

REVIEW ARTICLE

Antibacterial Potential of Pulp Capping Materials

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ABSTRACT

A critical analysis of the antibacterial effect of the main representatives of calcium silicate cements (CSCs) was made. An analysis of the most frequently used methods for screening the antibacterial activity of materials has been made. The inhibitory activity of CSCs against major types of microorganisms such as *Candida albicans*, *E. faecalis*, and strains *streptococcus* was evaluated and compared. The antibacterial effects of CSCs are not yet well and completely known because no evidence compares the antibacterial properties of bioceramic materials with a uniform methodological approach. It is important to provide standardization of testing methods for evaluating the antibacterial potential of the materials and different bacterial strains. To this stage, there are no reproducible and standardized methods for evaluating of antibacterial activity of CSCs.

Keywords: Calcium silicate cements; Bacterial inhibition activity; Microorganisms

1. Introduction

Biological treatment methods/or vital pulp therapy in dental practice are intended to preserve the vitality and functional integrity of the dental pulp (DP). They include various dental procedures such as indirect; direct pulp capping; and partial or complete pulpotomies. Indirect pulp capping is a procedure in deep carious decay that after complete caries tissue

removal and a minimum of 2 mm of remaining dentin thickness, followed by covering with an appropriate bioactive liner ^[1].

Direct pulp capping (DPC) is indicated in the early stages of inflammation of the dental pulp. This is a dental procedure involving the placement of a bioactive material in the place of communication of DP. The pathological communication may be

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the result of a deeply advanced carious process or as a result of traumatic or mechanical damage^[1]. A partial or vital amputation is a more invasive dental procedure in which the irreversibly changed coronal part of DP is partially or completely removed and the wound is covered with a suitable bioactive material^[1].

For the success of all these biological methods, it is important not only to work in aseptic conditions, but also to choose the appropriate material. The material, that stimulated DP enhanced dentinogenesis and the formation of dentinal bridge^[2]. The present bacteria and their by-products in deep caries are the main etiological factors for pulpal diseases and can induce severe inflammatory reactions in the pulp/dentin complex^[3]. The successful outcome of DPC is associated with the reason for the pulp exposure^[4]. According to Bjørndal et al. in the clinical situation of traumatic exposure to DP, DP is healthy and without present microflora. But following the deep decay there is a presence of diverse microflora^[4].

Deep carious lesion in dentin contain numerous bacteria such as *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.*^[4]. The complete removal of caries in deep carious decay is required for the biological methods to be successful^[5].

The smart bioactive material should possess a bioactive capacity and antibacterial potential so as to eliminate the effect of residual bacteria after necrotomy^[6]. Their main important properties to achieve the inhibitory effect on available microorganisms are associated with the release of calcium hydroxide and providing a highly alkaline antimicrobial environment^[7].

The first biomaterial introduced in the dental practice in 1930 by Hermann is calcium hydroxide^[1]. For many years this material was considered the gold standard for conducting biological treatments.

The development of knowledge of pulp biology led to the introduction of new smart bioactive materials such as calcium silicate-based cements (CSCs). Their chemical composition is an important factor that affects the properties of materials. By nature, these are hydraulic porous materials and the setting reaction of CSCs is a complex hydration

process. Calcium and hydroxyl ions are the basic components released by hydration when calcium silicate cements are mixed in water^[7]. The porous nature of cement facilitates the release of these ions during the hydration process^[8]. High pH results from the formation of calcium hydroxide during the hydration reaction^[9]. The high alkalinity is associated with the strong antimicrobial activity of CSCs^[10-12]. The release of Ca^{2+} ions increases the alkalizing activity emphasizing the bactericidal effect by suppressing the activity of osteoclasts and preventing further tissue damage^[13]. The bacteriostatic effect of calcium hydroxide is associated with damage to the cytoplasmic membranes of microorganisms^[14]. The bioactivity potential of CSCs is that they create a specific ability to control bacteria such as stopping their growth. They are bioactive materials with a variety of applications in dentistry such as direct pulp capping, perforation repair, retrograde root-end filling, regenerative therapy, etc.^[15].

The era of bioactive calcium-silicate cement in dental practice started in 1998 with the MTA Original-ProRoot[®] MTA (Dentsply, Tulsa Dental, Johnson City, TN, USA). This first cement was developed by the Torrebinead and Dr. White team and consisted of ordinary Portland cement and bismuth oxide. In 2002, the white form ProRoot MTA was introduced^[16]. The rapid development of materials science has led to the creation of a variety of new calcium silicate cements (CSCs) on the market. Four main generations of these materials are distinguished in clinical dental practice^[17].

Biodentine (Septodont, Saint-Maur-des-Fossés, France) is a third-generation CSCs, introduced in 2010 and developed on the basis of active biosilicate technology. This material is specifically indicated for vital pulp therapy^[17,18]. Biodentine is produced using an Active Biosilicate Technology. This means achieving the purity of the material^[19]. The powder of this cement has a similar composition to white ProRoot MTA, but the radiopacifying agent bismuth oxide is replaced by zirconium oxide in Biodentine. The liquid represents an aqueous solution of calcium chloride and a water-reducing agent^[20]. The main

properties of Biodentine™, have been extensively evaluated.

Resin modified calcium silicate cements (RMCSs) or hybrid cements are a new group of CSCs and belong to the fourth generation of materials. This type of material is a combination of calcium silicates and resin. RMCSs are mainly used in direct and indirect pulp capping [21]. The basic representative of this generation is TheraCal LC (Bisco Inc, Schamburg, IL, USA) This material was introduced in dental practice in 2017. According to the manufacturers, the chemical composition is represented by 45% Portland cement III type, 10% dicalcium silicate, a radiopaque substance from barium zirconate and 45% resin mainly from BisGMA, polyethylene glycol and photoinitiators [21].

Other resin modified calcium silicate cements have already been introduced in clinical dental practice such as Bright MTA etc. for which there is limited information [15]. According to Sung-Min et al., the organic component in this new hybrid material is represented by polyethylene glycol dimethacrylate (10–30%). The information presented considers physico-chemical properties but not its antibacterial effect [22].

2. Material and method

A literature search base was undertaken in three electronic databases: PubMed, Ebscohost and Google Scholar. The following keywords have been used: calcium silicate cements, direct pulp capping materials, and antibacterial activity.

A key factor in the success of biological methods is the high antibacterial potential of the pulp capping materials used. For this, it is extremely important to determine the antibacterial capacity of CSCs, and the materials indicated and used for them. The correct results depend on a good knowledge of the different methods for assessing antibacterial activity and their limitations and advantages.

There are a variety of in vitro methods for assessing the antibacterial activity of the materials [23]. The most commonly used method is the agar diffusion test. Its advantages over other tests are the ability

to evaluate varied numbers of microorganisms and materials, easy interpretation of the results and a more reproducible method [23–25]. The direct contact test evaluated the activity of freshly mixed materials while the Agar diffusion method analyzed only set agents [26]. The direct contact test is quantitative and is indicated for the analysis of insoluble materials [27]. However, the main advantages of the agar diffusion test are related to a simple procedure without the need for special equipment and a cheap method [23,28].

The serious limitations of the method are about the need for the tested and setting materials to have a diffusion gradient in agar and the obtained results cannot distinguish a bacteriostatic from a bactericidal effect of the tested materials [25,29,30]. According to Leonardo M et al., the diffusion gradient of the materials may depend on different factors such as the concentration of the tested material, agar viscosity, ion concentration in relation to the medium, used microorganisms etc. [31].

According to Funda Kont Cobankara et al., the advantages of the direct contact test are related to quantitative assay and reproducibility [25]. The bacterial inhibition activity of the agar diffusion test depends on the diffusion potential of the tested material [24,25,32]. An important advantage of the method is its ability to immediately assess the antibacterial activity of the material and the results are independent of the diffusion properties of the material [19,33]. Another method used to study the antibacterial effect of the materials is the tube dilution method. Through this method, the minimal inhibitory concentration of the material is recorded which leads to suppression of the growth of microorganisms by direct contact [34]. The Serious disadvantages of this method are errors in the preparation of solutions with different concentrations and the need for large amounts of reagents [23]. For correctly interpreted results after using different tests, it is important to assess the type form of the studied microorganisms. They can be studied in isolated form or organized in biofilms. In the isolate form, the cells are in suspension and they are more sensitive than those organized in biofilm [19].

The reason for differences in outcomes in comparative antibacterial studies is due to the fact is that most studies are on single isolated bacteria. In clinical situations, microorganisms are organized into a biofilm. Endodontic infections are polymicrobial. The induction of multispecies microcosm biofilm aimed to reproduce a real clinical situation. Depending on their application, CSCs are materials in contact with dentin and blood, for example, at DPC. The variations in the pH of host contact tissues could affect the physical and chemical properties such as microstructure change and antimicrobial properties of CSCs [35].

The main reason for differences in the reported results, regarding the antimicrobial effect of dental materials is the different methodologies and types of microorganisms.

Studies have been conducted on the antibacterial activity of CSCs, but the results obtained and reported in the dental literature are contradictory.

Several studies have found limited antimicrobial effects of MTA [36–38]. According to Torabinejad et al. MTA had no antibacterial activity against *S. faecalis*, *S. aureus* and *B. subtilis*. Similar data is reported by other researchers [37–39]. In contrast, others find that MTA has antibacterial activity against *E. faecalis* and *S. sanguis* by using a tube dilution test [10]. Similar data regarding the antibacterial activity of gray ProRoot MTA against *Staphylococcus aureus*, *Candia albicans* and *E. faecalis* were reported by Tanomaru-Filho M. et al. [40]. They found that gray ProRoot MTA has some antimicrobial effects against *Staphylococcus aureus* and *E. faecalis* but it is most pronounced towards *Candia albicans*.

According to Jerez-Olate et al., Biodentine has higher antibiofilm action for a prolonged aging period in the in vitro Modified Direct Contact Test than ProRoot MTA [41]. According to Vaki et al. and Jose et al., the agar diffusion test found a higher diameter of inhibition zones in the group of Biodentine in comparison to MTA [42,43].

Conflicting comparative data regarding antibacterial activity between Biodentine and ProRoot MTA have been reported. According to Bhavana V et al. [44],

Biodentine had higher antimicrobial activity against *E. faecalis*, *C. albicans*, *S. mutans* than MTA. Similar data concerning higher antimicrobial activity was found in Biodentine in comparison to MTA [42,43,45–48]. The latter author found that Biodentine has a higher antifungal capacity in *C. albicans* when compared to MTA. Species *Candida albicans* grow best when the pH of the environment is in the range of 3.0–8.0. According to Możyńska J. et al. [49], Biodentine has a more pronounced effect after 48 hours of incubation, against *Candida albicans* compared to ProRoot MTA. The pronounced antifungal effect of biomaterials is due to the strong alkaline reaction. It was found that Biodentine showed significantly higher pH and calcium ion release compared to ProRoot [50,51]. But Rubén Herrera-Trinidad et al. reported that the pH values and Ca release increased in the Biodentine group at 168 h [52].

However others found no statistically significant difference in antifungal efficacy for *C. albicans* between ProRoot MTA, and Biodentine [53]. In contrast, other authors found no difference in antibacterial activity against *E. faecalis* between them [52,54,55].

In a comparative study of the antibacterial activity between TheraCal LC, ProRoot MTA and Biodentine, after completing setting reactions against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis* by the agar-diffusion method none were found [56].

The data in the dental literature about the bacterial inhibition activity of resin modified calcium silicate cement (RMSCC) is limited. According to Poggio et al., TheraCal LC has some antibacterial effects against *S. mutans* while Biodentine is effective against *S. sanguis* when tested in an agar diffusion test [57]. The researchers were comparing the antimicrobial activity of different materials in vitro against *Streptococcus mutans*, *Streptococcus salivarius*, and *Streptococcus sanguis*. They found that only Dycal (calcium hydroxide) has an antibacterial effect against all studied strains of streptococcus.

In a comparative study between TheraCal LC

and Biodentine, the greatest antibacterial effect on *Streptococcus mutans* was found in both pulp capping materials^[58].

According to Akin D. et al.^[55], TheraCal LC, Pro-Root MTA and Biodentine did not have antibacterial activity against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis* after the completed setting reaction by the agar-diffusion method.

The data in the literature from a comparative study of antibacterial activity between TheraCal LC and Biodentine are contradictory. Farrugia et al. found that TheraCal LC showed more antibacterial effects than Biodentine using the direct contact test^[59]. In contrast, Fathy et al. reported that the antibacterial effects of Biodentine on *S. mutans*, were more effective than TheraCal LC^[60]. Similar results have been reported by other authors^[33].

However, the outcome of comparative studies varies from one to another depending on the used protocol and tested bacterial strains and whether these are isolated bacteria or organized in a biofilm. Overall, hydroxyl ions released upon hydration of CSCs lead to an alkaline pH in the surrounding environment with antimicrobial effects.

The antimicrobial potential of CSCs is directly related to their surface contact angle^[59]. A contact angle test is an indicator of the wetting behavior of a solid porous material (cement) and a liquid (water). Contact angle measurement is a reliable tool to better understand the interactions of hydraulic materials with the environment^[61–64]. The interactions of porous CSCs with the host environment can modulate their physico-chemical and antibacterial properties. The surface properties as modified by interactions with the environment may play a role in the inhibition of bacterial adherence and consequent biofilm formation or the pores may provide a medium that will enhance bacterial attachment^[62–65]. The contact angle reflects the interactions of fluids with solid surfaces, which depend on surface topography, hydrophobicity and wettability.

According to Farrugia, C. et al. a confocal laser scanning microscopy study^[59] found that Biodentine loses its antimicrobial activity after prolonged ex-

posure to biofilms. A Jardine, A.P. et al.^[66] reported that Biodentine was ineffective against multi-species biofilm^[66].

3. Conclusions

The antibacterial effects of CSCs are not yet well known because no evidence compares the antibacterial properties of bioceramic materials with a uniform methodological approach. It is important to standardize testing methods for evaluating the antibacterial potential of materials and different bacterial strains. To this stage, there are no reproducible and standardized methods for evaluating the antibacterial activity of CSCs.

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Independent work: author Ivanka Dimitrova.

Conflict of Interest

There is no conflict of interest.

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