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# *In Vitro* Fluoride Release and the Antibacterial Effect of Glass Ionomers Containing Chlorhexidine Gluconate

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### Clinical Relevance

Modification of inherent fluoride-containing materials with chlorhexidine provides an antibacterial and remineralizing varnish with potential anti-cariogenic properties.


## SUMMARY

**Fluoride release from glass-ionomers (GI) may be important for the prevention of secondary caries. The addition of chlorhexidine gluconate (CHX) to glass-ionomer cement (3%) adds an effect that enables it to be used as a varnish for the temporary coating of surfaces at risk for caries. This study investigated the fluoride release pattern and antibacterial effect of such a material. Glass-ionomer luting cement powder (Aqua-Cem) was mixed with water, 10% CHX or 10% CHX with 11% tartaric acid (TA), respectively, to test specimens (6 * 1.5 mm). After setting, the specimens were immersed in 10 ml deionized water and transferred to new vials after various intervals over a period of two months. The antibacterial effect towards mutans streptococci was assessed using agar diffusion. The fluoride release was measured after two hours and after shifting the specimens to new vials 10 times during the two-month period. The mean total fluoride release was 69.02, 50.64 and 48.56 µg/cm² from each specimen in the GI, GI-CHX and the GI-CHX-TA groups, respectively. For two-hour old specimens, the mean inhibition zone was 0, 50, 36 mm² in the GI, GI-CHX and GI-CHX-TA groups, respectively, and, after two months, 45 mm² in the GI-CHX group and 19 mm² in the GI-CHX-TA group. It can be concluded that the addition of CHX and CHX-TA adds antibacterial properties to GI and the release of fluoride is decreased.**



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## INTRODUCTION

Mutans streptococci (ms) are considered to be the most important group of bacteria initiating caries lesions,[1] even though this has been debated lately.[2] The number of salivary ms in the oral cavity is correlated to the formation of new caries lesions, and it is generally accepted that reducing the number of ms also reduces caries activity.[3-5] In this context, several different antimicro-

bial agents have been tested in plaque reduction,[5-8] and chlorhexidine (CHX) has been proven to be the most effective and safe agent.[9-10]

CHX has been shown to be the most suitable agent in reducing ms due to its increased susceptibility when compared to other oral microorganisms.[11] Since CHX is retained in oral structures from which it is slowly released, this is one reason that its antibacterial effect is significantly longer than other agents.[6,12]

The antibacterial effect of CHX is concentration-dependent.[13] In order to achieve maximum concentration of CHX at the tooth surfaces for a long period of time, CHX can be delivered as a varnish.[14-15] This approach has given the most promising results in the reduction of ms.[16] It has, however, been almost impossible to eradicate ms from the oral cavity for a longer period of time, and it has only been reported by Sandham and others.[14] Currently, there is only inconclusive evidence that CHX-varnishes containing the antibacterial substance thymol are effective against caries.[17]

In a caries-active population, however, CHX treatment alone does not totally eliminate caries activity.[18] In this case, further reduction demands additional measures, for example, increasing the resistance of tooth surfaces against acid producing bacteria using fluorides.[19] Glass ionomer cements, which are widely used as a restorative material, contain a high percentage of fluoride (10-20% in the powder fraction).[20] A portion of this fluoride can diffuse to the surrounding tooth structure. This is believed to contribute to the inhibition of secondary caries.[21] The fluoride release follows a pattern of intensive initial release, which decreases at a fast rate after approximately one day. Although the fluoride release gradually decreases, it can be observed (*in vitro*) for a long period of time (over three years).[22]

Combining glass ionomer cement with CHX was proposed by Jedrychowski and others.[23] Filling material containing 5% CHX had an antibacterial effect but also poor mechanical quality. The idea of incorporating CHX diacetate in materials used for restoration (and not only as a vehicle for a limited time drug treatment) has recently been reported,[24-25] while the current study addresses the effect of a material that is intended to have a limited time in the oral cavity in order to reduce ms and serve as a fluoride release varnish in patients with elevated caries risk.

Ribeiro and Ericson[26] showed a CHX dose-dependent antibacterial effect *in vitro* against ms that lasted up to 80 days when using a combination of GI and CHX. These authors also observed a deterioration of the material over time. The antibacterial effect was supposedly caused by the sustained release of CHX. They proposed use of the material in a varnish-like form as a CHX carrier. One of the primary motives for using GI cement as a vehicle was that GI cements are more difficult to remove in one piece, because of their inherent brittleness compared with polymer-based varnishes; GI cements also adhere well to tooth minerals.[27] *In vivo* experiments further showed an antibacterial effect against ms after coating tooth surfaces with GI containing 3.3% CHX. The ms reduction lasted for four weeks.[28] However, in a clinical investigation using such a material as temporary fissure varnish, no significant anticariogenic effect was seen by the addition of CHX to GI. Tartaric acid (TA) was added to the material to reduce the setting time.[29-31]

The current study investigated the fluoride release and antibacterial characteristics of GI *in vitro* after the addition of CHX and TA. The authors expected an increase in antibacterial effect and similar fluoride release properties after the addition of CHX and TA to GI when compared to GI alone.

## METHODS AND MATERIALS

### Glass Ionomer Cement (GI)

Aqua Cem (De Trey Dentsply, Konstanz, Germany) powder was mixed in a dappendish with de-ionized water in a powder/liquid (P/L) ratio of one scoop of powder to three drops of liquid in order to make a more varnish-like material (manufacturer's recommendation is 1:2 for this luting cement).

### CHX-containing Glass Ionomer Cement (GI-CHX)

Aqua Cem powder was mixed with a solution containing 10% CHX gluconate (Sterling Health AB, Solna, Sweden) in a P/L ratio as above.

### Tartaric Acid and CHX-containing Glass Ionomer Cement (GI-CHX-TA)

Aqua Cem powder was mixed with a 10% CHX gluconate solution and 11% tartaric acid in a P/L ratio as above.

The test materials were mixed with a microbrush in a dappendish, with a P/L ratio as above. This gave a final concentration of 2.5% (w/w) CHX.

### Test Specimens

Glass-ionomer cement (Aqua-Cem) was mixed as described above, and five cylinders with a diameter of 6 mm and a thickness of 1.5 mm were cast using a plastic mold for each material. The mixing time was one minute, the working time was two minutes and the setting time (at ambient temperature) was 10 minutes. After setting, each test specimen was placed in 10 ml deionized water (pH ~ 7) and stored at 37°C. The specimens were transferred to new vials with deionized water after 2, 4, 6, 8 and 24 hours, then after 4, 10, 15, 20 and 30 days. The total test time was 60 days. After transfer to new vials, the old solutions

were stored at -20°C in order to perform all fluoride concentration analyses in one session (n=5 in each group).

A second group of identical test specimens were made (n=5 in each group), as above, but with a thin coat of Vaseline (ACO, ACO hud AB, Stockholm, Sweden) applied after setting (GI-V, GI-CHX-V, and GI-CHX-TA-V).

### Measurement of Fluoride Concentration

Added to the 5 ml test solution was 0.5 ml of Tisab III (Orion Research Inc, Beverly, MA, USA) and measurement of the fluoride concentration was performed using a fluoride electrode (94095SC Orion Research Inc). Concentration readings after two minutes of measuring were registered for each sample.

### Bacterial Inhibition Test

To perform the bacterial inhibition test, a new set of identical test specimens were made. The first group of test specimens was placed on agar plates directly after setting. The second group were stored in deionized water at 37°C for two hours, then carefully dried using filter paper before placing them on agar plates. A third group was stored in deionized water at 37°C for 60 days, then placed on agar as above.

The antimicrobial effect was evaluated using an agar diffusion method similar to that of Ribeiro and Ericson.[26] Streptococcus mutans KPSK 232 was inoculated in Todd Hewitt broth (Difco Laboratories, Detroit, MI, USA) at 37°C anaerobically (95% $N_2$ + 5% $CO_2$) and cultured until an optical density of 1.0 at 650 nm was reached. After dilution of 1:500, 0.1 ml was spread over the mitis salivarius agar surface (Difco). After placing the test specimens on the agar surface, the agar plates were incubated at 37°C anaerobically for 48 hours. The inhibition areas were calculated from the mean diameter of two perpendicular diameters of the zone of inhibition minus the area of the test specimens.

### Statistical Methods

Fluoride concentration measurements were compared using Tukey's test for multiple comparisons and bacterial inhibition areas were compared using Student's *t*-test.

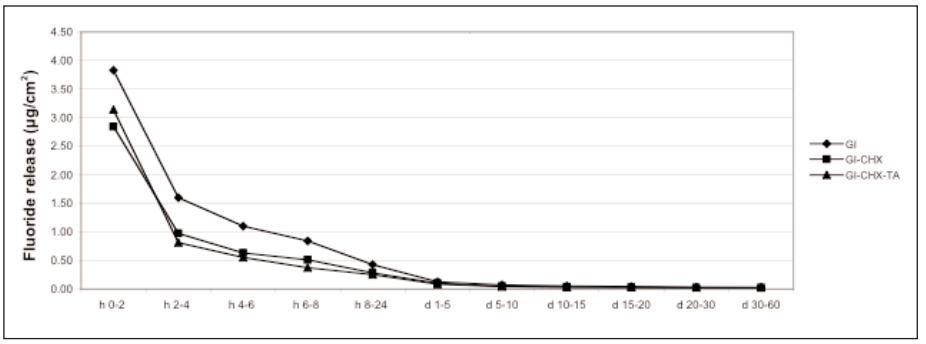


Figure 1. *Mean fluoride release (µg/cm²) from baseline to day 60 for the different materials (n=5 in each group).*

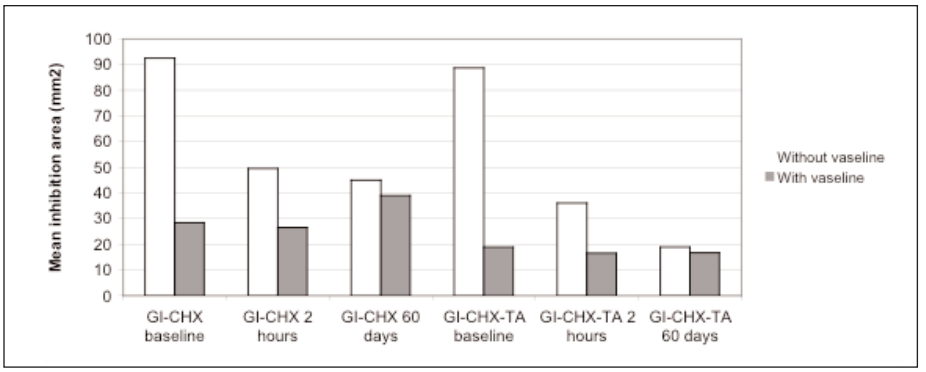


Figure 2. *Agar diffusion test. Mean inhibition areas (mm²) of S mutans growth, after two hours and after 60 days for the different materials (n=5 in each group). (GI alone did not inhibit bacterial growth.)*

## RESULTS

### Fluoride Release

The daily mean fluoride release decreased quickly during the first few days (Figure 1). After two hours, the fluoride release was different among all materials ($p<0.05$). The release leveled out after 10 days and decreased slowly until day 60. All the test specimens released measurable amounts of fluoride during the test periods.

Table 1 displays the mean fluoride release (µg/cm²) from the test specimens in the different groups from baseline to day 60. After 60 days, the mean cumulative fluoride release in the GI-CHX group was about 27% lower when compared to GI ($p<0.001$) and 30% lower in the GI-CHX-TA group ($p<0.001$) (Table 1). The addition of a Vaseline coat led to a further reduction in release. Comparing the same material with and without Vaseline, a small but not significant difference (up to 12%) in fluoride release was observed.

The antimicrobial properties of glass ionomer test specimens with the addition of CHX gluconate and with the addition of CHX gluconate and TA is presented in Figure 2. At baseline, there was no difference in bacterial inhibition between GI-CHX and GI-CHX-TA.

Table 1: *Cumulative Fluoride Release (μg/cm²) From Baseline Until Day 60*

| Time | GI | GI-V* | GI-CHX | GI-CHX-V* | GI-CHX-TA | GI-CHX-TA-V* |
|---|---|---|---|---|---|---|
| 2 hours | 7.66 ± 0.22 | 7.63 ± 0.22 | 5.69 ± 0.15 | 4.38 ± 0.22 | 6.28 ± 0.29 | 5.04 ± 0.19 |
| 4 hours | 10.86 ± 0.09 | 10.53 ± 0.45 | 7.64 ± 0.15 | 6.04 ± 0.25 | 7.89 ± 0.26 | 6.66 ± 0.29 |
| 6 hours | 13.07 ± 0.22 | 12.92 ± 0.61 | 8.91 ± 0.27 | 7.21 ± 0.20 | 9.00 ± 0.29 | 7.83 ± 0.28 |
| 8 hours | 14.75 ± 0.31 | 14.74 ± 0.48 | 9.93 ± 0.34 | 8.08 ± 0.20 | 9.74 ± 0.29 | 8.66 ± 0.32 |
| 24 hours | 21.58 ± 0.33 | 21.36 ± 0.58 | 14.45 ± 0.55 | 12.14 ± 0.40 | 13.74 ± 0.19 | 12.46 ± 0.23 |
| 5 days | 33.83 ± 0.49 | 32.84 ± 0.62 | 24.08 ± 0.92 | 20.56 ± 0.42 | 21.95 ± 0.67 | 19.97 ± 0.10 |
| 10 days | 42.31 ± 0.63 | 41.17 ± 0.73 | 29.87 ± 1.22 | 26.15 ± 0.41 | 27.10 ± 0.74 | 24.83 ± 0.34 |
| 15 days | 49.10 ± 0.71 | 47.38 ± 0.81 | 33.97 ± 1.45 | 29.98 ± 0.52 | 30.35 ± 0.86 | 28.27 ± 0.27 |
| 20 days | 54.50 ± 0.91 | 52.57 ± 0.86 | 37.58 ± 1.18 | 33.22 ± 0.46 | 33.65 ± 0.82 | 31.69 ± 0.28 |
| 30 days | 61.00 ± 0.93 | 58.20 ± 1.03 | 42.79 ± 1.32 | 37.52 ± 0.48 | 39.17 ± 1.19 | 36.44 ± 0.39 |
| 60 days | 69.02 ± 0.78 | 66.16 ± 1.54 | 50.64 ± 1.35 | 44.67 ± 0.69 | 48.56 ± 0.98 | 44.68 ± 1.00 |

*Means ± SD, n=5 in each group.*
**—with Vaseline*

However, after two hours and 60 days, CHX-TA generated less bacterial inhibition ($p<0.001$). The addition of a Vaseline coating significantly decreased the antibacterial effect at all times except for the 60-day old specimens ($p<0.001$).

## DISCUSSION

All the specimens released fluoride, which decreased with time, similar to what has been demonstrated previously.[22] It was observed in the group with CHX and TA that the total fluoride 60-day release was about 30% lower than that for the group with GI alone. This might be explained by the interaction between fluoride and the cationic CHX molecule, resulting in the precipitation of salts with lower solubility, leaving fluoride less available. Experiments mixing CHX with sodium monofluorphosphate resulted in the precipitation of insoluble salts.[32] However, in dentifrices containing 0.1% NaF and 2% CHX, the available fluoride is not reduced by adding CHX.[33] Furthermore, the combination of a CHX/thymol-containing varnish (Cervitec, 1% CHX) with a fluoride-containing varnish (Fluor Protector, 0.1% difluorosilane) was at least as effective as the CHX varnish alone in reducing the number of interdental ms three months after application.[34]

The clinical significance of 30% less fluoride release is not known but, comparing Ketac Fil and Vitremer, fluoride release from Vitremer was more than six times lower than Ketac Fil.[35] According to McComb and others,[21] these two materials were equally effective in inhibiting secondary caries. It is also not known whether the present material can be recharged with fluoride similar to other glass ionomers.[36]

Using completely cured specimens placed on inoculated agar plates, only the specimens containing CHX had an antibacterial effect in this study. In other studies, unset material applied directly into agar wells had an antibacterial effect.[26,37-38] This was probably due to the substantial diffusion of antibacterial components, as the setting reaction was disturbed and the setting incomplete.

The addition of TA (in order to reduce the setting time) resulted in a reduction of the antibacterial effect, apparently in contrast with earlier findings[37] in which the above mentioned well-technique was used. One reason for the reduction in antibacterial properties could be a reaction between CHX and TA, as the divalent cation CHX might react with the carboxyl groups of TA. This is supported by the observation in the laboratory by the authors of the current study that a gel was formed when mixing CHX and low concentrations of TA (below 8%) in an aqueous solution. In the current study, an excess of TA (11%) was used and no gel was formed. Similarly, CHX might also react with the polyacrylic acid and disturb the setting reaction, thus creating cement with less optimal chemical and mechanical properties.[23,26]

Coating the specimens with Vaseline, a common procedure to reduce water contamination during setting[39] before placing them in water had a small but significant effect on the cumulated fluoride release at 1, 20 and 60 days (Table 1).

A substantially greater effect of the Vaseline-coating than that on fluoride release was seen on the antibacterial properties. The inhibition zones decreased in both the GI-CHX and GI-CHX-TA groups, particular-

ly at baseline (Figure 2), but this effect decreased with time. This might be due to the Vaseline acting as a more effective diffusion barrier against the larger amphiphilic CHX-molecule, as it might interact with the non-polar petroleum jelly. After a few days, the Vaseline was probably worn off by specimen handling.

*In vitro*, a significant antibacterial effect of GI with CHX or CHX-TA can be expected for at least 60 days. GI alone has a demonstrated antibacterial effect in other studies,[24,40] but this has not been observed under test conditions in the current study. However, a moderate antibacterial effect has been observed with unset GI alone, provided it is applied to agar plates in an unset form, although it was low when compared to a similar test material (GI-CHX).[37] In the clinical situation, the retention time of such a varnish-like compound in the interproximal spaces or in deeper sections of a fissure might also be as long as 60 days. However, with dental hygiene habits using toothpicks or dental floss, this is not probable. Also, other factors, including material brittleness and susceptibility to erosion, might be clinically significant, particularly for smooth surfaces.

With the current study design, it is not known whether CHX is released from the surface alone or also from deeper sections of the specimens. If CHX is released from the surface only, an enhanced antibacterial effect could be expected in time, due to the erosion exposing a new surface to release CHX. This remains to be investigated.

The mechanical properties of the material depend on the concentration of CHX. According to Jedrychowski and others,[23] glass ionomer cement deteriorates after the addition of CHX to concentrations above 5%.

The anti-cariogenic effect of the material depends on a combination of retention time and amount of chlorhexidine and fluoride released from the material. The anti-cariogenic properties of glass ionomer have been demonstrated,[21] and it can be assumed that an additive effect of CHX can have a clinical relevance. However, using the current formulation,[31] no such effect could be demonstrated for fissure caries in a highly caries active population. Still, the potential benefits of adding CHX or other antibacterial compounds to GI need to be further elucidated.

## CONCLUSIONS

The addition of chlorhexidine gluconate and tartaric acid to glass ionomer luting cement renders a varnish that releases fluoride and exerts an antibacterial effect on mutans streptococci, *in vitro*. Fluoride release and antibacterial effect decrease with time but remain measurable after 60 days.

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