Long-Term Antibacterial Effects and Physical Properties of a Chlorhexidine-Containing Glass Ionomer Cement

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ABSTRACT
Statement of the Problem: Many regions in the world do not have electricity, water, or access to dental facilities that allows the treatment of caries with dental handpieces and rotary burs. For restorative techniques used in these regions, an antibacterial self-adherent glass ionomer material would contribute considerably.

Purpose: This study aimed to test if chlorhexidine diacetate (Fluka BioChemika, Buchs, Switzerland)- or chlorhexidine digluconate (Sigma-Aldrich, Steinheim, Germany)-added ChemFil Superior glass ionomer cement (Dentsply DeTrey, Konstanz, Germany) had any long-term antibacterial effect against certain oral bacteria and to test the new formulation’s physical properties.

Materials and Methods: ChemFil Superior was used as a control. Chlorhexidine diacetate (powder) was added to the powder and chlorhexidine digluconate (liquid) was mixed with the powder in order to obtain 0.5, 1.25, and 2.5% concentrations of the respective groups. Setting time, compressive strength, and acid erosion were tested according to ISO 9917-1. Working time, hardness, diametral tensile strength, and biaxial flexural strength were also determined. Long-term antimicrobial activity against S. mutans, L. acidophilus, and C. albicans were tested with the agar diffusion method. Analysis of variance (ANOVA) was used for comparison (p < 0.05).

Results: Regarding the immediate antibacterial effect for S. mutans, all the tested groups showed inhibitions of the strain compared with the control group (p < 0.05), with larger zones for the higher concentration groups and all the diacetates. For L. acidophilus, all the groups were effective compared with the control, but the greatest antibacterial effect was observed with the 2.5% diacetate group. The 2.5% group of chlorhexidine diacetate showed antibacterial activity up to 90 days against S. mutans and up to 60 days against L. acidophilus. The working and setting time, acid erosion test, diametral tensile strength, and biaxial flexural strength of the tested...
INTRODUCTION

Caries disease still remains a major public health problem despite the widespread use of fluoride and the decline in caries prevalence observed in the majority of highly industrialized countries.1 Caries epidemiology will still remain an indispensable part of dental public health because children of low socioeconomic status generally have higher disease levels, leading to an increase in caries prevalence as well. Furthermore, many regions in the world do not have electricity, water, or access to dental facilities that allows the treatment of cavities with dental handpieces and rotary burs. For field treatment in these regions, a new technique based on manual instrumentation, atraumatic restorative treatment (ART), was developed.2

The ART is a minimal-intervention approach where demineralized tooth tissues are removed using hand instruments and the cavity, including adjacent pits and fissures, are restored with adhesive restorative materials.3 Today, ART does not seem to be confined to places where electricity is absent. It is also accepted by patients with dental anxiety and by children in modern clinical settings, as the sound and pressure caused by rotary instruments is omitted and local anesthesia is not needed.4 At the beginning, conventional hand-mixed glass ionomer cements (GICs) were used with ART; later on, condensed glass ionomers with improved physical strength were produced especially for ART.2–4 Early studies applied in the field revealed the success of ART.4

However, dental hand instruments alone do not remove carious dentin as effectively as rotary burs, and cariogenic bacteria can survive incarceration under GIC restorations for up to 2 years.6–7 Consequently, cavities treated by ART may have residual infected dentin, and if a GIC is unable to arrest the carious process, the restoration could fail.8 For that purpose, the improvement of filling materials to overcome the problems caused by incomplete removal of infected dentin will be beneficial for increasing the success rate of ART further.

The chemistry of the setting reaction of all versions of glass ionomers is essentially an acid/base reaction, and the two major advantages of the material are an ion exchange adhesion and a continuing fluoride release. Recently, several hand-mixed conventional GICs have been manufactured specifically for the ART approach.9 Reports have shown that the newer, more viscous GICs release substantially less cumulative fluoride ions than less-viscous esthetic restorative GICs and resin-modified GICs.10–13 The effect of the lower fluoride ions release on the ability of the viscous GICs to inhibit dental caries in adjacent tooth tissues is not known. Moreover, the use of GIC as a

groups were not different from the control ChemFil group. However, the 1.25 and 2.5% groups of chlorhexidine diacetate had significantly lower compressive strengths than the control group. Lower hardness values were obtained with the 0.5 and 2.5% chlorhexidine digluconate groups in comparison with the control group.

CLINICAL SIGNIFICANCE

The results of this in vitro investigation demonstrated that chlorhexidine diacetate or digluconate added to the ChemFil Superior glass ionomer material can exhibit long-term antibacterial effects against *S. mutans* and *L. acidophilus* without compromising the physical properties of the material.

restorative material for the sealing-in of caries is also questioned because of the possible microleakage and limitations associated with their physical properties.\(^8\) Therapeutic benefit may therefore be gained by combining antibacterial agents with glass ionomer materials.

Recently, researchers modified filling materials such as composite resins, acrylic resins, and GICs by adding chlorhexidine (CHX) and quaternary ammonium compounds.\(^14\) Moreover, antiseptic agents have the potential to be used in combination with GICs to obtain an antibacterial restorative material. From the dental literature, it appears that CHX has been incorporated frequently into GIC materials, and all of the studies have shown an increased antibacterial effect in vitro.\(^15^{-}17\) However, the incorporation of antibacterial agents in restorative materials frequently results in changes in the physical properties,\(^14,15,18,19\) and it is critical that the type of restorative material show strong enough physical properties to resist occlusal load. Therefore, antibacterial GICs for use in the ART approach require an optimum amount of antibacterial agents, which should not jeopardize the basic properties of the parent materials.\(^15^-22\) It was shown that the incorporation of CHX dihydrochloride and CHX diacetate into GICs can increase the antimicrobial effect without seriously compromising the physical properties of the original material.\(^15,16\)

This study aimed to: (1) test if CHX diacetate- or CHX digluconate-added ChemFil Superior GIC had any long-term antibacterial effect, (2) test if these new formulations had similar physical properties compared with the parent material, and (3) determine the optimal concentration of CHX incorporation for obtaining antibacterial GICs for use with the ART approach.

**Materials and Methods**

CHX diacetate monohydrate (Lot 37/4204/1 24999, Fluka BioChemika, Buchs, Switzerland), which is commercially available as a solid substance, was added to a conventional glass ionomer powder, ChemFil Superior LY (Lot 0507001008, Dentsply DeTrey, Konstanz, Germany) in order to obtain three groups of 0.5, 1.25, and 2.5% concentrations (= content) of CHX in the GIC formulation. The same procedure was used with the CHX digluconate solution (Lot 084K0536, Sigma-Aldrich, Steinheim, Germany), which is available as an aqueous solution. The original ratio of powder/liquid for ChemFil Superior was 7.4 g: 1 g and was used as a reference. For the CHX diacetate group, 29.829 g of ChemFil powder was mixed with 0.171 g of CHX diacetate to obtain a 0.5% formulation. For the 2.5% diacetate formulation, 29.148 g of ChemFil powder was mixed with 0.852 g of CHX diacetate, and the half dose was used in order to obtain the 1.25% group. Three different GIC liquids, containing different amounts of CHX digluconate, were prepared. The ChemFil powder was then mixed with the undiluted CHX digluconate solution to obtain the 2.5% digluconate formulation. This solution was diluted 50% with distilled water to obtain the 1.25% formulation. For the last group, 4.190 g of CHX digluconate was added to 95.810 g of water to obtain the 0.5% CHX digluconate formulation. The groups tested are presented in Table 1.

**Agar-Diffusion Test**

The antibacterial activity was evaluated against *S. mutans* CCUG 6519 (Culture Collection, University of Göteborg, Sweden), *L. acidophilus* LA-CH-SDVS (CHR.HANS, Copenhagen, Denmark), and *C. albicans* ATCC 10231 (American Type Culture Collection, Rockville, MD, USA) using the agar-diffusion test. These microorganisms were chosen because *S. mutans* is the main bacteria responsible for caries formation, *L. acidophilus* is the principle bacteria related to caries progression, and *C. albicans* was recently isolated in 58 to 70% of the caries
in children\textsuperscript{23,24} and in root carious lesions in middle-aged and older adults.\textsuperscript{25}

All procedures were carried out under aseptic conditions in a laminar airflow cabinet. Seventy specimens were prepared for the six antibacterial material-added groups and the control (\(N = 10\)) for the initial antibacterial effect, and 70 more for testing the 24-hour antibacterial effect. For the long-lasting procedure, only five specimens were used for the tested groups.

All bacteria and yeast were cultivated overnight in specific culture mediums: tryptic soy broth for \textit{S. mutans}, Lactobacilli MRS broth for \textit{L. acidophilus}, and Sabouraud dextrose broth for \textit{C. albicans} (800.675.0908, Difco Laboratories, Detroit, MI, USA) at 37°C. The incubation for \textit{S. mutans} and \textit{L. acidophilus} had a 5% CO\(_2\) addition, whereas the one used for \textit{C. albicans} had no additions. The broth culture was diluted and grown to a density of 10\(^7\) colony forming units (cfu)/mL, confirmed by viable cell count.

Twenty-five milliliters of the respective culture mediums with agar (tryptic soy agar for \textit{S. mutans}, Lactobacilli MRS agar for \textit{L. acidophilus}, and Sabouraud dextrose agar for \textit{C. albicans}) were evenly distributed over the surface of 15-cm-diameter petri dishes to a thickness of 5 mm. For each petri dish, seven standardized wells with a diameter of 4 mm were punched into the agar with the blunt end of a sterile Pasteur pipette. Bacterial inoculation was made by a bent glass rod over the agar surfaces with 0.5 mL of the microorganism’s suspension (10\(^7\) cfu/mL).

The powder-liquid materials were mixed for 30 seconds with sterile metal spatulas to the given ratios and inserted in the wells within 1 minute with sterile dental instruments. For monitoring the immediate antibacterial effect of the tested groups (day 0), the plates were incubated (Sanyo CO\(_2\) incubator, MCO-17AIC, SANYO Electric Biomedical Co., Ltd., Osaka, Japan) at 37 ± 1°C for 48 hours to let the microorganisms grow, and then the diameters of the circular inhibition zones produced around the specimens (specimens + inhibition zones) were measured in millimeters with a digital caliper (Mitutoyo 0.02 mm 505-646-50 shockproof, Mitutoyo Corp., Tokyo, Japan) at three different points, and the mean was recorded as the “0”-day value. These specimens were then left in the same plates for five more days in the incubator (total of 7 days after insertion in the wells) and transferred to freshly inoculated plates and left there for 48 hours more to obtain the inhibition zones for day 7. The same initial procedure was repeated, with the specimens being left only for 24 hours in the incubator to obtain day 1 values. The specimens were then transferred to freshly inoculated plates and left for 48 hours for the growth of the microorganisms before the zone measurements of day 1.

The long-term antibacterial effect was carried out at 7, 14, 21, 30, 40, 50, 60, 70, 80, and 90 days on only the six tested groups because no antibacterial effect was observed in the control group at day 0. After the initial inhibition zone measurements (day 0 meaning immediate

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CF = ChemFil Superior; CHX = chlorhexidine.
effect), all the samples were incubated at 37 ± 1°C in their initial petri dishes until day 7. On that day, the respective culture mediums with fresh agar for the microorganisms were placed in new petri dishes, six standardized wells were punched into the agar, and new bacterial inoculation was made over the agar surfaces with 0.5 mL of the bacterial suspension (10⁷ cfu/mL). The glass ionomer specimens were taken out of their previous petri dishes and placed in the new wells. The plates were then incubated with active microorganisms at 37 ± 1°C for 24 hours, and the diameters of the inhibition zones around the specimens were measured in millimeters with a digital caliper the day after. This procedure was repeated with fresh agar plates inoculated with fresh microorganisms on all the control days.

For all seven groups, the setting time, compressive strength, and acid erosion were tested according to ISO 9917-1 standards.26 All specimens were mixed at a temperature of 23 ± 1°C and a relative humidity of 50 ± 10%. A fresh mix was prepared for each specimen.

Net Setting Time
The net setting time is the period of time measured from the end of mixing until the material has set. The test was undertaken in a climatic cabinet capable of being maintained at a temperature of 37 ± 1°C and a relative humidity of 95 ± 5%. A fresh mix was prepared for each specimen.

Compressive Strength
Five specimens per group were prepared using a split stainless-steel mold with inner dimensions of 6 ± 0.1-mm height and 4 ± 0.1-mm diameter. The molds were treated with a 5% solution of beeswax in volatile petroleum ether. The petroleum ether was given a few seconds to evaporate, and the residual beeswax gave a very thin layer to coat the surface of the mold. Within 60 seconds after the end of mixing, the GICs were packed into the split molds and covered with the plates. One hour later, the specimens were ground with wet 500-grit silicon carbide paper and stored at 37 ± 1°C in water for 24 hours. Prior to testing, the diameter of each specimen was determined using a micrometer gauge, and the specimens were placed with the flat ends up between the plates of the universal testing machine (Zwick machine, Z010, Zwick GmbH & Co., Ulm, Germany). A compressive load along the long axis was applied at a crosshead speed of 1 mm/min. The maximum force applied when the specimens fractured was recorded, and the compressive strength was calculated in N/mm² (MPa) according to the equation $CS = \frac{4F}{\pi d^2}$, where $F$ is the failure load and $d$ the diameter of the specimen.

Acid Erosion
Five specimens per group were prepared in conditioned 5 mm × 30-mm-diameter polymethyl methacrylate (PMMA) disks with a hole (5 ± 0.5 mm in diameter × 2 ± 0.5-mm deep) in the center. The PMMA disks were filled with the mixed cements and covered with separating sheets, pressed firmly together, and clamped. At 180 seconds after the end of mixing, the whole assembly was transferred to the cabinet maintained at 37 ± 1°C and a relative humidity of 95 ± 5%. After 24 hours, the clamps and the separating sheets were removed,
and the specimens were flattened wet with 1,200-grit abrasive paper. For each specimen, the initial depth at the center of the specimen was measured at five points using the edge of the specimen holder as a fixed reference plane. The height at the center of the specimen was subtracted from the average height of the specimen holder to obtain the Do value. The specimens were immersed horizontally into individual bottles containing 30 mL of the eroding solution (0.1 mol/L lactic acid/sodium lactate buffer solution adjusted to pH 2.74 ± 0.02) for 24 hours at 37°C. After the immersion period, the specimens were removed and rinsed with water. For each specimen, the depth at the center was measured again to obtain the depth of the GICs after erosion (Dt). The eroded depth, D (in mm), at the center of each specimen was calculated using the following equation:

\[ D = Dt - Do \]

Hardness27
A stainless-steel mold with inner dimensions of 6 ± 0.1-mm height and 3 ± 0.1-mm diameter was used for preparing the six samples per group. Within 60 seconds after the end of mixing, the GICs were packed into the conditioned molds slightly in excess and covered with strips. One hour after the end of mixing, the specimens were removed from their molds and stored at 37 ± 1°C in water prior to testing. After the storage time of 24 hours and 10 days, the Vickers hardness HV3 was measured by applying a load of 29.42 N on the samples for 30 seconds. Five indentation measurements were carried out and averaged for each specimen.

Diametral Tensile Strength28
Six cylindrical specimens (3 mm × 6 mm) per group were prepared using split metal molds. Within 60 seconds after the end of mixing, the GICs were packed into the conditioned split molds slightly in excess and covered with strips. All the assembly was then transferred into the climatic cabinet at 37 ± 1°C and 95 ± 5% relative humidity for 1 hour. After that period, the specimens were ground with wet 500-grit silicon carbide paper to flatten the surfaces, removed from their molds, and stored at 37 ± 1°C in water for 24 hours. Prior to testing, the diameter and thickness of each specimen was determined using a micrometer gauge. The specimens were placed on the universal testing machine (Zwick machine, Z010). The specimens were then loaded in compression to fail at a crosshead speed of 1.5 mm/min. The maximum force applied when the specimens fractured was recorded, and the diametral tensile strength was calculated in N/mm² (MPa) according to the equation

\[ DTS = \frac{2F}{\pi dt} \]

where \( F \) is the failure load, \( d \) the diameter, and \( t \) the thickness of the specimen.

Biaxial Flexural Strength29
Six shell-like specimens per group (20 mm × 1 mm) were prepared in Teflon molds. Within 60 seconds after the end of mixing, the GICs were packed into the ring molds slightly in excess, covered with strips, and clamped. All the assembly was then transferred into the climatic cabinet at 37 ± 1°C and 95 ± 5% relative humidity. After 1 hour, the specimens were ground with wet 500-grit silicon carbide paper to flatten the surfaces, removed from their molds, and stored at 37 ± 1°C in water for 24 hours. Prior to testing, the thickness was determined for each specimen, which was then placed horizontally on the universal testing machine (Zwick machine, Z010). The specimens were then loaded with 2 N/min at a crosshead speed of 1.5 mm/min. The maximum force applied when the specimens fractured was recorded, and the biaxial flexural strength was calculated in N/mm² (MPa) according to the equation below:

\[
\text{BiaxFS} = \frac{3F}{2\pi^2} \left[ (1 + \nu) \ln \left( \frac{d_s}{d} \right) + (1 - \nu) \left( \frac{d_s^2 + d_f^2}{2d^2} \right) \right]
\]
where $F$ is the failure load, $d_s$ the support ring diameter, $t$ the specimen’s thickness, $d_l$ the loading ring diameter, $d$ the specimen’s diameter, and $v$ the Poisson’s ratio.

**Working Time**

The working time, which is understood as the time at which it is possible to manipulate a dental material without an adverse effect on its properties, was measured by determining the viscosity over the time using a cycloviscosimeter (CycloViscos-E, Brabender, Duisburg, Germany). The material was mixed for 30 seconds and then transferred on the probe head, which was adjusted to 23°C. Exactly 60 seconds from the beginning of mixing, the measurement was started and the viscosity was recorded. Five mixtures per group were tested.

Statistical analysis was carried out by one-way analysis of variance (ANOVA) and Dunnet-C test for the immediate inhibition zones of the agar-diffusion test (day 0). One-way ANOVA, Dunnet-C, and Tukey posthoc test were carried out for all the physical properties except hardness. The two-way ANOVA with Tukey or Dunnet-C test was used for hardness, and $t$-test was used for comparing the different periods ($p < 0.05$).

**RESULTS**

**Agar-Diffusion Test**

For this test, only the immediate inhibition zones (day 0) against the strains of the different groups were compared statistically with each other. The zones were then checked for long-term antibacterial activity at 1, 7, 14, 21, 30, 40, 50, 60, 70, 80, and 90 days (Tables 2 and 3).

“Day 0” results showed no antibacterial effect of the conventional ChemFil Superior against all the tested strains. For *S. mutans*, the difference between all the groups and the control was significant ($p < 0.05$). The greatest inhibition

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<td>2.5% digluconate</td>
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“Day 0” means the immediate antibacterial effect, whereas “day 1” means the 24-hour effects.
zones were observed in all the diacetate groups and the 2.5% digluconate-added ChemFil Superior (Figure 1A). For \textit{L. acidophilus}, the difference between the groups was also significant while compared with the control ($p < 0.05$). The 2.5% diacetate-added ChemFil Superior group had the greatest inhibition zone against \textit{L. acidophilus}, followed by the 2.5% digluconate- and 1.25% diacetate-added groups (Figure 1B). None of the tested groups had an effect on \textit{C. albicans}.

As the control group of ChemFil Superior had no effect on the tested microorganisms after 24 hours, it was decided not to include this group in the long-term follow-up of the antibacterial effect. Figures 2 and 3 show the antibacterial effects of the effective groups after 1 week.

For \textit{S. mutans}, the inhibition zones' diameters diminished after “day 1” and stayed effective until the 70th day for the 0.5% groups and the 1.25% digluconate group. This effectiveness lasted until the 80th day for the 1.25% diacetate and the 2.5% digluconate groups, and up to the 90th day for the 2.5% diacetate groups (Table 2). For \textit{L. acidophilus}, the inhibition zones' diameters were similar during the first week for all the diacetate groups tested. The antibacterial activity of the 0.5% and 1.25% digluconate groups was effective until the 40th day, whereas the 2.5% digluconate group had some inhibition effects until day 50. All the samples of the 2.5% diacetate-added ChemFil Superior group and one of the 1.25% CHX diacetate showed effective antibacterial activity until the 60th day (Table 3). It was observed that the more CHX concentration added to the GICs, the more the long-lasting antibacterial effect against \textit{L. acidophilus}.

**Hardness**

The Vickers hardness was checked after 24 hours and after a longer

<table>
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<th>TABLE 3. LONG-TERM INHIBITION ZONES (IN MILLIMETERS) OF THE TESTED GROUPS AGAINST \textit{L. ACIDOPHILUS} ($N=5$).</th>
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| \textit{L. acidophilus} | \begin{tabular}{l} 0 \ 1 \ 7 \ 14 \ 21 \ 30 \ 40 \ 50 \ 60 \\
0.5% diacetate \\
24 & 20 & 20 & 14 & 13 & 10 & 10 & 8 & 8 & 0 \\
23 & 22 & 18 & 13 & 12 & 10 & 8 & 8 & 0 & 0 \\
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| 1.25% diacetate \\
26 & 24 & 20 & 16 & 14 & 10 & 10 & 8 & 8 & 0 \\
26 & 24 & 22 & 14 & 13 & 11 & 10 & 9 & 8 & 0 \\
25 & 23 & 20 & 14 & 12 & 11 & 8 & 0 & 0 & 0 \\
25 & 23 & 20 & 14 & 13 & 11 & 10 & 8 & 0 & 0 \\
26 & 24 & 22 & 16 & 14 & 12 & 11 & 9 & 0 & 0 |
| 2.5% diacetate \\
27 & 24 & 22 & 16 & 16 & 14 & 12 & 11 & 8 & 0 \\
28 & 26 & 23 & 18 & 15 & 13 & 12 & 10 & 8 & 0 \\
27 & 24 & 22 & 16 & 15 & 13 & 11 & 10 & 9 & 0 \\
27 & 25 & 22 & 16 & 15 & 13 & 11 & 10 & 9 & 0 \\
28 & 26 & 23 & 17 & 16 & 14 & 12 & 11 & 8 & 0 |
| 0.5% digluconate \\
22 & 16 & 12 & 9 & 8 & 8 & 8 & 0 & 0 & 0 \\
20 & 15 & 13 & 10 & 9 & 8 & 8 & 0 & 0 & 0 \\
21 & 15 & 13 & 10 & 9 & 8 & 0 & 0 & 0 & 0 \\
22 & 16 & 14 & 11 & 10 & 9 & 8 & 0 & 0 & 0 \\
20 & 15 & 12 & 9 & 9 & 8 & 0 & 0 & 0 & 0 |
| 1.25% digluconate \\
22 & 17 & 14 & 13 & 9 & 8 & 8 & 0 & 0 & 0 \\
22 & 18 & 15 & 12 & 10 & 9 & 8 & 0 & 0 & 0 \\
23 & 18 & 14 & 12 & 10 & 9 & 8 & 0 & 0 & 0 \\
23 & 18 & 15 & 12 & 10 & 9 & 8 & 0 & 0 & 0 \\
22 & 17 & 14 & 13 & 11 & 10 & 9 & 0 & 0 & 0 |
| 2.5% digluconate \\
26 & 20 & 16 & 12 & 11 & 10 & 9 & 8 & 0 & 0 \\
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25 & 21 & 16 & 11 & 10 & 11 & 10 & 0 & 0 & 0 \\
25 & 22 & 18 & 14 & 12 & 10 & 8 & 0 & 0 & 0 \\
26 & 23 & 19 & 15 & 13 & 11 & 9 & 8 & 0 & 0 |

“Day 0” means the immediate antibacterial effect, whereas “day 1” means the 24-hour effects.
storing time (10 days) in order to investigate if there was a leaching process of the CHX, which takes place mainly at the surface of the specimens. If CHX was leached out, this should have a negative influence on the hardness and would weaken the surface of the specimens over time. Moreover, if a substance is leached out of a dental material (here CHX), it will always lead to the weakening of the structure, especially at the surface as there is a very high mobility of the substance.

The differences between the 0.5 and 2.5% digluconate groups and the others were significant \((p < 0.05)\). At the 24-hour test, the 0.5 and 2.5% digluconate-added ChemFil Superior groups demonstrated significantly lower hardness than the control GIC \((p < 0.05)\). However, at the 10-day test, all of the tested groups, except the 2.5% digluconate, demonstrated hardness comparable to the original ChemFil Superior group. While comparing the first hardness value with that at the 10 days, it can be seen clearly that the hardness increased significantly with time for all the digluconate groups except the 1.25% group (Figure 4). Furthermore, because we did not see a difference between the test groups and the control in the hardness after 10 days, we could state that, if any leaching of CHX took place on the surfaces of the CHX-added groups,
it was negligible and did not influence the surface hardness of the specimens.

**Compressive Strength**
In comparison with the control GIC, 1.25% and 2.5% diacetate-added ChemFil Superior groups had significantly lower values ($p < 0.05$), whereas the other groups were not different (Figure 5).

**Diametral Tensile Strength**
Both 2.5% diacetate- and digluconate-added groups tended to have lower tensile values than the control GIC, but this difference was not statistically different (Figure 6).

**Biaxial Flexural Strength**
All of the tested groups had biaxial flexural strength comparable to the original ChemFil Superior glass ionomer group (Figure 7).

**Working Time**
The working time of all the CHX digluconate groups and the 2.5% CHX diacetate added ChemFil group was longer than the control material ($p < 0.05$) (Figure 8).

**Setting Time**
The setting time of all the experimental GICs was not different from the original ChemFil Superior material (Figure 9).

**Acid Erosion Test**
The conventional ChemFil Superior material and all the experimental groups had the same acid erosion value of 0.17 μ.

**Discussion**
The ability of dental materials to inhibit recurrent caries formation is
suggested that GICs may be antimicrobial because of fluoride release and/or acidity. It has been well established that fluoride is released from GICs and the material has a low pH while setting, but the results of previous investigations about the antibacterial effects of both fluoride and low pH are controversial. Furthermore, the reduction in bacterial counts obtained by placing conventional GICs in cavities is not reliable; therefore, antibacterial GICs would provide an alternative approach.

The concept of controlled-release therapeutic systems to deliver a predetermined amount of a drug for a specific period of time is not new, and the combination of antibacterial agents with restorative materials and, specifically, CHX has been investigated previously. In a recent study, Takahashi and colleagues showed with an HPLC test that there was very little CHX release from their experimental GIC formulations and concluded that a 1% CHX diacetate addition was optimal to give appropriate physical and antibacterial properties to Fuji IX (GC Corporation, Tokyo, Japan). Regarding the previous results, we selected CHX, which is antibacterial against caries-associated bacteria, as an antimicrobial, in the form of a powder and a liquid, to be incorporated into the conventional GIC, ChemFil Superior.

Figure 5. Mean and SD of the compressive strength of the different groups. The different letters indicate statistically different groups.

Figure 6. Mean and SD of the diametral tensile strength of the different groups. There were no differences between the groups.
Agar plate diffusion was the method of choice for this study because it allowed both set and unset materials to be assayed.\textsuperscript{38} Moreover, the process is relatively inexpensive and can be performed rapidly and easily with a large number of specimens. However, there are also some limitations with this test method.\textsuperscript{39} One of the main concerns is the inability of the method to distinguish between bacteriostatic and bactericidal effects, so the test does not provide any information about the viability of the test microorganisms within the inhibition zone. Moreover, the test does not simulate the clinical condition where multiple species of bacteria will be growing in complex biofilms.

It was observed that the materials had significantly more antibacterial effect while setting than when tested completely set.\textsuperscript{39,40} This could be partially explained by the effect that most dental materials seem to be bactericidal while setting, and their low pH during this period may also have an effect. Regarding these findings, we chose to use unset materials to be tested with the agar diffusion method for the initial antibacterial effects.

Previous studies using conventional GICs demonstrated conflicting results about the antibacterial effects observed by the addition of CHX; some reported that...
antimicrobial activity was dependent upon the concentration of the disinfectant added to GICs, and others indicated no dose-response effects. In our agar-diffusion tests, we found for all the tested groups that the sizes of inhibition zones produced against *S. mutans* and *L. acidophilus* were clearly dependent upon the concentration of the CHX incorporated to the GIC. Although monitored for a longer period, we could clearly see that the 2.5% CHX-added materials had longer effects compared with their low-concentration groups on both strains tested. Moreover, one might have had a bactericidal effect and the other a bacteriostatic effect, which cannot be determined with the method that we presently used.

This study demonstrated that the addition of CHX diacetate and CHX digluconate to ChemFil Superior resulted in a restorative material that had increased antibacterial properties over the conventional glass ionomer alone for *S. mutans* and *L. acidophilus*, but not for *C. albicans*. Effective antibacterial effects were noted over 2 months of the experiment and a decrease with time corresponding to a decrease in available CHX. The decrease in CHX may be a result of the loss of material by elution, or perhaps, as has been suggested by Ribeiro and Ericson, the decrease in CHX is related to the formation of insoluble salts with the GIC. However, the level of CHX in the microenvironment of the restoration may be sufficient to prevent secondary caries for extended periods of time. It has been suggested that as the concentration of CHX is decreased, less-sensitive microorganisms recolonize the tooth and prevent *S. mutans* from reestablishing itself on the tooth surface.

The ability of restorative dental materials to withstand functional forces is an important requirement for their long-term clinical performance. To be accepted clinically, modified materials must provide superior antimicrobial activity and display comparable physical properties such as tensile and shear bond strength when compared with conventional materials.

CHX digluconate, when added to glass ionomer, does inhibit the growth of *S. mutans*, but it may also result in a decrease in the mechanical properties of the parent material with high concentrations, as it does not contribute to the formation of the GIC structure. In addition, the mixing ratio of the powder and the liquid affects the mechanical properties of GICs; therefore, slight modifications in powder/liquid ratios by adding CHX diacetate or CHX digluconate to the powder or the liquid may

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**Figure 9.** Mean and SD of the setting time of the different groups. There were no differences between the groups.
have also contributed to the influences on mechanical strength and setting times.

The most commonly used strength value to characterize dental cements is compressive strength. However, such materials typically fail in flexure rather than in compression, and in recognition of this, there has been some work in recent years to characterize them in terms of biaxial flexure strength. This test was originally developed for very brittle materials such as ceramics, but dental cements, including GICs, have been considered sufficiently brittle for this test to be applied to them as well. Because of its usefulness as an indicator of flexural strength, and also as a straightforward comparison between similar materials, we have used it in the present study.

In our study, for both types of CHX-added materials, the diametral tensile strength, biaxial flexural strength, acid erosion test, and setting time results were similar to the original glass ionomer material. Although the hardness values for the 0.5 and 2.5% digluconate-added ChemFil Superior groups were lower than the control at 24 hours, at the 10-day values, the difference between the materials was not significant, except for the 2.5% digluconate group. Regarding compressive strength, high concentrations of diacetate additions resulted in lower values.

According to Sanders and colleagues, the decrease in the physical properties of the digluconate form of the CHX is related to the fact that it is a liquid and leaches out more rapidly than the powder or diacetate form of CHX. In our study, besides the 24-hour hardness test, we did not observe any decrease in the physical properties while using the low concentration of the CHX digluconate additive, whereas the decrease was significant for the 2.5% group at both time periods. Another similar study performed with CHX-added Fuji IX demonstrated that the incorporation of 1% CHX diacetate was optimal to provide antibacterial activities while not affecting mechanical properties, bonding abilities, or setting time.

Regarding our results on antibacterial properties, we found that both CHX derivates were effective in preventing the bacteria from growing. However, the CHX diacetate might be preferable to use for further development, as it is a more stable material, not prone to decomposition, and can be easily blended with glass ionomer powder. The CHX digluconate cannot be isolated in substance and can only be stable in diluted solutions. Furthermore, the stability of CHX solutions is adversely affected by exposure to higher temperatures or light, which may happen during storage of glass ionomer liquid.

On the other hand, the amount of CHX should be kept as low as possible, as the CHX does not contribute to the formation of the glass ionomer network, and therefore, high amounts of CHX would weaken the scaffold and compromise the physical properties of the antibacterial glass ionomer. According to all these facts and the results of the physical and microbiological tests, it would be more appropriate to use 1.25% diacetate additions for further development in antibacterial GICs. Furthermore, both CHX additives are classified as harmful and rather toxic [LD50 (mouse, oral) 2,515 mg/kg], and it is preferable to keep the amount of CHX as low as possible. Further studies to examine the benefits of the CHX-added antibacterial ChemFil in clinical situations should be performed.

CONCLUSIONS

1. The incorporation of CHX digluconate into ChemFil Superior glass ionomer liquid or CHX diacetate into the material’s powder has the ability to provide a long-term antimicrobial effect on S. mutans and L. acidophilus.

2. The new material’s immediate compressive strength for the 1.25 and 2.5% groups was lower, whereas the other physical properties of the material obtained were not compromised seriously.

3. The 2.5% diacetate-added ChemFil Superior was found to
be the most effective and longer-lasting antibacterial group against both tested strains. However, from a chemical point of view, the 1.25% diacetate-added ChemFil Superior would be a more appropriate material for further development.

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