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Laboratory Research

Antibacterial Activity and Physical Properties of Glass-ionomer Cements Containing Antibiotics

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Clinical Relevance

The incorporation of low quantity antibiotics into glass-ionomer cement can be effective for use with the ART approach.

SUMMARY

This study evaluated the antibacterial effects, physical properties and bonding strengths of conventional glass-ionomer cements (GICs) containing antibiotics and determined the optimal concentration of antibiotics addition for use with the ART approach. Fuji IX GIC was used as a control. Three antibiotic mixtures, ciprofloxacin, metronidazole and minocycline, were added to powdered GIC (Fuji IX) to obtain concentration ratios of 1.5, 3.0 and 4.5% w/w. The antibacterial activity of each GIC was evaluated against *Streptococcus mutans* or *Lactobacillus casei* using agar-diffusion methods. The release of antibiotics was analyzed by high-performance liquid chromatography (HPLC). The compressive strength and bonding strength to dentin were measured and compared with those of control samples. The results were analyzed using the Mann-Whitney test and Wilcoxon test. All tested groups showed a significantly greater inhibition of growth of the selected bacteria in comparison to the control groups ($p<0.01$). However, the 3% and 4.5% concentration ratios of antibiotics had significantly lower compressive strength and lower bond strength to dentin than the control group ($p=0.003$). The GIC-containing antibiotics

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were effective in inhibiting *S Mutans* and *L Casei*. The addition of a 1.5% antibiotic mixture was optimal to giving appropriate physical and bonding properties.

INTRODUCTION

Atraumatic Restorative Treatment (ART) is a minimally invasive technique in which hand instruments are used to clean carious dental tissues and stop the progression of caries.[1] The technique is suited for developing countries in which resources are scarce but is gaining acceptance in developed countries for the management of early childhood caries.[2] The straightforwardness and simplicity of ART and the relatively low cost compared to a treatment approach using rotary instruments are attractive advantages of this technique.[3]

During the ART procedures, dental hand instruments alone do not remove carious dentin as effectively as rotary burs, and cariogenic bacteria can survive remaining under restorations.[4-6] The caries process may progress in the course of time and cause failure of the restoration.[7] This problem may be solved with the use of dental materials that inhibit bacterial growth.

Conventional hand-mixed GIC is the most commonly recommended dental material for the ART approach.[8-9] The reason for choosing GIC is based on its self-curing and potential caries inhibition properties.[10-11] However, whether the caries-inhibitor influence is sufficient to completely arrest the caries process is doubtful.[7] Clinical studies show that residual bacteria located under a GIC restoration are viable for up to two years.[45] Therapeutic benefit may therefore be gained when combining antibacterial agents with GIC materials.[12] For increasing the success rate of ART, many researchers incorporated antibacterial agents (for example, chlorhexidine and antibiotics) into GIC to increase its antibacterial effects.[12-18] The incorporation of chlorhexidine or its derivatives into GIC improved the antimicrobial effect of the GIC on cariogenic microorganisms.[12,14-18] Additionally, GIC-containing antibiotics were recommended for the treatment of carious lesions, reducing the total number of viable bacteria, while preserving dentin tissue and pulpal vitality.[13] However, incorporation of chlorhexidine or its derivatives decreased the physical properties of GIC.[14-16,18] Therefore, the particular antibacterial agent selected and its quantity are important for incorporation into GICs. These additives should not jeopardize the basic properties of the materials.[14] The effect of combining antibiotics with GICs on cariogenic microorganisms has previously been investigated.[13] The dental literature, however, lacks data related to the basic properties and bonding strengths of GICs to which antibiotics are incorporated.

This study evaluated the antibacterial effects, physical properties and bonding strengths of GICs containing an antibiotic mixture (ciprofloxacin, metronidazole and minocycline) and determined the optimal concentration of antibiotics necessary to produce anticariogenic action.

METHODS AND MATERIALS

Preparation of Antibacterial Cement

A conventional restorative GIC (Fuji IX, GC, Tokyo, Japan) was used as the control. In experimental groups, three antibiotics (Ciprofloxacin, Eczacibasi, Istanbul, Turkey; Metronidazole, Eczacibasi and Minocycline, Fako, Istanbul, Turkey) were added into the powder of the control GIC at 1.5, 3.0 and 4.5% w/w (Table 1).

Antimicrobial Activity Screening Tests

The antimicrobial effects against *Streptococcus mutans* HF676 (a local strain obtained from the School of Dentistry, Hacettepe University, Ankara, Turkey) or *Lactobacillus casei* ATCC27139 of unset and set cements were evaluated with agar well diffusion and agar diffusion tests, respectively.[14] Briefly, with some modifications, the strains stored at -20°C were cultured on blood agar (Merck, Darmstadt, Germany) and Lactobacilli MRS agar plates (for *L casei*, Difco Laboratories, Sparks, MD, MI, USA) at 37°C for 24 hours in 5% CO_2. Single colonies from plates were transferred into BHI broth (Merck) and Lactobacilli MRS broth (for *L casei*, Difco Laboratories) and incubated at 37°C, for 24 hours. Suspensions of the strains were prepared in PBS at ca $1.5x10^8$ organisms/ml by using McFarland 0.5 turbidity tube were flood-inoculated onto the surface of BHI agar plates. Before replacement of the set and unset specimens, the surface of the plates were air dried by leaving them at 37°C for 15 minutes.

The set disc-shaped specimens (10 mm in diameters, 2 mm thick) were prepared by mixing powder and liquid from each group (P/L ratio: 3.6/1). After setting at room temperature for 30 minutes, the specimens were

Table 1

Groups	The Composition of the Control and Experimental GIC (total weighing: 10 g)	Additives (w/w %)
Control	10 g of GIC	-------
Group I	50 mg of ciprofloxacin, 50 mg of metronidazole, 50 mg of minocycline, and 9,850 g of GIC	1.5%
Group II	100 mg of ciprofloxacin, 100 mg of metronidazole, 100 mg of minocycline, and 9,700 g of GIC	3.0%
Group III	150 mg of ciprofloxacin, 150 mg of metronidazole, 150 mg of minocycline, and 9,550 g of GIC	4.5%

placed onto BHI agar plates. All the specimens were then sterilized with UV before the experiment. For unset specimens, 10 mm-diameter wells were cut from the agar by using sterile glass-made pipettes attached to a vacuum pump and filled with paste using a syringe. After the incubation at 37°C for 48 hours, inhibition zones around the specimens were measured. The sizes of the inhibition zones were calculated by subtracting 10 mm (diameter of wells) from the average diameter of the zones for each specimen and control. Five specimens were tested for each group.

Release of Antibacterial Drugs

The molds were made of Teflon and the internal dimensions were 2 mm high and 10 mm in diameter. The round-shaped GIC specimens were prepared (P/L ratio: 3.6:1) and allowed to set for 30 minutes. All the samples were dissolved with distilled 2.5 mL water and stored at 20°C for 24 hours and 7 days. Sample concentration analyses were achieved with the Agilent 1100 series high performance liquid chromatography (HPLC, Agilent 1100, Waldbron, Germany) system. The chromatographic reversed-phase column used for analysis was an HI ODS column (HiChrom Prep 20 C18 ODS 4.6x25 HiChrom, Berkshire, UK). Acetonitril and 10 milimoles of phosphate buffer solution (pH=2.6) (60:40) were used for the mobile phase at a flow rate of 1 mL/minute and the detector wavelengths were performed at 268 nm (for minocycline), 280 nm (for ciprofloxacin) and 315 nm (for metronidazole) for each sample. Six specimens were tested for each experimental group.

Compressive Strength

The compressive strength testing was conducted according to methods described in EN-ISO 9917 (Dental Water-based Cements, 1991). The molds were made of Teflon and the internal dimensions were 6 mm high and 4 mm in diameter. The cylindrically-shaped GIC specimens were prepared (P/L ratio: 3.6:1), and stored at 37 ± 1°C in 100% humidity for 60 minutes after mixing. Ten specimens were made and randomly divided into two groups of five. The specimens were then stored in distilled water for 24 hours and 7 days. The strength of the specimen was measured by applying a compressive load using an Instron universal testing machine (Lloyd Instruments Ltd, Foreham, Hampshire, England) at a crosshead speed of 1 mm/minute^{-1}. The results were reported as an average of five replications.

Bond Strength to Dentin

Occlusal dentin specimens were obtained from 30 human molars, then the dentin surface was polished flat with 200-, 400- and 600-grit silicon carbide papers to expose the flat surface. The dentin surface was conditioned with a polyacrylic acid (Cavity Conditioner; pH: 1.65) for 10 seconds following rinsing of the conditioner with air-water spray for 10 seconds. After completion of the surface procedures, the powder and liquid of each cement were mixed (P/L ratio : 3.6/1) and placed into the center of the prepared dentin surface by packing the material into cylindrically-shaped plastic tubes with an internal diameter of 3 mm and a height of 4 mm. After storing the specimens at 37°C and 100% humidity for 24 hours, the shear bond strength was measured using an Instron universal testing machine (Lloyd Instruments Ltd) at a crosshead speed of 1 mm/minute^{-1}. Five specimens were tested for each group.

Statistical Analysis

Statistically significant differences among the groups were performed by means of the Kruskal-Wallis variance analysis, including the Bonferroni adjusted Mann-Whitney U test. The intra-group measurements, over time, were analyzed by using the Wilcoxon test for compressive strength test.

RESULTS

Antimicrobial Activity Screening Tests

The mean values (mm) of the growth inhibition zones for control and experimental groups are shown in Figure 1a and 1b. No inhibition zone existed for either bacterial species tested in the set specimens of the control groups, while unset specimens of the control groups exhibited zones of growth inhibition. However, a large inhibition zone was produced when tested against both bacteria with set or unset specimens of the experimental groups (Groups I, II and III). In set specimens, the size of the inhibition zones was significantly smaller than in the unset specimens against all bacteria. Additionally, the size of the inhibition zones was dependent upon the amount of added antibiotic mixture.

When *S mutans* and *L casei* were tested, significant differences existed in the size of the inhibition zones produced among the control and experimental groups in set specimens (for *S mutans*, p=0.001 and for *L casei*, p=0.003). Significant differences in the size of the inhibition zones produced among all the groups were observed in testing with *S mutans* and *L casei* in unset specimens (p<0.0005).

Release of Antibacterial Drugs

Release of the antibiotics metronidazole, ciprofloxacin and minocycline from the experimental GICs after 24 hours and 7 days is shown in Table 2. The amount of antibiotic released increased as the amount of antibiotic added increased. The levels of antibiotics released at 7 days were greater than at 24 hours for all the experimental groups. Significant differences were observed among the groups of experimental GICs (p<0.0005 for each group).

Figure 1: *Results of antibacterial activity test for set and unset specimens.*

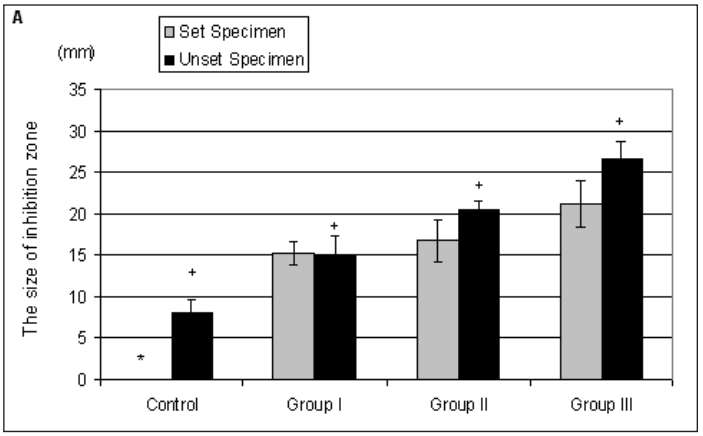


Figure 1A: *Streptococcus mutans HF676.*

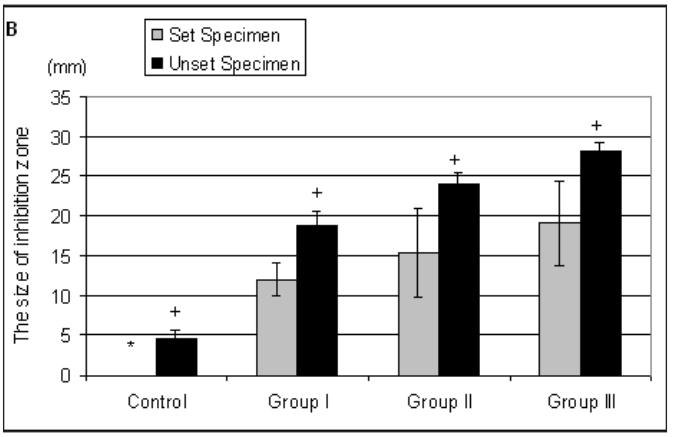


Figure 1B: Lactobacillus casei *ATCC27139.*

The vertical bar indicates standard deviation for three replicates. (*) Control group vs other groups for set specimens. (+) Significantly different each group vs other group for unset specimen.

Table 2: *Concentrations (mg/ml) of antibiotics released from experimental GICs*

Groups and added antibiotic ratios (w/w %)	after 24 hours Mean ± SD	after 7 days Mean ± SD
Group I (1.5%)	0.01004 ± 0.01409	0.02952 ± 0.03819
Group II (3.0%)	0.02981 ± 0.04304	0.07980 ± 0.06475
Group III (4.5%)	0.06853 ± 0.09776	0.15770 ± 0.14079

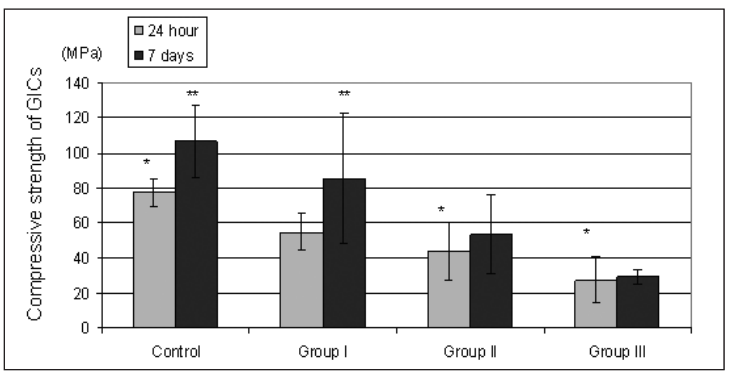


Figure 2. *Compressive strengths of control and experimental GICs after 24 hours or 7 days. The vertical bar indicates standard deviation for five replicates. *different groups **Control Group and Group I vs Group III.*

Compressive Strength

The mean compressive strength of the control and experimental groups after 24 hours and 7 days of storage in water is shown in Figure 2. The compressive strength values at 7 days were greater than at 24 hours for all groups tested (p=0.003). The experimental groups showed lower compressive strength when compared with the control group at 24 hours and 7 days. However, no significant differences existed between the control and Group I at 24 hours and 7 days. No significant differences were observed among the experimental groups at 24 hours. However, a significant difference was observed between Group I and Group III after 7 days.

Bond Strength to Dentin

The shear bonding strengths for the control and experimental groups are shown in Figure 3 (p=0.003). The shear bonding strengths of Groups II and III to dentin were significantly lower than that of the control group (Figure 3). No difference in bonding strength existed between the control and Group I.

DISCUSSION

The effects of GIC on cariogenic bacteria are known, probably resulting from the release of fluoride, but this information is not reliable.[4,19] According to Vermeersch and others,[20] the low pH of GICs, while setting, may contribute more to their antibacterial properties than their fluoride-leaching capabilities. Additionally, Yap and others[21] reported that there was no antibacterial activity despite the presence of fluoride in the agar around the set materials. According to the results of the current study, for pure GICs (control group), the set specimens did not produce bacterial inhibition, although the unset specimens demonstrated antibacterial activity. These results supported previous studies.[20-21] The antibacterial effects of unset control specimens may be correlated with decreasing pH during the setting reaction. Therefore, the influence of fluoride on the antimicrobial properties of GICs may be limited, especially after the setting reaction is completed.

Knowing that a large number and variety of bacteria play a role in caries development,[22] the use of a mixture of antibiotics is probably a better choice than the use of

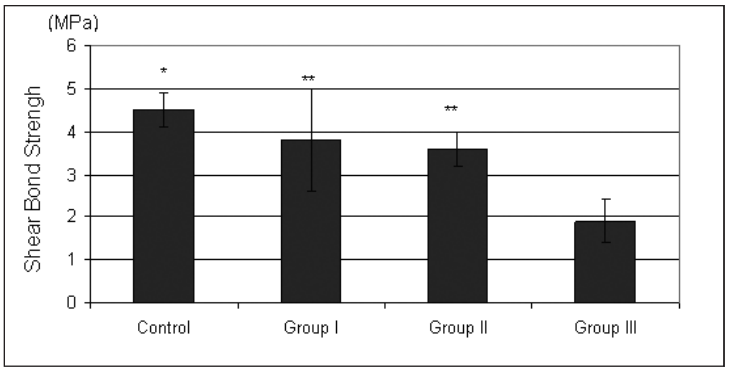


Figure 3. *Dentin bond strengths of control and experimental GICs. The vertical bar indicates standard deviation. *Control Group vs Group I and Group III****

a single antibiotic, resulting from a broader spectrum of action. A wide spectrum of antibiotic activity would minimize the possibility of selecting resistant strains, while the use of a single antibiotic would increase that possibility.[13] Pinherio and others[13] suggested that a GIC-containing antibiotic mixture may be used for the treatment of carious lesions, reducing total viable bacteria. Sato and others[23] investigated the efficiency of ternary drug combinations (*metronidazole*, *ciprofloxacin* and *minocycline*) *in vivo* and found, as *in vitro*, that this approach was very effective in the sterilization of caries lesions, necrotic pulps and infected root dentin of deciduous teeth. Hoshino and others[24] performed an *in vitro* study, testing the antibacterial efficacy of these drugs, alone, and in combination, against the bacteria of infected dentin, infected pulps and periapical lesions. Independently, none of the drugs resulted in complete elimination of bacteria. However, in combination, these drugs were able to consistently sterilize all the samples. These previous studies were taken into consideration in the current study. Therefore, metronidazole, ciprofloxacin and minocycline were the preferred mixture of antibiotics tested in this research.

The results of the current study demonstrated that the GIC-containing antibiotic mixture was effective in inhibiting bacterial growth. Both set and unset specimens containing antibiotics exhibited inhibitory effects against *S mutans* and *L casei* compared with the control specimens. Also, all the antibiotic-containing set specimens showed less antibacterial activity than the unset specimens against the bacteria tested. These results can be explained by understanding that setting GIC materials are more soluble and, therefore, better able to diffuse in agar gel than in the set materials. For all the groups examined, the agar-diffusion tests showed that the size of the inhibition zones produced in the presence of *S mutans* and *L Casei* were dependent upon the quantity of the antibiotic incorporated to the GIC.

The antibiotic compounds were solids that were easily mixed with the GIC powder. However, in this study, it was observed that, increasing the concentration of the antibiotics had increasingly adverse effects on the physical properties of the mixture. When compared with the control specimens, the compressive strength of the groups having concentration ratios of 3% and 4.5% was significantly lower; whereas, the compressive strength of the group having a concentration ratio of 1.5% was not compromised seriously at 24 hours or after 7 days. One possible explanation for these results is that the increasing concentration of antibiotic powders may decrease the reaction between the glass particles and liquid cement, thereby increasing the number of unreacted particles in the structure. Also, the powered antibiotic particles, which are added into the GIC, easily absorb water. The absorption of water can decrease the compressive strength of the GIC. As a result, the low compressive strength of GICs may hamper the use of these materials in posterior teeth for the ART approach.

The capacity of GIC to bond chemically to enamel and dentin, as well as its fluoride-releasing property, is very important. Mount[25] reported that the antibacterial capacity of GIC may be associated with the adhesion of these materials to the cavity by an ion-exchange layer, thereby isolating the caries lesion from the oral environment and rendering bacterial nutrition more difficult. In the current study, the results of the dentin bond strength test demonstrated a similar trend to that of the compressive strength test results. The GICs containing 1.5% antibiotics produced bonding strengths similar to those of the control GICs, but a significant reduction in bonding strength was observed for GICs containing 3% and 4.5% antibiotics. Presumably, the lower bonding strength results from interference in the polar and ionic attraction between the carboxylate and inorganic ions with dentin.

The release of antibiotics was monitored using HPLC analysis and was observed to change in relation to concentration and time. The antibiotic release at 24 hours was lower than after one week. Therefore, GICs containing antibiotics may have a long-term effect on cariogenic bacteria compared with the pure Fuji IX GIC.

GICs containing antibiotics should be considered not only for their caries-inhibitory properties, but also for their safety. The incorporation of antibiotics into GICs may increase the risk of side effects or the development of resistance to the drugs over time. Therefore, for use with the ART approach, until the long-term clinical effects of these products are investigated,

antibiotic-added GICs can be placed as a base material under GICs to which antibiotics are not added.

CONCLUSIONS

The results of this *in vitro* investigation demonstrated that experimental GICs containing antibiotic mixtures are effective in inhibiting bacteria associated with caries. The addition of the antibiotic mixture at a concentration ratio of 1.5% was optimal for achieving expected physical and bonding properties. However, the long-term pharmacological and clinical effects of antibiotic-containing GICs should be investigated in future studies.

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