Antibacterial Activity and Physical Properties of Conventional Glass-ionomer Cements Containing Chlorhexidine Diacetate/Cetrimide Mixtures

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ABSTRACT

Statement of the Problem: Hand excavation instruments are not as efficient as that with rotary burs in atraumatic restorative treatment (ART).

Purpose: To evaluate the antibacterial activity (ABA), microhardness numbers (VHN), and cumulative fluoride releasing (CFR) patterns of conventional GICs (Fuji IX (FX) and Ketac Molar (KM)) containing chlorhexidine diacetate/cetrimide mixtures (2.5%/2.5%) (AB).

Materials and Methods: The powders of ABs were added to powders of FX and KM selected as experimental groups (EXPs). FX and KM were assigned as controls (CNTs). ABA against S.mutans (MS) and L.casei (LB) were examined between 1–90 days. VHN were calculated after 24 hours and CFR patterns measured between 1–30 days. Analysis of variance was used for comparison ($p < 0.05$)

Results: Differences were found between EXPs regarding MS levels at days 1, 7 and 60 as well as for LB at all time periods ($p < 0.05$). VHN decreased in EXPs compared to CNTs ($p < 0.05$), and no differences were found between EXPs ($p > 0.05$). CFR patterns tended to decrease in EXPs compared to the CNTs, but no differences were found between EXPs ($p > 0.05$)

CLINICAL SIGNIFICANCE

These mixtures could be recommended for ART procedures to provide beneficial antibacterial effects without seriously deteriorating the physical properties of selected GICs.

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INTRODUCTION

Atraumatic Restorative Treatment (ART) is known as a minimally invasive procedure involving excavation of the carious dentine by using hand instruments and sealing the cavities with restorative materials such as glass-ionomer cement (GIC).[1–3] This technique is widely used in underdeveloped countries where electricity and technological dental equipment are lacking, or in developed ones to manage early childhood caries with reduced dental anxiety levels.[4] Because ART techniques are performed under such conditions, it is difficult to eliminate bacteria adequately, which is also valid for the rotary instruments.[5,6] Thus, under the GIC restorations, secondary caries leading to the restoration failure may progress over time. Moreover, when conventional hand-mixed and fluoride-containing GICs are used for sealing cavities, it is questionable whether a caries inhibition process would occur under the restorations.[7] To overcome this problem, several studies aiming to incorporate antibacterial agents to the GICs have been reported.[8–18] By this means, the incorporation of cationic disinfectants such as chlorhexidine (CHX) or cetrimide (CT) to GICs in various concentrations (1–5%) had exhibited favorable antibacterial effects on certain microorganisms under in vitro conditions.[11,14] Moreover, it was demonstrated that CHX-GIC combinations had shown increased susceptibility in reducing *Mutans Streptococci* (MS),[11,15–17] whereas CT-GIC combinations had exerted increased susceptibility in *Lactobacillius* (LB)[11] species. Therefore, it is important to acquire particular antibacterial property that should not jeopardize the main characteristics of GICs.

The critical point is that antibacterial-GIC combinations should have optimum surface properties to resist occlusal loads.[8,10,15,16] However, when antibacterial materials are incorporated to the GICs, many researchers reported alterations of restorative materials regarding the physical properties, and it is generally accepted in the literature that the physical properties of GICs are deteriorated with the addition of antibacterial agents.[10,15–20] Furthermore, it has been emphasized recently that, if any antibacterial agent is incorporated, the fluoride-releasing pattern of GIC is decreased.[17] Authors explained this with the interactions between cationic molecules and fluoride, which results with the precipitation of less soluble salts. Since the fluoride-releasing pattern is important for the inhibition of secondary caries in tooth structure,[21–23] it is expected from the antibacterial-GIC combinations to have similar fluoride-releasing properties compared to the GIC alone.[17]

This study aimed to evaluate the antibacterial activity, microhardness numbers, and cumulative fluoride-releasing patterns of two different conventional GICs containing chlorhexidine diacetate/cetrimide mixtures.

MATERIALS AND METHODS

Conventional glass-ionomer cements; Fuji IX (FX) (GC, Tokyo, Japan) and Ketac Molar (KM) (3M ESPE, Seefeld, Germany) were used as control groups. Chlorhexidine diacetate (2.5%) (Serva, Heidelberg, Germany)/2.5% Cetrimide (Serva) mixture was incorporated into the powder of conventional GICs and served as experimental groups: FX + antibacterial (AB), KM + antibacterial (AB).

Agar-Diffusion Test

The antibacterial effects against MS and LB of set specimens were assessed with agar-diffusion tests. The strains stored at –20°C were cultured on blood agar (Merck, Darmstadt, Germany) and *Lactobacilli* MRS agar plates (for *L. casei*, Difco Lab., MI, USA) at 37°C for 24 hours in 5% CO_2. Single colonies from plates were transferred into Brain Hearth Infusion (BHI) broth (Merck) and *Lactobacilli* MRS broth (for *L. casei*, Difco Lab.) and incubated at 37°C, for

24 hours. Suspensions of the strains were prepared in phosphate-buffered saline at *c.* 1.5×10^8 organisms/mL by using McFarland 0.5 turbidity tubes and then were flood-inoculated onto the surface of BHI agar plates. Before replacement of the set specimens, the surface of the plates was air dried by leaving them at 37°C for 15 minutes.

The set disc-shaped specimens (10 mm in diameter; 2 mm depth) were mixed. Three specimens were used for all groups. The paste was then put into a mold, covered with a glass slide, and allowed to set at room temperature. All the specimens were then sterilized with UV before the experiment. The set disc-shaped specimens were placed onto BHI agar plates. Inhibition zones around the specimens were calculated at three different points of the zones for each specimen and control. The specimens were then transferred onto the freshly prepared test agar plates and stored at 2–4°C. The procedures were repeated at 1, 7, 15, 30, 60, and 90 days.

Microhardness Test

A total of 20 glass ionomer samples 5 mm in diameter and 2 mm deep were prepared. The glass ionomer samples were prepared according to the manufacturers' directions, and a polyester strip was used to cover the cement for 7 minutes until the initial reaction was completed. Slight pressure was applied and the bulk of extruded excess cement was removed. After the completion of setting reaction, samples were placed into the plastic tubes containing distilled water and stored at 37°C for 24 hours. After 24 hours, Vickers microhardness (HMV-700, Shimadzu, Tokyo, Japan) numbers (VHN) measurements were carried out on the top of the surface of each specimen. Vickers diamond indentations were performed under a load of 300 g and 15 seconds. Three indentations were carried out and averaged for each specimen. The diagonal length impressions were measured, and the hardness number H was calculated according to the standard formula $H = 1.854\ P/d$.[2]

Cumulative Fluoride Measurements

Glass ionomer samples of 5 mm in diameter and 2 mm in depth were prepared for fluoride determination. Samples were stored in distilled water at 37°C for 24 hours. The potential measurements were carried out at room temperature with an ionmeter (ELIT 9808, London, UK). The ionmeter was connected to a personal computer with an Athlon AMD Processor. Fluoride concentrations were determined electrochemically with fluoride (ELIT 8221) ion-selective electrode and Ag/AgCl reference electrode (ELIT 001N). Added to the 5 mL test solution (distilled water) was 0.5 mL of Tisab solution (58 g of sodium chloride, 57 mL of glacial acetic acid, and approximately 150 mL of 6 M NaOH in a volume of 1,000 mL)[24] in order to maintain pH 5.0 and to eliminate the interference effect of complexing ions. A calibration curve was produced with the values of the known standards from which the values of the test samples were calculated. Each assay was performed in triplicate to check the reliability of the procedure. The cumulative fluoride concentrations were evaluated at 1, 7, 15, and 30 days by renewing batch procedure.

Statistical data analysis was performed with SPSS for 13.0 Windows (SPSS Inc., Chicago, IL, USA). Kruskall-Wallis and Mann-Whitney *U*-tests were used both for agar-diffusion test and cumulative fluoride-releasing measurements, and one-way analysis of variance–Turkey's post hoc tests for VHN at a significance level of $p < 0.05$.

RESULTS

Agar-Diffusion Test

No inhibition zones were detected in the control groups (FX and KM) during the study period. The antimicrobial activity of tested FX + AB and KM + AB combinations on *S. mutans* and *L. casei* are given in Tables 1 and 2. Significant differences were found between the experimental groups regarding

TABLE 1. INHIBITION ZONES (MM) OF *S. MUTANS* DURING THE STUDY PERIOD.

Groups	Inhibition zones of MS (mm)																	
	1 day			7 days			15 days			30 days			60 days			90 days		
FX + AB	13*	13*	13*	14*	14*	14*	13	13	13	13	13	13	13*	13*	13*	13	13	13
KM + AB	12*	12*	12*	13*	13*	13*	13	13	12	14	14	13	14*	14*	14*	14	14	13

*$p < 0.05$.

TABLE 2. INHIBITION ZONES (MM) OF *L. CASEI* DURING THE STUDY PERIOD.

Groups	Inhibition zones of LB (mm)																	
	1 day			7 days			15 days			30 days			60 days			90 days		
FX + AB	18*	18*	18*	14*	14*	14*	14*	14*	14*	14*	14*	13*	12*	12*	12*	12*	12*	12*
KM + AB	19*	19*	19*	19*	19*	19*	16*	16*	16*	16*	16*	15*	14*	14*	14*	14*	14*	14*

*$p < 0.05$.

TABLE 3. VICKERS MICROHARDNESS (VHN) VALUES OF THE GROUPS (MEAN ± SD).

Groups	VHN (mean ± SD)	VHN change (%) compared to their controls
FX	66.18† ± 6.92	—
KM	64.64† ± 3.92	—
FX + AB	55.18‡ ± 4.32	(–16%)
KM + AB	47.44‡ ± 0.89	(–26%)

*In the columns, values with different superscript letters are significantly different ($p < 0.05$).

MS levels at 1, 7, and 60 days ($p < 0.05$). KM + AB had shown better antibacterial effect compared to the FX + AB regarding LB levels at all time periods ($p < 0.05$). Both of these combinations (FX + AB and KM + AB) had exhibited continuous antibacterial effect up to 90 days.

Microhardness Test

VHN values were significantly decreased in experimental groups compared to controls in the following order FX > KM > FX + AB > KM + AB ($p < 0.05$). The % VHN changes were determined as (–16%) in FX + AB and (–26%) in KM + AB groups compared to their controls. However, significant differences were not found between FX-KM and FX + AB-KM + AB ($p > 0.05$). Additionally, FX + AB had exhibited higher VHN values compared to the KM + AB (Table 3).

Cumulative Fluoride Measurements

Figure 1 illustrates the cumulative fluoride-releasing amounts of the tested GIC samples. Up to 30 days, cumulative fluoride ion concentrations could be measured. Overall, the experimental groups (FX + AB and KM + AB) released less cumulative fluoride ion concentrations compared to the controls (FX and KM) at all time periods, but no significant differences were found ($p > 0.05$). The cumulative fluoride-releasing pattern was determined as FX > KM > KM + AB > FX + AB at 1 and 7 days, respectively, whereas at 15 and 30 days, it

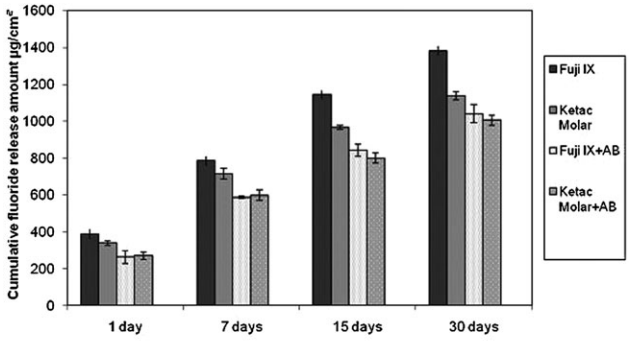

Figure 1. Cumulative fluoride-releasing amounts (µg/cm²).

was found as FX > KM > FX + AB > KM + AB.

DISCUSSION

Our study confirmed that two conventional GIC + antibacterial mixtures ([FX + AB] [KM + AB]) had exhibited continuous antibacterial effect up to 90 days against the MS and LB bacteria without seriously deteriorating surface hardness and fluoride-releasing patterns of selected cements.

In this study, the well-known agar-diffusion test was used to determine the antibacterial action of GIC + AB mixtures. Although this test could be used both for set and unset materials, some limitations reported because of its inability to provide any information about the viability of the test microorganisms.[11,14] However, recent studies in which the antibacterial-GIC combinations have been tested on different microorganisms have shown that this still seems to have potential advantages, such as less expensive properties and its realistic and rapid characterization for determining the antibacterial effects.[11,14–18]

Previous studies claimed that the anticariogenic properties of GICs could be related to their fluoride-releasing capacities.[25,26] Moreover, when GICs were placed freshly onto the dentin, they could exhibit antibacterial actions with their low pH features instead of fluoride-releasing properties.[27,28] Considering the set specimens, investigators reported that there was no antibacterial activity in the agar around the materials.[11,15–18,28] Similarly, in this study the set specimens were used and control groups did not produce any antimicrobial actions parallel to the findings of previous studies.

Overall, CHX has been widely used to enhance the antibacterial activity of GICs, since it may be easily mixed with the powder of GICs.[8–12,15–17,20] It was demonstrated in the literature that, when CHX was added to the GICs alone, setting specimens showed significant antibacterial effects on the MS levels in agar-diffusion tests. This is because MS was considered to be a relatively more sensitive species to the CHX than the other bacteria.[11,15,16] Investigators highly recommended the usage of the CHX diacetate form particularly between 1 to 5% final concentrations to obtain optimum antibacterial effects without jeopardizing the basic physical properties of the GICs.[9–11,15,16] Consistent with this, we also used the diacetate form of CHX in this study. Moreover, it was observed from the results of such studies that the antibacterial effectiveness of GIC-CHX combinations decreased to between 60 and 90 days, dependent upon the effect of additive concentrations on the selected microorganisms.[10,11,16,17] Except for the MS, LB was determined as the most resistant bacteria under the GIC restorations.[5,6,26] Besides CHX, CT resulted in better antibacterial effectiveness on the LB species in different studies. Thus, the incorporation of CT to the GICs would have beneficial effect on eliminating LB species.[11,14]

Dentin carious lesions possess wide microflora; it is clear that a

mixture of antimicrobial agents that can be effective against all the microorganisms is needed.[13,18] Pons and colleagues[29] investigated the combined effects of CHX, CT, and benzalkonium chloride (BC) cationic disinfectants against the *Staphylococcus aerus* and *Escherichia coli* bacteria and reported that these combinations had shown synergistic or additive properties. On the other hand, Botelho[30] indicated that no combination of antibacterial agents appeared to be superior to any other for cationic disinfectants (CHX, CT, BC, and cetylpyridinium chloride) against the MS, LB, and *Actinomyces* species. However, he also reported that it might be beneficial to use combined antibacterial agents that have a broader range of activity against an ecosystem of bacteria than using an individual agent. Recently, the idea of the combined usage of CHX + CT as root canal irrigants has been reported to exhibit a more powerful antibacterial activity in dental practice.[31] In an effort to gain specific and continuous antibacterial effectiveness on complex microorganisms under the GICs, incorporation of CHX + CT mixtures should be carefully evaluated for increasing the clinical success rate of ART procedures. Because of the previously mentioned reasons, we selected CHX + CT mixtures with certain conventional GICs at a total of 5% final concentrations.

When CHX diacetate was added to the FX at 1, 2, or 4% concentrations, decreasing antimicrobial trends were observed on MS and LB microorganisms during the study periods.[11] Regarding the inhibition zone results of the current study, FX + AB showed acceptable antimicrobial action compared to the previous investigations.[11,15] No data was available in the literature about the antimicrobial effect of KM + antibacterial combinations. Nevertheless, significant differences were observed between FX + AB and KM + AB at 1, 7, and 60 days against MS. Furthermore, against the LB species, KM + AB caused significantly different and superior antibacterial properties at all time periods. These results may be explained by the inherent potency of the selected GICs at these periods.[11] Moreover, both of these exhibited continuous antibacterial effect up to 90 days.

Generally, investigators determined the anticariogenic properties of GIC-antibacterial combinations between 1 and 90 days.[9–11,14–18] From the literature, no data were available about the antibacterial effectiveness beyond the 90-day period. Additionally, the antimicrobial effect of the GIC-antibacterial combinations depends on the retention time and the amount of antibacterials released from the cement.[13,17] Indeed, the GICs that were placed on the cavities should be assumed to retain as long as possible in ART procedures.[1,3,4] Thus, considering the previously mentioned studies,[9–11,14–18] the longer antibacterial properties should be taken into account for enhancing the retention capacities and the antibacterial success of GIC-antibacterial combinations in future ART procedures. Nevertheless, analyses of the antibacterial effects up to 90 days might be considered as an acceptable period.

In a pilot study, we also investigated the antibacterial effect of individual combinations with certain GICs and observed that when they were used alone, the antimicrobial action lasted up to between 60 and 90 days on both MS and LB species, distinctly. These findings highly extrapolated that the usage of CHX + CT mixtures with conventional FX and principally with KM showed a beneficial antibacterial effect by constituting synergistic interactions.

Although the GIC + antibacterial combinations have potential benefits over the microorganisms, the addition of these agents may have a role in the basic physical properties of GICs. Previous findings revealed that incorporating antimicrobials to the GICs has reduced the physico-mechanical performance of the GICs.[10,15–18,20] However, it is expected from the

GIC + antibacterial combinations to provide clear antibacterial effect without jeopardizing the physical properties.

In addition, the mixing ratio of the powder and the liquid affects the mechanical properties of GICs.[8,10,15,16,18] Investigators highly mentioned that obtaining slight modifications in powder/liquid ratios would aid the GIC + antibacterial combinations to gain comparable physical properties versus the additive-free ones.[10,15,16,18,20] Thus, the antibacterial additive concentration may have a crucial role on GICs' mechanical performance. Therefore, we used a total of 5% final AB concentrations to minimize and tolerate the adverse effects on the physical performance of GICs.

Since surface hardness is an important factor that correlates well with wear, abrasion resistance, and compressive strength tests, it can be used as an indication of likely long-term durability of materials.[32,33] Recently, microhardness testing has been suggested to be a valuable method to detect the surface alterations of GICs, as it provides more accurate data to assess the setting reaction characteristics of GICs having influence on their optimal long-term clinical performance.[34] Thus, this test was performed for antibacterial-GIC combinations to compare the alterations in surface hardness between the control (additive-free) and experimental groups.[10,16] In a previous study, Sanders and colleagues[10] found that the antibacterial-resin modified GIC combinations exhibited less Knoop microhardness (KHN) values at the concentration of 5% at 24 hours compared to the control (additive-free) group, but no significant differences were indicated. However, after 6 weeks from the initial setting reaction, they reported that both groups showed increased microhardness values but this increase was significantly clearer in the control group. In this study, KHN changes declined in the resin modified GIC-CHX groups at the level of –6.8% at 24 hours and –1% at 6 weeks after the initial setting reaction. Nevertheless, they concluded that, at neither 24-hour nor 6-week periods, the physical properties altered seriously. These findings indicated that the surface hardness could have been altered by the CHX particles during the setting reaction.[10,16]

In another study, Türkün and colleagues[16] tested different CHX formulations (digluconate and diacetate) at 0.5, 1, 1.25, and 2.5% concentrations with the resin-modified GIC and found only significantly decreased VHN values in 0.5% CHX digluconate (–37.2%) and 2.5% CHX digluconate (–58.9%) groups compared to the control after 24 hours from setting. However, in the 2.5% CHX diacetate group, the declines were found at the –2% level, and no significant differences were found compared to their control. This group exhibited acceptable VHN values during the study periods. But somehow, in our study, no significant differences were found between the control groups (FX-KM). We also found similar values compared to the previous studies for the typical VHN values of FX and KM conventional GICs.[33–35]As expected, FX + AB and KM + AB had exhibited significantly lower VHN values compared to controls after 24 hours of setting reaction. Moreover, the % VHN changes were determined as (–16%) in FX + AB and (–26%) in KM + AB groups compared to their controls. In this manner, FX + AB resulted at greater VHN values compared to the KM + AB, but these were not significantly different. These results may probably be related to the sensitivity of certain antibacterials to the conventional tested GICs. Considering the previously mentioned studies[10,16] and within the in vitro limitations of this study, our findings could be interpreted as acceptable, particularly in the case of the use of these antibacterials at this concentration. Although significantly reduced hardness values were found

comparable to the controls in GIC + AB combinations, they were able to be measured and did not seriously affect the VHN values particularly in the FX + AB group. All these reports and our findings may give an indication about the incorporation of these antibacterials to the GICs resulting at softened but measurable surfaces compared to their controls.

Nevertheless, superficial microhardness measurements cannot reliably detect the setting reaction occurring in the bulk of the material[35] and cannot always explain the real longevity of GICs because of certain factors such as saliva, pH changes, food, liquids, and masticatory functions in the oral environment.[36] Despite the fact that the decreases in microhardness may cause the abrasion of the material resulting in lower resistance to the occlusal forces,[37] using other mechanical tests in addition to the microhardness measurements may provide comprehensive results for the tested combinations in this area.[10,16] Moreover, obtaining therapeutic antibacterial properties from these materials might overcome the disadvantages of the altered mechanical features in terms of constituting appropriate combinations, and these beneficial effects should not to be overlooked as mentioned previously.[10,15,16,18]

Fluoride-releasing properties of GICs are important for improving resistance of tooth surfaces against the acid-producing bacteria.[17,22,23] Particularly in the initial phase, GICs release more fluoride that tends to be reduced during the ongoing setting reaction, which is the well-known fluoride-releasing behavior of the GICs.[23] In a previous study, researchers demonstrated the decreasing fluoride-releasing pattern in correlation with the time in GIC (additive-free) or GIC + CHX combinations. They also found lower fluoride levels in GIC-CHX combination groups compared to the additive-free controls but did not report any significant differences.[17]

It is important to take into consideration that different methodologies used in the studies, including specimen size, media used to measure fluoride release and uptake, and quantity of media used to measure fluoride release, are responsible for the numerous differences. Thus, comparisons between the materials should be made considering the behavior of materials rather than the absolute amount of released fluoride (in absolute terms).[38] In this study, cumulative fluoride-releasing amounts were tested by using batch systems with distilled water in vitro. However, in this study and many others, examining the release of fluoride ions from fluoride-containing materials results in limitations in evaluating the dynamics of fluoride ions emanating from these materials.[39] Therefore, to mimic the real oral environment conditions, pH-cycling or in situ systems may be studied for determining the accurate fluoride-releasing patterns of GIC-antibacterial combinations.

Moreover, it can be concluded that both FX and KM released higher fluoride amounts in each period, but these were not significantly different compared to the FX + AB and KM + AB. This may be attributed to the interaction between CHX/CT molecules and fluoride resulting in precipitation salts that caused lower amounts.[17] Furthermore, in future studies, the combination of antibacterials and the GICs should be carefully evaluated regarding their fluoride-releasing alterations to gain better anticariogenic effects.

CONCLUSIONS

In view of the results of the present study, the addition of CHX diacetate/CT at 5% concentration to the conventional FX and KM GICs may constitute an antibacterial effect against the MS and LB bacteria up to 90 days without seriously deteriorating their surface hardness and fluoride-releasing properties. These combinations could be an alternative for the

ART procedures to provide beneficial antibacterial effects.

DISCLOSURE

The authors do not have any financial interest in the companies whose materials are included in this article.

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