of the antimicrobial agent removed is not sufficient, the zone of inhibition will not be produced. In general, larger zones correlate with the concentration and/or potency of the tested bactericide. This also proposes the susceptibility of the tested bacteria to a specific antimicrobial agent, and the size of the inhibition zone can be measured using a graduated ruler [16–18].

The bacterial strains used throughout the experiments were *S. mutans*, oral Lactobacilli, and *E. fecalis*. These microorganisms play an important role in dental biofilm formation and in the etiology and progression of caries. *S. mutans* is one of the bacteria most frequently engaged in dental caries. It efficiently degrades fermentable carbohydrates into acids, which can demineralize tooth tissues [19–21].

Fransson et al. (2014) consummated that *E. faecalis* reduced the activity of odontoblast-like cells, and it had an inhibitory effect on collagen-1 production. Thus,

it decreases the ability of odontoblasts to induce the synthesis of tertiary dentine [22]. *E. faecalis* has also been shown to be a highly resistant bacteria in the root canal system, and it plays major role in endodontic treatment failures [23].

The results of this study revealed the effective antibacterial activity of experimental new calcium-based capping material, which showed larger growth inhibition zones of tested bacteria compared to MTA and Biodentine. This may be associated to the liberation of ions and alkalinity of new capping material.

The experimental cement consisted of 70% CaO, 25% MgO, 3% hydroxyappetite (HA), 1.5% bismuth oxide, and 0.5% calcium acetate. Calcium-based cement has an antibacterial effect, and it is a possible candidate for use in pulp capping and cavity lining [24]. Li et al. (1998) showed that HA has antibacterial properties against cariogenic bacteria. They concluded that it would be best to harness the antibacterial properties of HA by using it as a base in the treatment of carious cavities in order to inhibit residual cariogenic bacteria[25].

Tin-oo et al. (2007) found that tubes containing 200 mg or more of HA completely inhibited *S. mutans*, and no bacterial growth was seen. This finding has shown that HA displays solid antibacterial properties. The bacterial growth inhibition could be related to magnesium ions or active oxygen released by MgO into the medium [26].

Sawai et al. (1995a) showed that MgO, CaO, and ZnO exhibited strong antibacterial activity. They found that MgO and CaO powders displayed bactericidal effects against Gram-positive and Gram-negative bacteria, while ZnO powder inhibited the growth of Gram-positive bacteria more strongly than Gram-negative bacteria[27].

The polycationic structure of chitosan CH is necessary for antibacterial activity. Electrostatic interactions between the polycationic structure and the predominantly anionic components of the microorganisms' surface (such as Gram-negative lipopolysaccharide and cell surface proteins) play a primary role in antibacterial activity, because environmental pH is below the pKa of CH and its derivatives [28].

Liu et al. (2006) found that the antibacterial activity of low molecular weight (MW) CH against *Escherichia coli* is higher than that of the high MW CH with the same degree of deacetylation [29]. *R. dentocariosa* was originally isolated from dental plaques and caries. It is found in the oral cavity and pharynx of humans where it forms a portion of the normal microflora[30]. *R. dentocariosa* was seen in over 30% of healthy individuals by an investigation of throat swabs [31]. Furthermore, it can also be extracted from respiratory tract specimens as part of the normal oral flora [29].

Biodentine showed some initial bacterial inhibition, but this was significantly lower than that of the new cement based capping material and MTA, which displayed a large spread in data. The fact that Biodentine revealed zones of inhibition implies that Biodentine itself has some definite antimicrobial effects. This might be related to Biodentine's high pH [32]. Because *E. faecalis* can live in alkaline environments, this may play a role for the smaller inhibition zone of Biodentine against *E. faecalis*. Perhaps the inherent and persistent alkalinity of Biodentine is just enough to smash *E. faecalis* [33]. Biodentine powder is composed mainly of tricalcium silicate, calcium carbonate, and zirconium oxide as a radio-pacifier. Meanwhile, liquid Biodentine contains calcium chloride as the setting accelerator and water as the reducing agent. The addition of up to 30% calcium carbonate, calcium sulfate, and calcium chloride resulted in an improvement in the physical properties of tricalcium silicate cement. It also improved the degradability and bioactivity of the resultant material. Calcium silicate hydrate gel, calcium hydroxide and unreacted tricalcium silicate are resulted from the hydration of tricalcium silicate. The antibacterial and anti-inflammatory properties of the calcium hydroxide produced from the tricalcium silicate hydration, mainly due to the high (alkaline) pH of the surrounding environment after it dissolves [34–35].

Conclusions

Within the limitations of the experimental methods employed in the present study, a number of conclusions can be seen. All tested materials have antibacterial properties against the tested bacteria. Furthermore, the experimental new calcium-based capping material cement showed remarkable antibacterial activity against the bacteria tested, which was comparable to that of MTA and better than Biodentine.

Conflict of Interest: Not

Ethical Clearance: The study was approved by the Ethics Committee of the College of Dentistry, Hawler Medical University, Kurdistan Region, Irbil-Iraq.

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