Evaluation of Antimicrobial Effectiveness of Dental Cement Materials on Growth of Different Bacterial Strains

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Background: The aim of this study was to evaluate the antimicrobial effectiveness of dental cement materials for the prevention of bacterial growth, which can cause failure of fixed cementation.

Material/Methods: We developed an agar diffusion disk test in-house to evaluate the antibacterial properties of 3 commercially available dental cement materials (Ketac, Harvard FLB, and Panavia SA Universal Dual Resin cements) compared with a negative control. The materials were tested for the inhibition against Streptococcus mutans (ATCC 10449), Streptococcus salivarius (ATCC 25975), Enterococcus faecalis (ATCC 29212), and Lactobacillus acidophilus (ATCC 4356). The antimicrobial effectiveness of materials was expressed as the diameters of the inhibition zones around the disk.

Results: Overall, 240 specimens were tested. All cement materials showed antimicrobial effectiveness. Different microbial strains reacted differently to the different dental cements (all P<0.001). The bacterial strain of S. mutans showed the largest zones of inhibition of all cement, and E. faecalis was less susceptible. Statistically significant differences were observed when comparing different materials among each other (P<0.001). The fluoride-releasing material Panavia was significantly superior to the other 2 materials (P<0.001).

Conclusions: Fluoride-releasing dental cements showed antimicrobial properties, showing resin cements having superior antimicrobial effects when compared with conventional glass ionomer or zinc phosphate cements.

Keywords: Dental Caries • Dental Cements

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Background

Dental cement materials are used in fixed prosthodontics and act as luting agents for dental crowns and bridges to natural teeth or implants [1]. Therefore, retention of fixed partial dentures relies on the adaptation of prosthetic material to the tooth surface by the dental cement material. To cement the crown to the tooth, there is a space left between the crown and tooth, called the “cement gap”. This gap is the main reason for failed cementation, as the margin can ensure the environment for colonization of microorganisms, which can create bacterial biofilm and cause periodontitis, periimplantitis, and secondary caries [2-4]. Owing to the different sources of dental cements, previous studies investigated not only their chemical composition, physical properties, and application, but also their antimicrobial effectiveness [5]. Resolving antimicrobial effectiveness led to a range of solutions, including minimizing the cement gap, improving oral hygiene, developing dental cement that is less susceptible for adherence of bacteria and thus biofilm development, and, finally, developing cements that have an antimicrobial effect [6-9]. Studies showed differences in antimicrobial effects, and there is no consensus on which material shows superiority in this matter [10-14].

To date, zinc phosphate is the material that has been most widely used for luting. It has a low solubility; however, the retention relies entirely on mechanical retention. Zinc polycarboxylate cement, by contrast, shows proper adhesion due to the chemical interaction with enamel and stainless steel. However, its solubility is relatively high, which can be considered a disadvantage [15]. Therefore, glass ionomer cements have become more popular as luting and cementation agents [16]. Glass ionomer cements have lower solubility in saliva, have higher strength for compression, and form ionic bonds with stainless steel in prosthetic materials [15]. However, the key disadvantages of glass ionomer cement are the sensitivity to moisture during its setting and time limitation to achieve maximum bond strength [16,17]. Next, resin-modified glass ionomer was developed as an advanced glass ionomer hybrid material with the intention to preserve low solubility, the ability to chelate via the acid–base reaction to enamel and metal, moisture tolerance, and high strength. The potential limitation of this material is its questionable fluoride release [16,18].

The evolution is clearly seen in the development of cements. In addition to all mentioned properties, such as high retention, strength, and easy removal, an ideal dental cement material should also inhibit the development of bacterial plaques [19]. For instance, secondary caries with the formation of bacterial biofilm is the main reason for failed fixed partial dentures. Secondary caries occur due to the colonization of bacteria at locations with low oxygen. Cariogenic bacteria such as Streptococcus mutans or Lactobacillus acidophilus produce acids from metabolized carbohydrates, which then dissolve the calcium phosphate mineral content in enamel and dentin, and lead to the creation of cavities [20]. The antibacterial effectiveness of dental cement materials has been established previously [21-23], and the effects are related to their antimicrobial properties due to a low pH or the release of fluoride. However, the literature contains contrary data regarding which class of cement has the best antimicrobial effect [22-25]. Therefore, the aim of our study was to evaluate the antimicrobial properties of different commercially available dental cement materials using a disk agar diffusion method for the prevention of bacterial growth, which is the common cause of secondary caries.

Material and Methods

Dental cement materials were analyzed for their antimicrobial effects using a disk diffusion method. Three different dental materials were tested: glass ionomer cement (3M Ketac Cem Easymix), phosphate cement (Harvard FLB cement), and fluoride-releasing resin cement (Panavia SA Universal Dual Resin cement) (Table 1). The cements were divided into 4 subgroups, according to the bacterial strains. Blank paper disks were used as a negative control.

Bacterial Samples

The antimicrobial performance of cement materials was tested against S. mutans (ATCC 10449), S. salivarius (ATCC 25975), E. faecalis (ATCC 29212), and L. acidophilus (ATCC 4356). The frozen ATCC microbial strains were dissolved and subsequently inoculated onto the blood agar plates. The inoculated plates were incubated anaerobically with an atmosphere of 5% CO₂, 10% H₂, and 85% N₂ for 2 days at 37°C. The atmosphere was created using the Anaeroxomat System (MART Microbiology BV, Netherlands). The bacterial growth was confirmed with morphological identification (colony shape, color, thickness, hemolysis on agar plate) and Gram staining.

Creation of Disk Diffusion Assay and Antimicrobial Testing

The antimicrobial effects of the cements were measured with the agar diffusion test. A drop of material was directly applied into the plastic mold in a shape of a disk, which was created to ensure the standardized quantity of each material. Using molds, the error in the amount of material applied to microbial agar could affect false results. The disks of each material were prepared using approximately 10 mg of material. For each material, a new mold was used to prevent cross-contamination between materials. Disks were immediately placed on freshly inoculated plates with brain heart infusion (BHI) agar, using an aseptic technique in the aseptic chamber. Each plate
contained negative control, which was represented by an empty paper disk in the middle of the agar plate.

Bacterial suspensions were prepared to a concentration of 0.5 MacFarland standard. BHI agar plates were inoculated with the respective bacterial strains, with a cotton swab, using an aseptic technique. The disk diffusion method of antimicrobial testing was used, and disks were placed onto the hardened BHI agar.

After inoculation, the agar plates were incubated at 37°C for 72 h under anaerobic conditions to allow sufficient growth of the bacteria. After incubation, the diameters of the zone of inhibition on microbial growth around each disk were measured in millimeters, using digital calipers.

**Statistical Analysis**

Statistical analyses were performed using the software package SPSS version 21 (IBM Corp, Armonk, NY, USA). One-way ANOVA was used to assess differences between normally distributed mean diameters of zones of inhibition. The Kruskal-Wallis test was used when the data were not normally distributed. Statistical significance was set at $P<0.05$.

**Results**

Overall, 240 specimens were tested after being divided into 3 groups according to cement type. The effectiveness of each cement material is presented in Table 2 as mean inhibition zones in millimeters and accompanied standard deviations.

All tested cements showed antimicrobial effectiveness. Following that, different microorganisms were found to have reacted differently to different dental cement materials ($P<0.001$). The bacterial strain of *S. mutans* showed the largest zones of inhibition among all cements. This was followed by *S. salivarius*, *L. acidophilus*, and finally *E. faecalis*. Thus, all dental cement materials showed antimicrobial effectiveness against all 4 microorganisms.

Pairwise comparisons showed that with Ketac cement there was no difference between *E. faecalis* and *L. acidophilus*. With Harvard cement, *S. salivarius* and *L. acidophilus* showed no statistically significant difference. With Panavia, there was no statistically significant difference between *S. mutans* and *L. acidophilus*. The marginal means are displayed in Figure 1.
Our study showed similar results whereby resin cement showed
the best antimicrobial effect, followed by zinc phosphate cement
and glass ionomer cement. Here, it must be mentioned
that all cements performed extremely well, showing superi-
or zones of inhibition when compared with the negative con-
trol. This performance was observed with all 4 bacterial strains.

The agar diffusion method in the present study showed the
antimicrobial effects of different cements over time showed some
antimicrobial effects at the beginning but no significant differ-
ences in the antibacterial effects for zinc phosphate cement
when compared with the control in later time points. Also,
the glass ionomer cement Ketac showed no difference when
compared with the control. The reasons for these contradic-
tory results could be the study methodology. In our study, we
used the agar diffusion method, whereas Unosson et al [19]
relied on measuring the viability through the direct contact
test, based on the turbidimetric determination of continuous
bacterial outgrowth from the material under investigation.

Moreover, the time of evaluation also might play a crucial role.
The agar diffusion method in the present study showed the
antimicrobial effects 48 to 72 h after the incubation of bac-
teria, and in the other study, the authors evaluated the effect
over a longer period.

Nevertheless, conventional cements create a low pH and there-
fore an acidic environment, which should limit the growth of mi-
croorganisms. However, with glass ionomer and zinc phosphate
cements, low pH can cause pulpal irritation [31]. Moreover, a
slightly acidic environment cannot inhibit the growth of bac-
teria. For instance, S. mutans can still grow at a pH of approxi-
mately 5 [32]. In the present study, we did not measure the
performance of cements for the creation of low pH. However,
Unosson et al [19] did measure pH and reported that none
of the tested cements inhibited bacterial growth at a pH of
around 6. After some time, cements lose their acidic proper-
ties, so such an environment does not last long. Therefore,
the main antimicrobial properties come from fluoride or the
release of zinc [24].

Studies have shown that glass ionomers have antimicrobi-
al properties against S. mutans and against plaque develop-
ment and have protective features for secondary caries, which

Figure 1. Estimated marginal means of inhibition zones for
respective material compared between the tested microorganisms.

- Statistically significant differences were observed among the
different materials ($P<0.001$). The fluoride-releasing materi-
al Panavia was significantly superior to all other materials
($P<0.001$). The phosphate cement Harvard showed the sec-
ond-best performance, but the difference was not statistical-
ly significant when compared with the glass ionomer Ketac
($P=0.289$). Moreover, no zones of inhibitions were detected in
any of the negative controls; thus, all differences in cement
materials were statistically significant when compared with
the negative control (all $P=0.001$).

Discussion

We assessed 3 different commercial dental cements for their
antimicrobial effectiveness against the 4 bacterial strains that
most frequently create bacterial biofilm and lead to failure
of cementation in fixed prosthodontics. The assessment was
performed by the agar diffusion method, as this method was
confirmed as appropriate for the determination of antimicro-
bial properties in other studies [21,26].

The retention of dental cements is often related to their prop-
erties and is determined by their resistance in the creation of
bacterial biofilm and their avoidance of the creation of sec-
ondary caries [27-29]. Cements that have an antimicrobial ef-
fect can thus have a longer retention time of restorations. It is
inevitable at some point that patients will develop early bac-
terial plaques, as the intraoral space of restoration between

the cement and teeth is generally an ideal environment for
bacterial growth [30].

The three dental materials tested were representative of glass
ionomer cement (Ketac), zinc phosphate cement (Harvard FLB),
and fluoride-releasing resin cement (Panavia SA Universal Dual
Resin). Previous reports showed that glass ionomer cements
have an antimicrobial effect, most likely due to their low pH or
fluoride release [21-24]. There are also reports indicating that
polycarboxylate cements and zinc phosphate cements have
superior effects [23], which are also attributed to low pH. Our
study showed similar results whereby resin cement showed
the best antimicrobial effect, followed by zinc phosphate ce-
ment and glass ionomer cement. Here, it must be mentioned
that all cements performed extremely well, showing superi-
or zones of inhibition when compared with the negative con-
trol. This performance was observed with all 4 bacterial strains.

Figure 1.
are mainly attributed to continuous fluoride release [9,33-35]. However, the antibacterial properties of glass ionomer cements differ from one material to another. The fluoride release can drop significantly with long-term usage [36]. This explains why the 2 fluoride-releasing agents in our study might have differed in antimicrobial effectiveness, with Panavia (despite being resin cement) performing better than Ketac. This result suggests that further studies are needed to examine the levels of fluoride release. Moreover, the zinc phosphate Harvard cement also showed good antimicrobial activity. Its effectiveness was attributed to the low pH and release of ions that inhibit the growth of bacteria [37-39]. The growth of S. mutans was found significantly decreased at a low pH in previous reports [40]. In a study by Dastjerdiea et al, the inhibition of S. mutans growth was detected as unchanged from day 2 up to day 7 [36]. In this case, the increase of pH after setting the material could explain the reduced antimicrobial effect. However, the results of Dastjerdiea et al [36] were contrary to our results, that glass ionomer cement had superior antimicrobial activity when compared with zinc phosphate cement. This might be explained by the combined effect of low pH and the fluoride release of glass ionomer [40]. However, we would like to highlight that these differences are very much related to the material, and the same material was not used in these studies. Other studies might have different conclusions, although the properties between different cements can be similar. In some experiments, the antibacterial activity measured with different cements might be correlated with the higher zinc or fluoride release rates, as observed in other studies.

Due to their ability to release zinc ions, zinc-containing materials can inhibit the growth of bacteria [38]. Zinc is an inhibitor of multiple activities in the bacterial cell in glycolysis, transmembrane proton translocation, and acid tolerance [41]. Its antimicrobial action is similar to the action of fluoride, but zinc works better in neutral pH (while the inhibitory potency of fluoride for glycolysis is much greater at acidic pH values). Zinc can also enhance proton permeability of bacterial cell membranes [41]. It reduces proton-extruding ATPases, and ATP synthesis in glycolyzing cells because it can inhibit the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenases and pyruvate kinase, as well as the metabolism of phosphoenolpyruvate [42]. In our study, the activity of zinc was confirmed, and glass ionomer showed a bit less of the antimicrobial effectiveness, when compared with resin cement and zinc phosphate cement. However, fluoride release has been confirmed as an effective antibacterial agent also in other dental products [24]. Whether the effect was more related to creating an acidic environment or to fluoride release was not established in the present study. Regarding this matter, there are no established correlations between acidity or fluoride release and antibacterial properties. Rather, it has been proven that fluoride has a direct effect on the proliferation of bacteria, negatively affecting its growth [19]. Fluoride ions F- or HF directly inhibit the enzymes enolase, urease, phosphatases, and heme-catalase, which are involved in metabolism of bacterial cells. Moreover, HF is a transmembrane proton carrier and disturbs the membrane of the bacterial cell and overloads the proton-extruding ATPases, and eventually causes cell starvation [32].

In conclusion, the release of fluoride and zinc has an antimicrobial effect. It has been proposed that the ideal release from dental cements should be initially rich, then followed by a stable, lower release [36]. Of course, the releases might vary between different materials. We found that resin cement showed better antimicrobial effectiveness and has also been shown to have better, or at least a similar, release profile when compared with conventional glass ionomer cement [29,43,44].

Nevertheless, our study had limitations. The agar diffusion method that was used is semi-quantitative and depends on solubility and diffusion properties of the tested cement materials, so this might have impaired the results of material with lower solubility. In the present study, the performance of low pH, which also contributes to an antimicrobial effect, was not tested. It would be interesting to analyze the correlation between these 2 variables in the future. In addition, the levels of fluoride and zinc released might be of interest, as in the studies in the literature, there might be different levels of antimicrobial ability. Furthermore, this study was small and evaluated only 3 different materials. We encourage others to conduct additional studies and assess antimicrobial effectiveness of more agents within glass ionomer, zinc phosphate, and resin cements. Our study was conducted using in vitro conditions and could not simulate the physiological conditions of the oral cavity.

**Conclusions**

Antimicrobial properties in cement materials are desirable. Glass ionomer and resin cements released fluoride with a similar profile but showed slight differences in their performance of inhibition zones, which was most likely due to different levels of fluoride release. Meanwhile, zinc phosphate cement released zinc ions and also showed reliable antimicrobial ability in the inhibition of bacteria growth. Resin cements were statistically significantly better and might be applicable in clinical practice as the preferred dental cement material to reduce the probability of plaque formation and thus protect teeth from bacterial infections.

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All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission.
Declaration of Figures’ Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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