Antimicrobial effects of glass ionomer cements containing bioactive glass (S53P4) on oral micro-organisms in vitro

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The antimicrobial effects of two glass-ionomer cements (GICs), GC Fuji II and Fuji II LC, mixed with different amounts (0 wt%, 10 wt% and 30 wt% of the total powder weight) of bioactive glass (BAG), S53P4, on *Streptococcus mutans* and *Candida albicans* were studied in vitro. The growth inhibition was tested using agar diffusion. The materials were also studied in a liquid media. The effect of the material extracts on acid production was studied using cell suspensions. The antimicrobial activity of the materials was examined by incubating the cell suspensions with the material powder. In the agar diffusion test, only the GICs containing 30 wt% BAG inhibited the growth of *S. mutans*. When the materials were tested in culture medium, no inhibitory effects on *S. mutans* were detected. The only materials to inhibit acid production of *S. mutans* were the GIC extracts without added BAG. Furthermore, they also had antibacterial activity against *S. mutans* when tested as powders. We found very few effects of the tested materials on *C. albicans*. The only material with an antimicrobial effect on *C. albicans* was BAG when incubated in a suspension with *C. albicans*. This is the first time that this effect has been demonstrated for *C. albicans*. By adding BAG to GICs the structure of the material becomes more brittle than the structure of GICs without BAG. Thus, in addition to the composition of the tested materials, their structure may also have influenced the results. In summary, commercially available GICs and GIC disks containing 30 wt% of BAG exerted antibacterial effects on *S. mutans*. BAG exerted antimicrobial effects on both *S. mutans* and *C. albicans*. □ *Bioactive glass; Candida albicans; glass-ionomer cements; Streptococcus mutans*

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Glass-ionomer cements (GICs) have been in use for nearly 30 years in clinical dentistry. They are beneficial in that damaged dentin can be replaced with an adhesive, anticariogenic and biologically compatible cement (1). Several studies have demonstrated that GICs can inhibit microbial growth in agar diffusion tests (2–4). It has also been demonstrated in vivo that GICs have antibacterial activity (5). The plaque formed on GIC restorations may be less likely to induce recurrent caries than the plaque formed on amalgam restorations (5). Of many in vitro studies demonstrating that fluoride is released from GICs (6), some suggest that the antibacterial activity of GICs is based on the released fluoride ion resulting in high fluoride concentration and/or on the low initial pH of the cement (3, 7). The capacity to release F-ions improves remineralization and reduces the solubility of dentin and enamel. Even though the amount of fluoride released has been shown to decrease significantly with time (6), the capacity of bonding and releasing F-ions aids the functioning of GICs as a long-term F-reservoir in the changing oral environment (6). These findings are supported by an in vivo experiment in which the application of GIC restorations increased the fluoride concentration in saliva for a considerable period of time (8).

Bioactive glasses (BAGs), first introduced by Hench et al. (9), are surface-active glasses that bond chemically to bone minerals. The bonding of BAGs to living bones is achieved through a bone-like apatite layer forming on their surface in the body environment (9). Owing to their strong bond with living bone, BAGs have been used as bone substitute materials in many different clinical conditions, e.g. in orthopedics and in dentistry (10). In the literature, there are only a few publications concerning the antimicrobial properties of BAGs. In an in vitro BAG-paste study (11), it is reported that BAGs have a broad antibacterial effect on oral microorganisms, including *Streptococcus mutans*. On the other hand, in vitro studies have been carried out with an agar plate model in which BAG did not show any inhibitory action, for example, to periodontal pathogens (12, 13). In a recently published article (14) it was revealed that adding Ag to BAGs produces a marked bacteriostatic effect on *Escherichia coli*; in addition, a significant reduction in bacterial growth was detected. This effect was believed to be based on the Ag of the material, as BAG without Ag was not shown to possess antibacterial properties (14).

Studies carried out in vivo and in vitro have identified *S. mutans* as one of the most important microorganisms in the etiology of dental caries (15). *S. mutans* is found to be associated with the initiation of human dental caries (15). *Candida albicans* is generally considered the predominant cause of oral fungal infections (16), but it has also been

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Table 1. Contents of different powders in wt% and the powder weight to liquid weight (1 g) ratio for each test specimen when using the level scoops recommended by the manufacturer of the GICs used in this experiment

Specimen code	GC Fuji II	Fuji II LC	S53P4	Powder/g
GI	100			2.7
GI10BAG	90		10	1.7
GI30BAG	70		30	1.8
LC		100		3.2
LC10BAG		90	10	2.2
LC30BAG		70	30	2.5
BAG			100	–

related to caries (17). Both *S. mutans* and *C. albicans* can grow in a low pH (18). The conditions in which these pathogens are successful also support the accumulation of cariogenic plaque. A reduction in the number of these microorganisms at the tooth-restoration interface could reduce the accumulation of cariogenic plaque and thus influence the incidence of dental caries.

Our previous in vitro study showed that adding BAGs to GICs did not prevent the release of fluoride from GIC materials; on the contrary, by adding 30 wt% of BAG to GIC the release of fluoride was enhanced (19). In addition to F-ions, the BAG containing GICs also released Ca, P, and Si ions. This indicates that the materials studied here may not only have good potential to reduce the accumulation of cariogenic plaque, but also to mineralize hard tissues.

The aim of this in vitro study was to evaluate the possible antimicrobial properties of two different types of GICs containing BAGs against two common oral pathogens: *S. mutans* and *C. albicans*.

Materials and methods

Two different kinds of commercially available GIC were used in the experiments: conventional cure GIC (code: GI) (batch no. 206251, GC Fuji II, GC Corporation, Tokyo, Japan) and resin-modified light-curing GIC (code: LCGI) (batch no. 205011, Fuji II LC, GC Corporation, Tokyo, Japan). The material consisted of powder and liquid.

A commercially available bioactive glass (code: BAG) (S53P4, batch no. ABMS53-7-00, Vivoxid Ltd, Turku, Finland) was used in this experiment. The composition of the BAG by weight is SiO_2 53%, Na_2O 23%, CaO 20%, and P_2O_5 4%.

The BAG powder with a particle size of <45 μm (average particle size, 20 μm) was added to the GIC powder. Two different ratios of BAG and GIC powder (10 wt% and 30 wt%) were used. GIC powders without BAG particles were used in the fabrication of control specimens. BAG powder and microbial suspension without added material powder were also used as controls. Description of the GIC/BAG powder ratio and powder to liquid ratio is given in Table 1.

BAG and GIC powders were measured in 20 mL Falcon plastic test tubes and mixed in a Coulter mixer (Luton, UK) for 10 min to even the filler particle distribution. The powder was then mixed with polyacrylic acid of GI and diacrylate resin-polyacrylic acid mixture of LCGI in accordance with the manufacturer's instructions. The mixed materials were packed into metallic molds and gently compressed between glass plates to form discoid specimens (thickness 1.0 mm, diameter 5.5 mm). The specimens made from LCGI cement were cured with a visible light-curing device (ESPE Elipar Highlight, Seefeld, Germany) (470 nm wavelength, light intensity 690 mW/cm^2) for 40 s. All the specimens were prepared at room temperature (21 ± 1°C) and relative humidity of 55%.

The microorganisms used were *Streptococcus mutans*, ATCC 25175, and *Candida albicans*, ATCC 28366 (American Type Culture Collection, MD, Rockville, USA).

Growth inhibition on agar plates

In the growth inhibition experiments, the inhibition of *S. mutans* was investigated with Mitis Salivarius (MS) agar plates (Becton Dickinson, Sparks, Md., USA), and that of *C. albicans* with blood agar plates (Oriola, Espoo, Finland). The precultivations of both microorganisms were performed overnight at 37°C in Brain Heart Infusion (BHI, Unipath Ltd, Basingstoke, Hampshire, UK). After approximately 18 h of growth, the cells were washed once in sterile saline. The suspensions were adjusted with saline to an optical density of approximately 0.5 (A_{660}). After the precultivations, the microorganisms were swabbed onto the agar plates with a sterile cotton applicator. Each test specimen and solid BAG disks (thickness 1.0 mm, side length 5 mm) were placed on the agar plates, immediately after plating of the microorganisms. *S. mutans* was cultured for 2 d at 37°C in an atmosphere of 93% air and 7% CO_2. *C. albicans* was cultured for 2 d at 37°C in air. All the materials were allowed to interact with the two different oral pathogens. The tests were repeated twice.

Growth inhibition in culture medium

For the growth experiments, the test specimens and a solid BAG disk were immersed individually for 3 d in test tubes in 20 mL of distilled water in a water bath with a shaking volume of 70 rpm and at a constant temperature of 37°C (Grant OLS 200, Cambridge, UK). After immersion, they were put into a cuvette with 900 μL of BHI growth medium (37°C), and 100 μL of the microorganisms cultivated overnight and adjusted with saline to an optical density of approximately 0.5 (A_{660}), was added to the mixture. The test specimens and a solid BAG disk without immersion were used as controls. The microorganisms were cultured without agitation at 37°C for up to 6 h for *S. mutans* and up to 8.5 h for *C. albicans*. The growth was followed at A_{660}, and the pH of the culture was assessed at

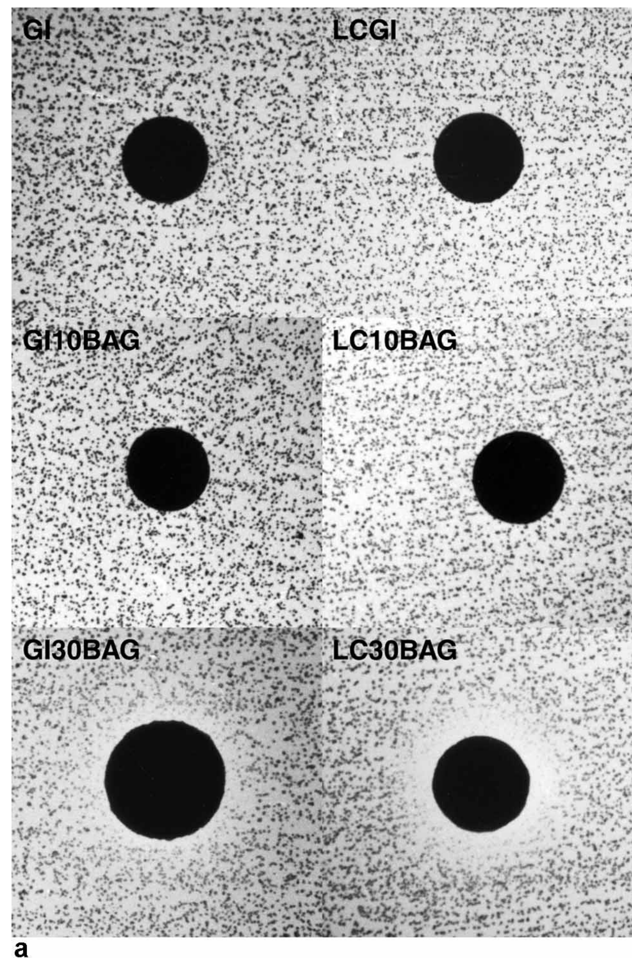
the end of the experiment. The experiment was performed with three parallels, and was repeated twice. The material GI30BAG was excluded for the cyvette experiment because the disks broke during the immersion period.

Acid production

For the acid production experiments, the test specimens and a solid BAG disk were immersed individually for 14 d in test tubes in 20 mL of distilled water in a water bath with a shaking volume of 70 rpm and a constant temperature of 37°C (Grant OLS 200, Cambridge, UK); the solutions or 'extracts' were used after centrifugation (12,000*g*, 10 min) to test their effects on acid production from glucose in *S. mutans* or *C. albicans* suspensions. The overnight, approximately 18 h, precultivated microorganisms were washed once in saline and suspended in A_{660} with fermentation minimum medium (FMM) (20). To delete endogenous sugars the supernatant was preincubated at a constant temperature of 37°C for 15 min. The acid production experiments were started by adding 350 μL of the 14 d specimen incubation supernatant to 600 μL of the bacterial suspension. After that, 50 μL glucose was added to the mixture. The final volume of the incubation mixture was 1 mL and the cell density 1.0 (A_{660}). The pH of the bacterial suspension was followed electrometrically (PHM80 Portable pH-Meter, Radiometer A/S, Copenhagen, Denmark) for up to 15 min. The experiment was performed with one parallel and it was repeated twice.

Antimicrobial activity of material slurries

The antimicrobial effects of the ground-material powders were studied by incubating each microbe in a suspension with the test materials, thus creating a slurry. The microorganisms were first precultivated overnight as described under the growth inhibition on agar plates experiment. The test specimens were homogenized to powder form with a MM-type Retsch-laboratory mixer (Retsch GmbH & Co, Haan, Germany); BAG was used in powder form as such. The ground-material powders were combined with the microbial suspension using a material powder and microbial suspension ratio of 50 mg and 30 μL. Each test material slurry was first vortexed for thorough mixing for 15 min at room temperature in Eppendorf tubes (Sarstedt, Germany), followed by incubation without agitation for 60 min at 37°C. For assessment of viability as CFUs, the incubation was stopped by adding 470 μL saline, followed by vortexing and sonication for 10 s to detach the microorganisms from the material powders. From this mixture, a 100 μL sample was taken immediately after the sonication and added to 900 μL of Tryptic Soy Broth (Difco Laboratories, Detroit, Mich., USA) with 10% glycerol (v/v), and this was the sample that was cultivated. Assessment of the viability of the microbial suspensions was performed on agar plates by cultivating 10 μL samples from the suspension diluted



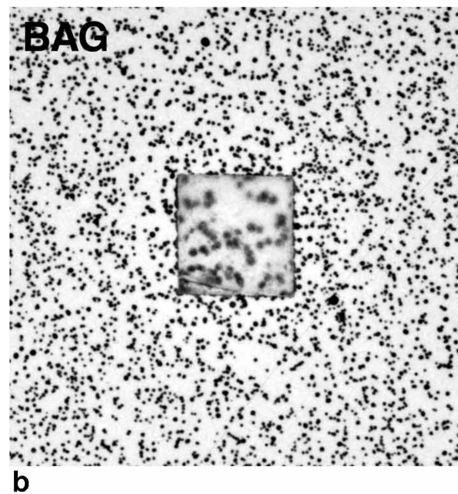


Fig. 1. Growth inhibition of *Streptococcus mutans* in the agar diffusion test. (a) GI = GC Fuji II cement, GI10BAG = GC Fuji II cement with 10 wt% of BAG, GI30BAG = GC Fuji II cement with 30 wt% of BAG, LCGI = Fuji II LC, LC10BAG = Fuji II LC with 10 wt% of BAG, LC30BAG = Fuji II LC with 30 wt% of BAG. (b) BAG = Bioactive glass.

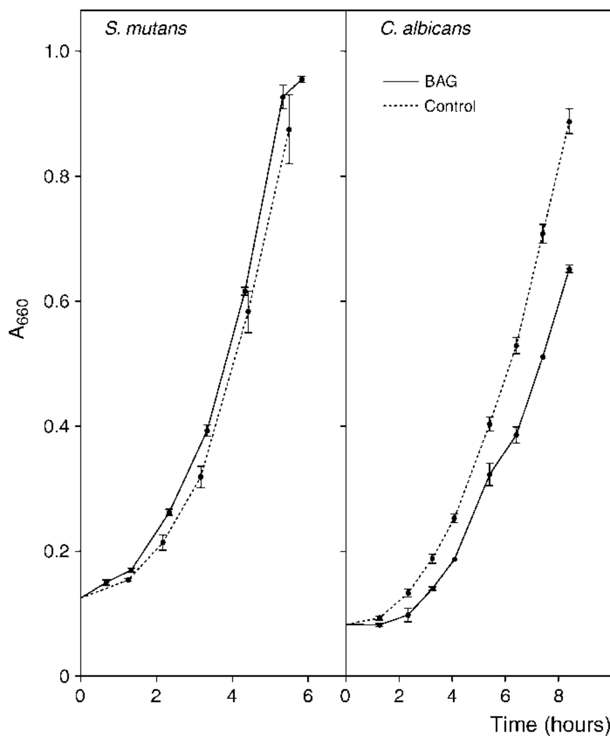


Fig. 2. Growth curve for *Streptococcus mutans* and *Candida albicans* in the presence of and without bioactive glass (BAG, non-immersed). The growth was monitored at A_{660}. The values shown are means ($\pm s$); ($n = 3$).

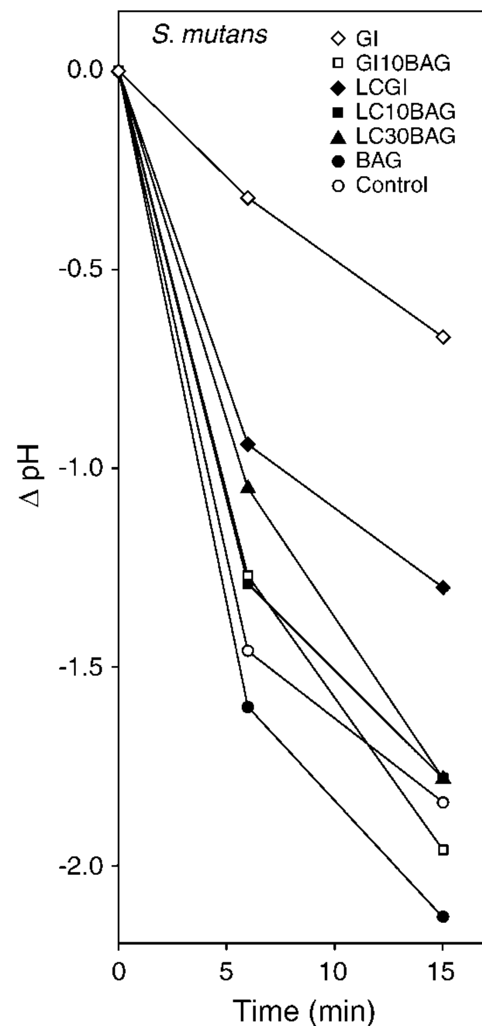


Fig. 3. The effects of water extracts of the study materials on glucose-induced acid production from a *Streptococcus mutans* suspension. GI = GC Fuji II cement, GI10BAG = GC Fuji II cement with 10 wt% of BAG, GI30BAG = GC Fuji II cement with 30 wt% of BAG, LCGI = Fuji II LC, LC10BAG = Fuji II LC with 10 wt% of BAG, LC30BAG = Fuji II LC with 30 wt% of BAG and BAG = Bioactive glass. Water was used as control.

serially with saline. The undiluted suspension was also cultivated using 10 µL samples. *S. mutans* was cultivated on MS agar overnight at 37°C aerobically in an atmosphere consisting of 74% N_2, 19% O_2, and 7% CO_2. *C. albicans* was cultivated on a Yeast nitrogen base (6.7 g) and dextrose (5 g) (Difco Laboratories, Detroit, Mich., USA) in accordance with the manufacturer's instructions. The experiment was performed with three parallels and was repeated twice.

Results

Growth inhibition on agar plates

Both GICs with 30 wt% of BAG (GI30BAG and LC30BAG) inhibited the growth of *S. mutans* (Fig. 1). No other materials tested had any influence on the growth of *S. mutans* (Fig. 1). As can be seen in Fig. 1, GI30BAG absorbed water from the agar during the 48 h culturing period. The studied materials did not inhibit the growth of *C. albicans*.

Growth inhibition in culture medium

The growth of *S. mutans* in the presence of and without BAG is shown in Fig. 2. None of the study materials, immersed or non-immersed, inhibited the growth of *S. mutans* (not shown). The materials had no effect on the end pH values of the *S. mutans* cultures. The only material to inhibit the growth of *C. albicans* was the non-immersed BAG (Fig. 2). The other materials, immersed or non-immersed, had no effect on its growth (not shown). BAG was the only material to significantly elevate the pH values of the *C. albicans* culture medium.

Acid production

The effect of the 14 d material extracts on the acid production of *S. mutans* is shown in Fig. 3. The only extracts of the materials which inhibited the acid production were those of GI and LCGI. For GI, the

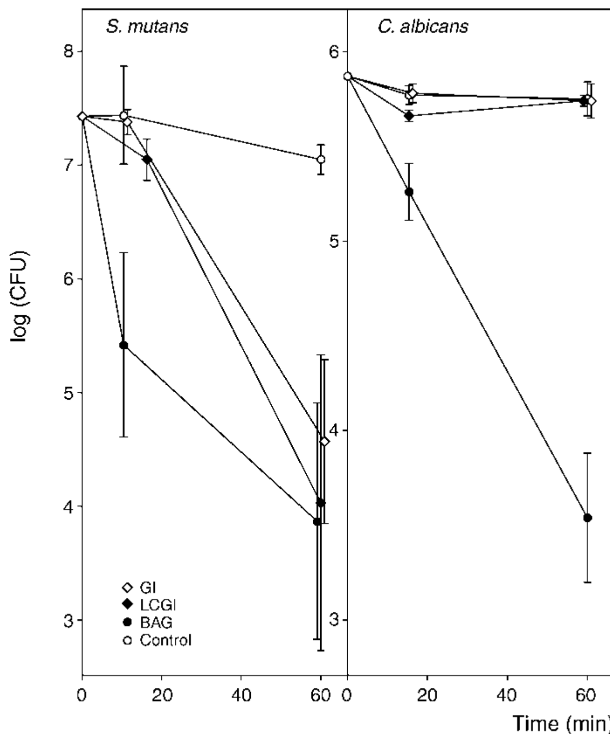


Fig. 4. The effects of powdered bioactive glass (BAG), GC Fuji II cement (GI), Fuji II LC cement (LCGI) and no added material (control), on viable counts of *Streptococcus mutans* (left) and *Candida albicans* (right) in suspension. The values shown are means; $n = 3$ ($\pm s$). CFU = colony forming units.

inhibition was stronger than for LCGI. The acid production of *C. albicans* was not inhibited by any of the material extracts tested.

Antimicrobial activity of material slurries

A major decrease in the viable counts of *S. mutans* was seen with BAG alone. A major decrease in the number of viable counts of *S. mutans* was seen within 10 min, as the number of viable counts decreased from 7.5×10^6 to 5.5×10^5. Within 60 min, the viable counts decreased further, to 1×10^4. This was the maximal inhibition achieved with the materials tested (Fig. 4). A decrease in viable counts was also seen with the materials GI and LCGI. With these materials, a decrease in the viability of *S. mutans* occurred from 10 min (7.5×10^6) to 60 min (4.7×10^4), and the decrease in number was almost the same with both materials (Fig. 4). The GICs containing BAG did not show any influence on the viability of *S. mutans* (not shown).

A major decrease in the viable counts of *C. albicans* was seen with BAG. With this material, *C. albicans* showed an almost linearly decreasing loss of viability from the beginning of the experiment (1×10^6) to the end (3.5×10^3) (Fig. 4). *C. albicans* was found not to lose its viability in contact with any of the other test materials, although a slight decrease from 1×10^6 to 5.8×10^5 was seen with the LCGI (Fig. 4). The GICs containing BAG did not have any influence on the viability of *C. albicans* (not shown).

Discussion

The ability of GICs to inhibit *S. mutans* has been related to either their low initial pH (7) or to the release of fluoride in an aqueous environment (3, 7, 21). They have also inhibited the growth of *S. mutans* in vivo (8, 22). In our agar diffusion tests, only the GICs with 30 wt% of BAG inhibited the growth of *S. mutans*. Our findings cannot be the result of low pH: the BAG should counteract the pH decrease in the GIC when it is placed in an aqueous environment (11). In fact, the pH of all GICs containing BAG in water suspensions was 0.6–2.8 pH units higher than the pH values of the corresponding GICs without BAG (23). The factor affecting our findings is the different composition and structure of the materials tested. In a previous study (19) the disks made of GI30BAG were found to be brittle, which might have been one reason for the increased release of Ca, P, Si, and F ions. Therefore, even though GICs containing BAG contain less fluoride than the corresponding GICs, the fluoride in them might have been released more easily than the fluoride in the GICs without BAG. Adding 30 wt% BAG to the material increased the release of fluoride when compared to the GICs, while the GICs with 10 wt% of BAG released the same amount of fluoride as the GICs (19). In the growth inhibition experiments in culture media, no inhibition was detected, probably because the concentration of fluoride did not reach growth-inhibiting levels because of the diluting effect of the culture medium itself.

When the materials were immersed in water for 2 weeks and the extracts were tested against acid production of *S. mutans*, only the GIC extracts without BAG (GI and LCGI) inhibited the acid production. As discussed above, this effect cannot be directly related to the amount of fluoride released, since in our earlier studies (19) the GICs with 30 wt% of BAG released the highest amounts of fluoride. The most likely explanation for this finding is that calcium released from BAG reacts with fluoride ion forming calcium fluoride during the long (2 weeks) immersion period, and thus fluoride is not in an active form that is able to inhibit the metabolism of *S. mutans*. However, in a clinical environment calcium fluoride may remain in plaque and function as a fluoride-ion reservoir.

The only material slurries that showed any antibacterial effect against *S. mutans* were the GICs without BAG (GI and LCGI), and BAG as such. In a previous study (21), the antibacterial activities of conventional and resin-reinforced GICs were found to be similar. Our results confirm this finding. In our experiment, the materials were studied in slurry form. Because it was not dependent on the structural characteristics of the test specimens, the fluoride was easily released when the test used excluded the influence of surface structure and increased the reactivity of the whole

sample, thus allowing each cement granule to affect individually. Also the BAG powder achieved a major decrease in viable counts of *S. mutans*. This is in accordance with earlier findings (11). The antibacterial properties of BAG towards *S. mutans* in this study are probably related to the high level of pH maintained by this material (11). Since GIC counteracts the change in pH caused by the BAG, no antimicrobial effect on *S. mutans* could be detected for the GICs containing BAG. Furthermore, the amount of BAG that we used in the GICs was not sufficient to achieve the same results as BAG as such did.

We found very few inhibitory effects on *C. albicans* from the tested materials. The only material with some antimicrobial effect on *C. albicans* was the BAG. In the growth inhibition experiments, this is probably related to the ability of the BAG to increase the pH of the growth medium, since no inhibitory effects were seen in the agar diffusion tests. However, for BAG, we demonstrated a significant decrease in the viability of *C. albicans* in the slurry experiment; this result was probably related to the high pH of the paste (11). On comparing agar diffusion test and slurry assays, it can be assumed that in the agar diffusion test the BAG as disks is a material interacting less with the surroundings than the BAG in a powder form. Furthermore, it can be concluded that the growth inhibition is bonded to the pH values since the BAG in powder form can increase the pH values to a higher level than the BAG disks do. Thus the experimental conditions were clearly different in these two tests. It has been proposed that the concentration of fluoride released from GICs is insufficient to inhibit the growth of *C. albicans,* even though it has been shown that *C. albicans* is inhibited by fluoride (24). Our results are in accordance with this finding.

To summarize, within the limitations of this study, it can be concluded that the commercially available GICs and the GIC disks containing 30 wt% of BAG, exerted antibacterial effects on *S. mutans*. BAG exerted antimicrobial effects on both *S. mutans* and *C. albicans*.

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