



Bellis, C., Nobbs, A., O'Sullivan, D., Holder, J., & Barbour, M. (2016). Glass ionomer cements functionalised with a concentrated paste of chlorhexidine hexametaphosphate provides dose-dependent chlorhexidine release over at least 14 months. *Journal of Dentistry*, 45, 53-58. <https://doi.org/10.1016/j.jdent.2015.12.009>

Peer reviewed version

Link to published version (if available):
[10.1016/j.jdent.2015.12.009](https://doi.org/10.1016/j.jdent.2015.12.009)

[Link to publication record in Explore Bristol Research](#)
PDF-document

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Glass ionomer cements functionalised with a concentrated paste of chlorhexidine hexametaphosphate provides dose-dependent chlorhexidine release over at least 14 months

Abstract

Objectives

The aim of this study was to create prototype glass ionomer cements (GICs) incorporating a concentrated paste of chlorhexidine-hexametaphosphate (CHX-HMP), and to investigate the long-term release of soluble chlorhexidine and the mechanical properties of the cements. The purpose is the design of a glass ionomer with sustained anticaries efficacy.

Methods

CHX-HMP paste was prepared by mixing equimolar solutions of chlorhexidine digluconate and sodium hexametaphosphate, adjusting ionic strength, decanting and centrifuging. CHX-HMP paste was incorporated into a commercial GIC in substitution for glass powder at 0.00, 0.17, 0.34, 0.85 and 1.70% by mass CHX-HMP. Soluble chlorhexidine release into artificial saliva was observed over 436 days using absorbance at 255 nm. Diametral tensile and compressive strength were measured after 7 days' setting (37°C, 100% humidity) and tensile strength after 436 days' aging in artificial saliva. 0.34% CHX-HMP GICs were tested for their ability to inhibit growth of *Streptococcus mutans in vitro*.

Results

GICs supplemented with CHX-HMP exhibited a sustained dose-dependent release of soluble chlorhexidine. Diametral tensile strength of new specimens was unaffected up to and including 0.85% CHX-HMP, and individual values of tensile strength were unaffected by aging, but the proportion of CHX-HMP required to adversely affect tensile strength was lower after aging, at 0.34%. Compressive strength was adversely affected by CHX-HMP at substitutions of 0.85% CHX-HMP and above.

Conclusions

Supplementing a GIC with CHX-HMP paste resulted in a cement which released soluble chlorhexidine for over 14 months in a dose dependent manner. 0.17% and 0.34% CHX-HMP did not adversely affect strength at baseline, and 0.17% CHX-HMP did not affect strength after aging. 0.34% CHX-HMP GICs inhibited growth of *S. mutans* at a mean distance of 2.34 mm from the specimen, whereas control (0%) GICs did not inhibit bacterial growth.

Clinical Significance

Although GICs release fluoride *in vivo*, there is inconclusive evidence regarding any clinical anticaries effect. In this study, GICs supplemented with a paste of chlorhexidine-hexametaphosphate (CHX-HMP) exhibited a sustained release of chlorhexidine over at least 14 months, and small additions of CHX-HMP did not adversely affect strength.

Keywords: glass ionomer; restorative; antimicrobial; chlorhexidine; biomaterials

Introduction

Glass ionomer cements (GICs) are used for a number of purposes, including as direct restorative materials, lining and luting materials, adhesives, and in atraumatic restorative therapy. GIC restorations typically have shorter lifetimes than composites or amalgams (1-3), although the reasons for this are complex and, of course, the materials are not selected at random but are chosen by the clinician according to clinical need. When a GIC does fail, there are a number of potential reasons, one of which is secondary caries; this is responsible for 25% of failures of GIC-lined restorations after 18 years of clinical service (4) and around 18% of GIC failures over a range of 0.1-23 years (5).

GICs leach fluoride into the oral environment. This results in elevated fluoride concentrations close to the restoration, and thus there is an hypothesis that this may reduce dental caries in the local area owing to the interaction of the fluoride ion with the hydroxyapatite in the enamel and dentine. This hypothesis is broadly supported by *in vitro* data, but *in situ* and clinical studies of caries incidence in the vicinity of fluoride-releasing restoratives do not show consistent results (6). At the current time it is not possible to conclude whether fluoride release from GICs provides lasting protection against dental caries (6, 7).

Chlorhexidine (CHX) is a biocide with broad spectrum efficacy against a wide range of microbes. Its main application in dentistry is as a topical agent, usually in oral care products, products for treatment of periodontal disease, and varnishes. CHX is efficacious against the microbes implicated in dental caries, and is used in a number of products designed to protect the dentition against decay. However, the CHX salts in currently available commercial form have poor retention *in situ*, providing typically a few hours of antimicrobial function. One commercial material used for treatment of periodontal disease provides some sustained CHX release, but this is a short-term effect with 80% of the CHX released within the first 2 days, and a very slow release over the following 3-4 weeks (8).

There have been a number of attempts to incorporate CHX into GICs, with the aim of creating a restorative material that offers lasting protection against caries. GICs doped with CHX diacetate and CHX digluconate have been reported, and these inhibited growth of *Streptococcus mutans* and *Lactobacillus acidophilus*, but there was also some deterioration of mechanical properties and the antimicrobial effects were limited to the first 40-90 days of the study, with no bactericidal effect observed after this time (9). There are also reports of an increase in porosity and setting time and a reduction in hardness and tensile bond strength when GICs are doped with CHX digluconate (10, 11). CHX diacetate and CHX hydrochloride have also been incorporated into GICs, and these too inhibited growth of caries-causing organisms, but CHX release was only observed for 24 h so it is not clear how long this effect would be sustained (12). CHX diacetate doped resin modified GICs exhibited some sustained release of CHX, although this reached completion in 14-21 days (13). The release profiles of soluble CHX from GICs doped with these conventional salts of CHX exhibit a high initial release followed by little or no sustained release, and this perhaps explains the cytotoxic effects observed against fibroblasts when GICs were doped with 1 % CHX diacetate (14). However, supplementation of a resin-modified GIC with CHX digluconate at modest concentrations (1.25%) had no adverse effects on osteoblasts *in vitro* and resulted in an elimination of *Streptococcus mutans* populations following indirect pulp treatment *in vivo* (15), suggesting a potential clinical benefit of a CHX-functionalised restorative material.

We have previously reported the use of a novel salt of CHX: CHX-hexametaphosphate (CHX-HMP) (16). CHX-HMP has a lower solubility than CHX digluconate or CHX diacetate and, when used as a coating or dopant, can confer a sustained release of CHX that persists for at least three months (17). We have described the use of CHX-HMP as a filler for GICs (18). In that study, large clusters of CHX-HMP particles were used, and the size of these large particles, which were formed due to the production process, is likely to account for the adverse effects on the mechanical properties observed. The aim of the study described here was to investigate the use of CHX-HMP particles as GIC fillers but using a new preparation method which omits the drying process which creates the large aggregates and instead

uses a process of ionic strength adjustment and centrifugation to sequester the particles. CHX release was probed over a clinically relevant timescale of over one year, and both compressive and tensile strength were investigated; the latter was measured also after 14 months' aging to determine if the modification of the cement adversely affects long-term mechanical properties.

The hypothesis was that the prototype cements incorporating a concentrated paste of CHX-HMP particles would confer a more sustained CHX release, sufficient to inhibit growth of cariogenic microbes, coupled with less adverse effects on mechanical properties in comparison to large, dry aggregates of CHX-HMP or conventional salts of CHX such as digluconate or diacetate.

Methods

Preparation of CHX-HMP paste

Aqueous 10 mM solutions of CHX digluconate and sodium HMP were prepared. 100 mL of each solution were combined in a glass beaker under ambient laboratory conditions. The suspension created was stirred vigorously for approximately 1 min, then 30 mL 1 M potassium chloride was added. Stirring continued for a further 1 min before the preparation was allowed to settle for 24 h. The precipitate settled at the bottom of the flask and the supernatant was gently discarded leaving a concentrated suspension of the precipitate. This suspension was then centrifuged at 4760 g for 30 min. The supernatant was again discarded and the pellet of paste was removed from the centrifuge tubes using a spatula and used immediately.

Preparation of specimens

A commercially available GIC, Diamond Carve™ (Kemdent Ltd, Purton, UK), was used as the base material to create the experimental cements. This GIC comprises a powder, consisting of alumina-silica based glass filler particles which contain calcium fluoride and other minor salt components and freeze dried poly(vinyl)phosphonic acid, and a liquid, which contains polyacrylic and tartaric acids. The

manufacturers' instructions indicate that the powder and liquid should be mixed in a 4:1 ratio by mass to create the finished cement.

The water content of the paste was established to allow the concentration of the liquid component of the GIC to be adjusted to account for the additional water in the CHX-HMP paste. CHX-HMP paste was weighed as freshly prepared, then stored at 37°C and weighed periodically until the mass of the powder was constant, indicating that the available water had evaporated (24 h). This revealed a composition of 83% water and 17% CHX-HMP particles. The GIC liquid component was thus prepared at a concentration that resulted in the standard final concentration when diluted by the paste. The paste was substituted for the overall mass at 0, 0.17, 0.34, 0.85 and 1.70 % by mass of CHX-HMP (0, 1, 2, 5 and 10% by mass of the paste). The paste was mixed into the liquid first and then the powder was added to the paste-liquid combination. Mixing of the specimens was completed in 40-50 s and packing into the moulds took a further 10 s, such that all manipulation of the cement was completed within 1 min.

GICs were packed, using a stainless steel spatula, into stainless steel moulds with dimensions of 6 mm height and 4 mm diameter (for compressive strength determination) or 4 mm height and 6 mm diameter (for measurement of diametral tensile strength and elution of CHX). The moulds were lined with a thin layer of petroleum jelly to aid removal of the set cement. Immediately after packing, the moulds were placed between two sheets of acetate and a 2 kg weight placed on top of the specimens on a flat surface in order to ensure even distribution of the cement. After 5 minutes the specimens were sanded using a P120 grit sanding disc (Hermes, Hamburg, Germany) to remove excess material and were then placed into small, sealed plastic vessels containing wet tissue paper packed into the lid to achieve 100% humidity without direct contact with water. Specimens were stored at 37°C for 7 days prior to any further testing to ensure the setting process had reached completion.

Compressive strength (CS) testing

CS was measured by applying a compressive force to the flat surface of the cylindrical specimens using a universal testing machine (Zwick/Roell Universal Testing Machine, Zwick/Roell, Leominster, Herefordshire, UK) and recording the load at fracture. Specimens were examined after fracture for evidence of flaws on the internal or external surfaces and data from flawed specimens were rejected. Load at fracture L_F was used with diameter D to calculate CS according to the relationship $CS = 4L/\pi D^2$. The load was used in conjunction with the average diameter of the specimen. Specimen dimensions were measured three times using a digital micrometer. $N = 24$ specimens per group were used. Data were analysed using a one-way ANOVA followed by a Tukey Honestly Significant Difference post-hoc test.

Diametral tensile strength (DTS) testing

DTS was measured by applying a compressive force to the curved sides of the cylindrical specimen using a universal testing machine and recording the load at fracture. Specimens were examined after fracture for evidence of flaws on the internal or external surfaces and data from specimens found to be flawed were rejected. The load at fracture L_F was used in conjunction with the average diameter D and height h of the specimens to calculate DTS according to the relationship $DTS = 2L/\pi Dh$. Specimen dimensions were measured three times using a digital micrometer.

$N = 24$ specimens per group were used for the “new” specimens; these were prepared, allowed to set for 7 days then tested immediately. Specimens were also tested after 436 days’ elution, to establish the diametral tensile strength of “aged” specimens. $N = 15$ specimens were used for this test. Data were analysed using one-way ANOVA followed by Tukey Honestly Significant Difference post-hoc tests.

Characterisation of CHX release

GIC specimens were weighed using a precision balance then placed in individual cuvettes (Z637157-100EA, Sigma-Aldrich, Gillingham, UK) transparent to ultraviolet (UV) light. 1.5 mL artificial saliva was added to each cuvette. The artificial saliva was composed of 0.9 mM CaCl₂, 0.2 mM MgCl₂, 4.0 mM KH₂PO₄, 30.0 mM KCl, 20.0 mM HEPES buffer, titrated to pH of 6.8. The cuvettes were sealed with tightly-fitting lids (SEMA2533, VWR, Lutterworth, UK) and then placed onto an orbital shaker (SSM1, Stuart, Staffordshire, UK) at 150 rpm and readings taken initially once a day, and less frequently as CHX release decelerated. Artificial saliva was refreshed at two-week intervals to avoid any decrease in CHX release that could be attributed to saturation of the artificial saliva with respect to CHX salts. Adsorption of light at wavelength 255 nm was measured at regular intervals using a spectrophotometer (Hitachi U-1900, Hitachi, Japan) and calibration standards of 5 – 50 µM CHX used as references to establish CHX release from the GICs into the artificial saliva (19). This was converted to µmoles CHX released per unit surface area for each specimen and normalised by subtracting the mean reading for the 0% substitution, correcting for other eluents of the GIC such as the polyacrylic acid (18). N = 15 specimens per group were used.

Microbiological testing

Streptococcus mutans GS-5 was cultivated anaerobically at 37 °C on BHYN agar (per litre: 37 g Brain Heart Infusion, 5 g yeast extract, 5 g Neopeptone, 15 g agar). Suspension cultures were grown in BHY medium (per litre: 37 g Brain Heart Infusion, 5 g yeast extract) in sealed bottles and incubated stationary at 37 °C for 16 h. Bacterial cells were washed once with phosphate-buffered saline (PBS) by alternate centrifugation (5000 *g*, 7 min) and suspension, and suspended in PBS at OD₆₀₀ 1.0 (approximately 2 x 10⁸ cells/ml). A lawn of bacterial growth was generated by spreading 100 µl of adjusted *S. mutans* suspension onto a BHYN agar plate, which was then incubated anaerobically at 37 °C for 16 h.

The GIC specimens were made using the method described above in a 0% (control) and a 0.34% substitution. These specimens were prepared using a sterile spatula and moulds in a biological safety cabinet (ESCO airstream, ESCO micro pte ltd, Singapore). The specimens were left to mature in a moist environment for 7 days at 37 °C, then placed onto the bacterial lawn plates (with minimal force to ensure no movement once in the incubator). Lawn plates were incubated for 16 h at 37 °C under anaerobic conditions and the zones of inhibition (clearance) measured by diameter.

Results

Compressive strength

Rejection of specimens with internal voids, imperfections or non-linear force-distance curves resulted in final n per specimen group of 16-21 (mean n = 19). CS data are shown in Figure 1. There was no statistically significant difference between CS of control, 0.17 or 0.34% CHX-HMP specimens. 0.85 CHX-HMP had significantly lower CS than control, 0.17 or 0.34% specimens, and 1.70% CHX-HMP had statistically significantly lower CS than all other groups.

Diametral tensile strength

Rejection of specimens with internal voids, imperfections or non-linear force-distance curves resulted in final n per specimen group of 18-24 (mean n = 21). DTS data are shown in Figure 2. The only group that was statistically significantly different from the control was the 1.70 % CHX-HMP, which had a reduced DTS ($p < 0.0001$). 0.17 and 0.34% CHX-HMP had a numerically higher DTS than control specimens but this was not statistically significant ($p = 0.981$ and 0.638 respectively).

Diametral tensile strength of aged specimens

Rejection of specimens with internal voids, imperfections or non-linear force-distance curves resulted in final n per specimen group of 10-13 (mean n = 11.6) for specimens aged for 436 days. DTS data are shown in Figure 3. It can be seen that there were numerical changes in DTS compared to baseline

values, but these were not statistically significant. For the aged specimens, 0% and 0.17% formed a homogeneous group, 0.34% was significantly lower than 0 and 0.17%, and 0.85% and 1.70% were significantly lower still.

Characterisation of CHX release

Elution of soluble CHX from CHX-HMP doped GICs normalised to control specimens are shown in Figure 4 and Figure 5. Figure 4 shows mean cumulative CHX elution for specimens with 1.70% CHX-HMP at all time points measured. The purpose of displaying the data in this way is to illustrate the smooth curve, indicating that the practice of refreshing the artificial saliva elution medium at 14 day intervals was sufficient to prevent saturation with respect to CHX salts from inhibiting the release of further CHX. There is a slight suggestion that there is a step in CHX concentration between day 28 and day 29 (ie before and after a saliva change) but no other such step changes or accelerations of CHX elution are observed.

In Figure 5, data from day 14x is shown illustrating CHX release as a function of time and dose of CHX-HMP. A dose response is observed, with greater CHX-HMP substitution in the GIC resulting in a greater, and faster, release of CHX-HMP throughout the experimental period. All specimens groups were still releasing CHX at the conclusion of the experiment, with the greatest release observed for the higher CHX-HMP substitutions.

The mean cumulative CHX release at each 14x day time was compared to the lowest substitution, to establish the relationship between CHX-HMP dose and CHX release. There was a non-linear relationship between CHX-HMP dose and CHX release; while the ratio of CHX-HMP concentration for 0.17, 0.34, 0.85 and 1.70 % CHX-HMP was 1:2:5:10, the ratio of CHX release was 1:4.6:12.3:30.6.

Microbiological testing

0.34% CHX-HMP GICs exhibited zones of inhibition on the lawn of *Streptococcus mutans*. The mean zone of inhibition was 2.34 mm (standard deviation 1.19 mm) from the periphery of the specimen. The control GICs displayed no zones of inhibition.

Discussion

CHX-HMP particles in a concentrated aqueous paste were prepared and incorporated into a commercial GIC, using a bespoke polyacid formulation to correct for the water fraction of the CHX-HMP paste. The experimental GICs exhibited a sustained release of aqueous CHX into artificial saliva. This CHX release continued for the duration of the experiment (436 days), and although all decelerated over the course of the experiment, even the lowest substitution (0.17%) had a gradient >0 at the conclusion of the experiment. For the higher substitutions there was still a steady and substantial release of CHX when the experiment concluded.

The CHX release observed from GICs supplemented with CHX-HMP was sustained for much longer than that achieved using CHX digluconate or CHX diacetate as the dopant (9). This is likely to be owing to the low solubility of CHX-HMP; the CHX salt is added as a solid filler which is incorporated into the GIC structure, only releasing its soluble CHX payload as some of the particles gradually dissociate. This is in contrast to the approach of supplementing a GIC with CHX digluconate or diacetate; in this case the CHX is added either already in solution (digluconate) or as a solid, but highly soluble, filler. It is not surprising that these approaches lead to a rapid release of CHX in contrast to CHX-HMP.

CHX release was dose-dependent, with a non-linear relationship; the greater the CHX-HMP in the GIC, the more the CHX release, but CHX release increased faster than CHX-HMP dose. This may reflect a less stable structure for the higher substitutions; with the higher dopings of CHX-HMP, the particles disrupt the setting process of the GIC, meaning that the GIC is more porous and the CHX-HMP particles embedded deep within the cement structure are “accessible” to the artificial saliva and contribute to

the CHX release. In contrast, those with lower dopings of CHX-HMP have an effective setting process and the particles that contribute to the CHX release are only those close to the GIC's external surface. This is supported by the mechanical property data (discussed below) which indicates that the higher dopings of CHX-HMP adversely affect the material's strength.

The presence of soluble CHX in the elution medium was insufficient during the experiments to restrict any further CHX release owing to the large solution:surface area ratio, the agitation of the reaction vessels (cuvettes) and the regular changes of artificial saliva. It should be noted that the conditions used in this study are not an accurate representation of the clinical scenario; much higher shear conditions and larger volumes of saliva were used in this study than would be in contact with the GIC at the margins of a restoration. As such it is likely that the *rate* of CHX release would be slower *in vivo*, because local low shear forces and small fluid volumes would result in a local saturation with respect to CHX. That is to say, it is likely that CHX concentrations at the restoration-tooth interface would be higher than reported here, as there is little shear or diffusion to remove the CHX, and that these locally high CHX concentrations would likely slow the subsequent release of CHX owing to saturation effects. Thus the true duration of CHX release from these cements could only be determined from a study with a more accurate depiction of the clinical conditions, but it is hypothesised to be longer than that observed here.

DTS of the GICs was not statistically significantly affected by addition of CHX-HMP, compared to the unmodified cement, up to and including 0.85% CHX-HMP (Figure 2). There was a numerical rise in DTS up to 0.34% CHX-HMP and then a fall with the lowest value recorded for 1.70% CHX-HMP, but only the 1.70% CHX-HMP group differed from the control to a statistically significant degree. DTS of specimens aged for 436 days indicated no statistically significant changes compared to the baseline values. This is consistent with previously published data, indicating that while flexural strength of GICs typically decreases after laboratory aging, by as much as 55% (20), compressive and tensile strengths tend to remain similar or increase (21), as was observed here. Although there were increases in DTS

for control and 0.17 % CHX-HMP and a decrease in DTS for 0.34% CHX-HMP, these were not statistically significant (Figure 3). One outcome of this was that the substitution of CHX-HMP required to affect a reduction in DTS was lower with aged specimens; 0.34% CHX-HMP specimens had a statistically significantly lower DTS than control specimens after 436 days' aging, as compared to 1.70% for new specimens. This development can be attributed to the increase in DTS for control specimens, as well as the decrease in DTS for specimens with 0.34% CHX-HMP, associated with aging.

CS of GICs was affected by addition of CHX-HMP; cements with 0.84 and 1.70% CHX-HMP had statistically significantly reduced CS compared with control, but those with 0.17 and 0.34% were equivalent to the unmodified cement. CS is acknowledged as one of the more sensitive properties as regards modifications to GIC formulations (22) and thus it is not surprising that this parameter was affected by CHX-HMP addition more than DTS.

The lower strengths of the GICs with the higher substitutions of CHX-HMP can be explained by observing that, in increasing the doping of CHX-HMP, the ratio of polyacid to glass is altered, with less glass to account for the greater proportion of CHX-HMP. This will disrupt the GIC setting process as fewer glass particles and thus fewer bi- and trivalent ions are available to cross-link the polyacid matrix. Thus those specimens with more CHX-HMP are likely to have a less complete setting reaction, and this is likely to explain the reduced strength for these specimens.

The 0.34% CHX-HMP GIC was tested for its ability to inhibit the growth of oral pathogen *Streptococcus mutans in vitro*. This GIC formulation was selected on the basis that it was the highest substitution of CHX-HMP that did not have an adverse effect on initial mechanical properties. It was observed that these GICs inhibited growth of the microorganism to a mean distance of 2.34 mm from the periphery of the specimen, whereas control GICs displayed no zone of inhibition. This indicates that, *in vitro* and utilising a simple, single species model, the concentration of CHX released by the 0.34% CHX-HMP GIC was sufficient to inhibit growth of this cariogenic organism.

The strength data, particularly the DTS of the aged specimens combined with the CS data, suggests that if such a GIC were to find clinical application, the lower substitutions of CHX-HMP paste would be more suitable, as these have no negative impact on mechanical properties. The 0.34% CHX-HMP GIC, which was the highest concentration of CHX-HMP while not adversely affecting mechanical properties at baseline, inhibited growth of *Streptococcus mutans* in an in vitro zone of inhibition model. Noting the comments above regarding the experimental conditions, the next step should be to determine whether this cement can inhibit the growth of caries-causing microorganisms under flow conditions more representative of the clinical environment of a GIC.

Conclusions

CHX-HMP particles in a concentrated paste were incorporated into a commercial GIC, with adjustment of the acid concentration in the commercial GIC to account for the water in the paste. Higher substitutions of CHX-HMP paste (0.85 and 1.70% CHX-HMP by mass, or 5 and 10% paste by mass) had a negative impact on compressive strength, but lower substitutions had no significant impact on compressive strength. None of the prototype cements displayed a reduction in diametral tensile strength at baseline; DTS after 436 days aging was not statistically significantly different when comparing specimens with and without aging, although the substitutions of 0.34% and higher were significantly weaker than the control cement after aging. All of the prototype cements exhibited a sustained release of soluble CHX over the 436 day experimental period.

Figures

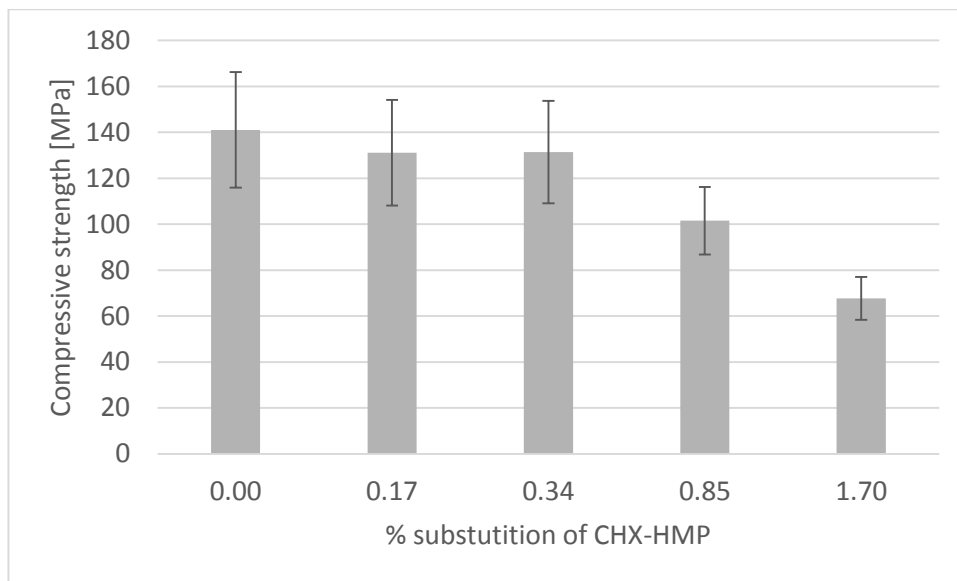


Figure 1. CS of GIC specimens as a function of CHX-HMP substitution. Error bars represent standard deviations.

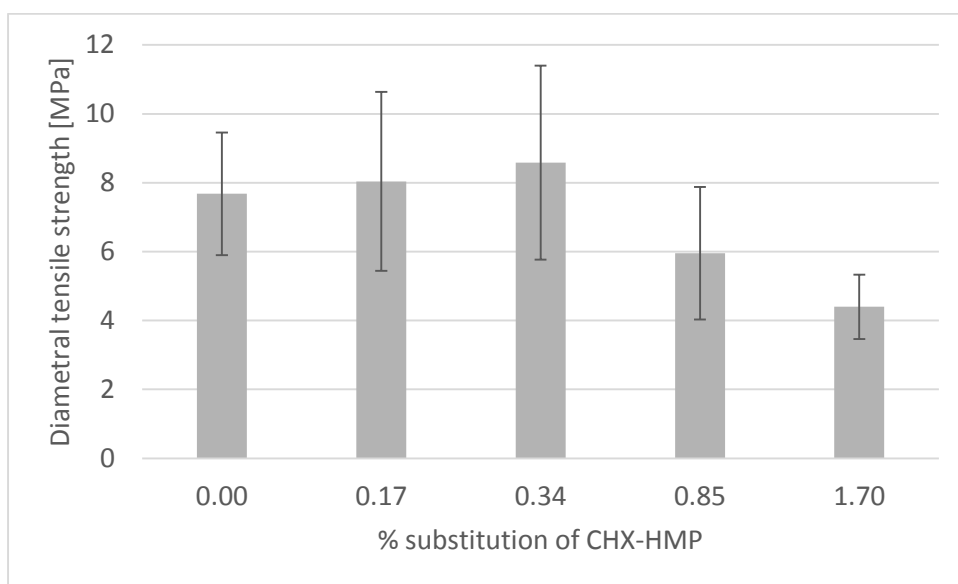


Figure 2. DTS of GIC specimens as a function of CHX-HMP substitution. Error bars represent standard deviations.

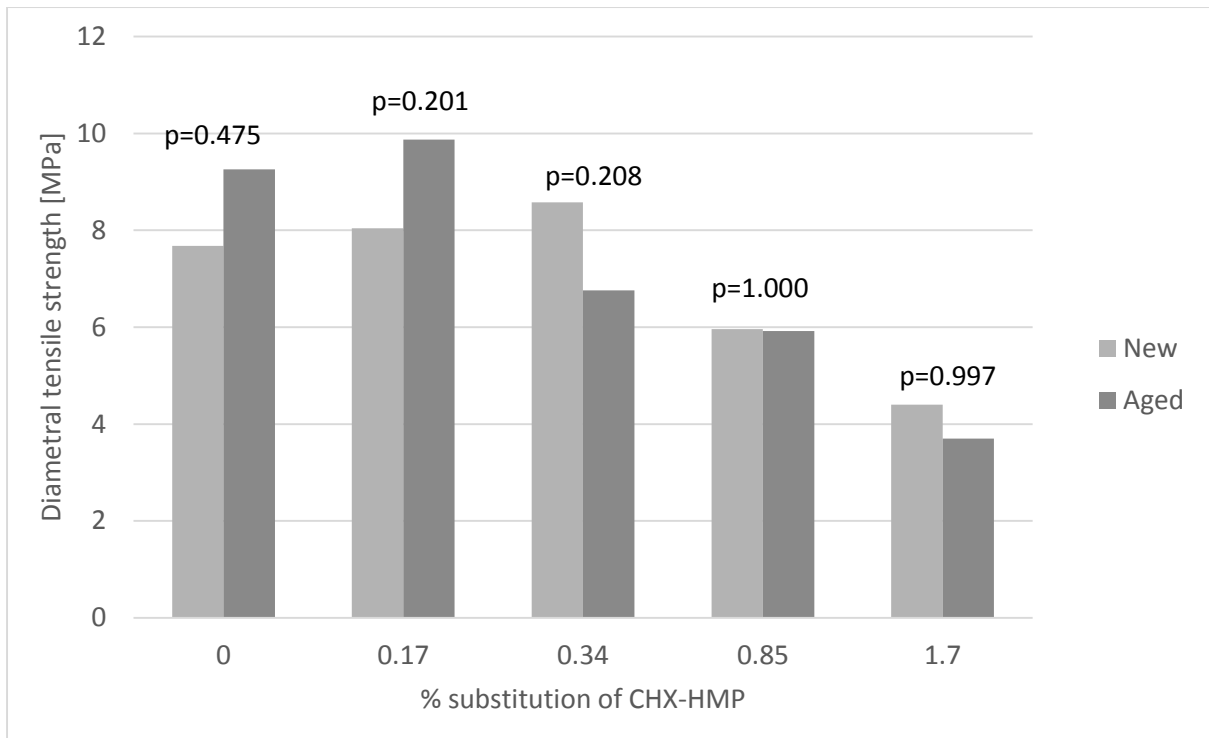


Figure 3. Diametral tensile strength of newly prepared GIC specimens (“new”) and specimens immersed in artificial saliva, refreshed fortnightly, for 436 days (“aged”). p values refer to comparisons of new and aged specimens for a given % substitution of CHX-HMP; although numerical differences were observed these were not statistically significant.

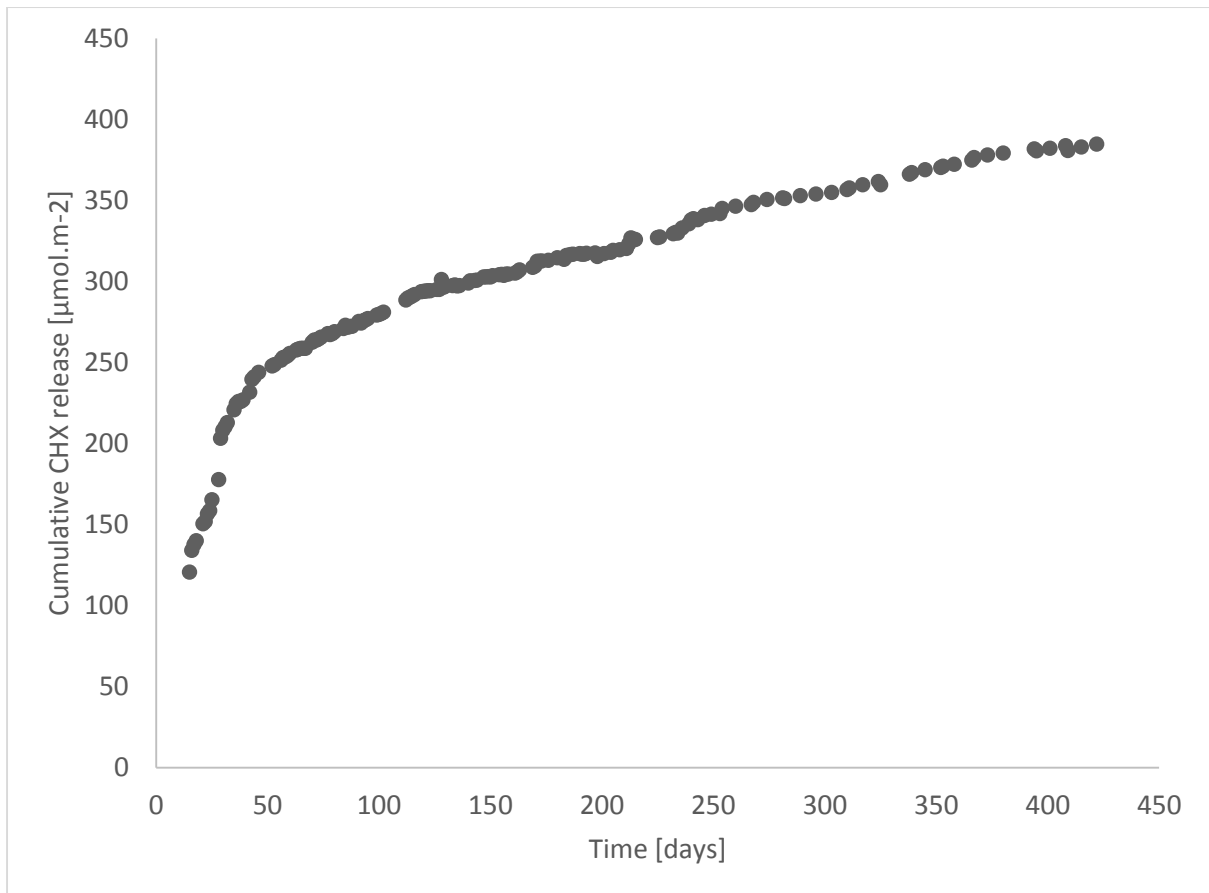


Figure 4. Cumulative CHX release from GIC specimens containing 1.70% CHX-HMP in substitution for the powder component. This figure shows data points from all sampling days, indicating the smooth transition between the 14 day periods, and no local saturation existing prior to artificial saliva change, with one possible exception where there is a step change from day 28 (before saliva change) to day 29 (after saliva change) indicating the possibility of some minor inhibition of CHX release at this single time point.

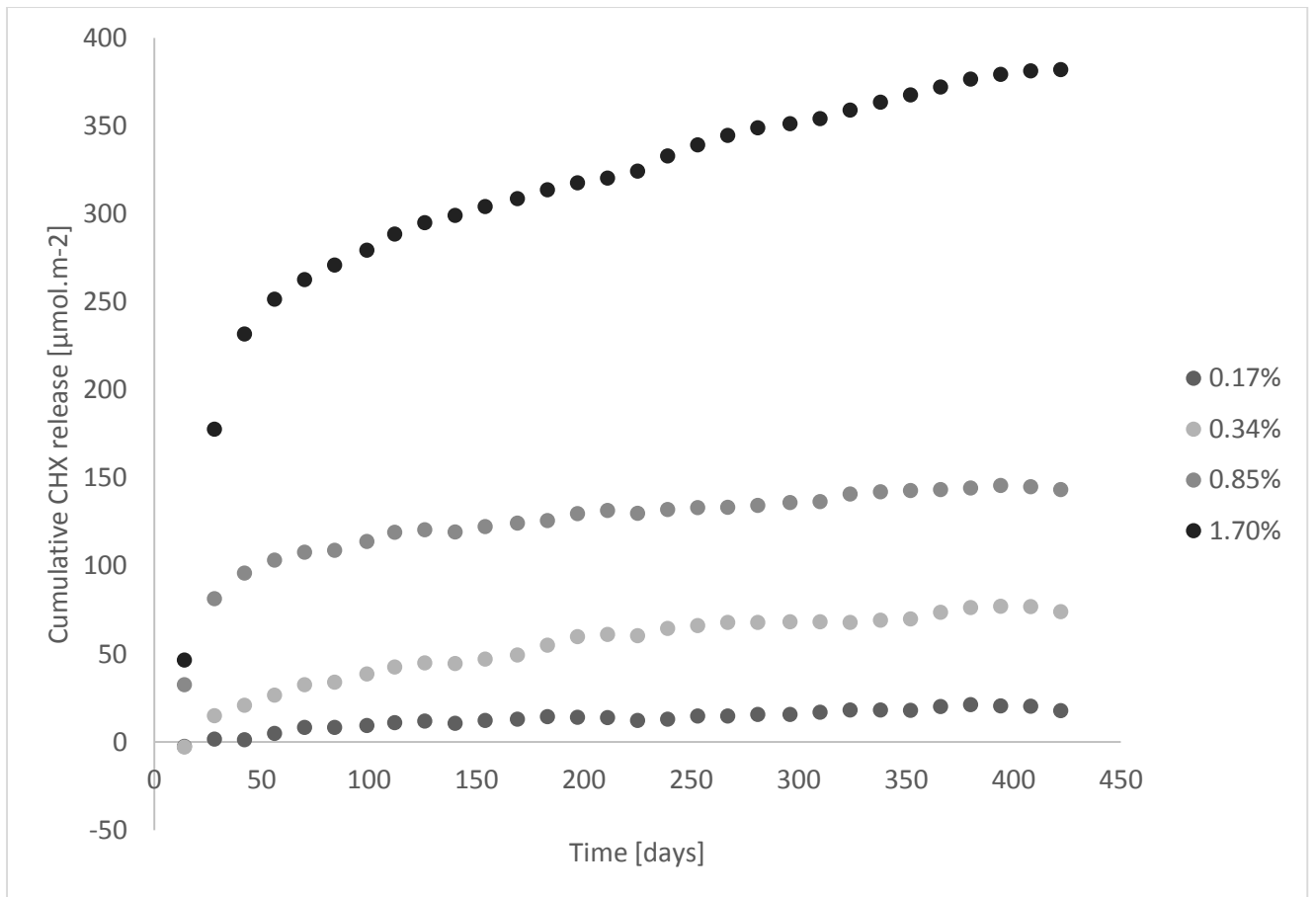


Figure 5. Cumulative CHX release from GIC specimens as a function of CHX-HMP substitution for the powder component of the cement. Data is presented at 14 day intervals.

Bibliography

1. Pinto Gdos S, Oliveira LJ, Romano AR, ScharDOSim LR, Bonow ML, Pacce M, Correa MB, Demarco FF, Torriani DD. Longevity of posterior restorations in primary teeth: results from a paediatric dental clinic. *Journal of Dentistry* 2014; **42**: 1248-54.
2. Burke FJ, Lucarotti PS. How long do direct restorations placed within the general dental services in England and Wales survive? *British Dental Journal* 2009; **206**: E2; discussion 26-7.
3. Hurley E, Da Mata C, Stewart C, Kinirons M. A study of primary teeth restored by intracoronal restorations in children participating in an undergraduate teaching programme at Cork University Dental School and Hospital, Ireland. *European Journal of Paediatric Dentistry* 2015; **16**: 78-82.
4. van de Sande FH, Rodolpho PA, Basso GR, Patias R, da Rosa QF, Demarco FF, Opdam NJ, Cenci MS. 18-year survival of posterior composite resin restorations with and without glass ionomer cement as base. *Dental Materials* 2015; **31**: 669-75.
5. Namgung C, Rho YJ, Jin BH, Lim BS, Cho BH. A retrospective clinical study of cervical restorations: longevity and failure-prognostic variables. *Operative Dentistry* 2013; **38**: 376-85.
6. Wiegand A, Buchalla W, Attin T. Review on fluoride-releasing restorative materials-- fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. *Dental Materials* 2007; **23**: 343-62.
7. Yengopal V, Harneker SY, Patel N, Siegfried N. Dental fillings for the treatment of caries in the primary dentition. *Cochrane Database of Systematic Reviews* 2009: CD004483.

8. Tabary N, Chai F, Blanchemain N, Neut C, Pauchet L, Bertini S, Delcourt-Debruyne E, Hildeband HF, Martel B. A chlorhexidine-loaded biodegradable cellulosic device for periodontal pockets treatment. *Acta Biomaterialia* 2014; **10**: 318-29.
9. Turkun LS, Turkun M, Ertugrul F, Ates M, Brugger S. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. *Journal of Esthetics and Restorative Dentistry* 2008; **20**: 29-44; discussion 45.
10. Marti LM, Mata MD, Ferraz-Santos B, Azevedo ER, Giro EM, Zuanon AC. Addition of chlorhexidine gluconate to a glass ionomer cement: a study on mechanical, physical and antibacterial properties. *Brazilian Dental Journal* 2014; **25**: 33-37.
11. Marti LM, Becci AC, Spolidorio DM, Brighenti FL, Giro EM, Zuanon AC. Incorporation of chlorhexidine gluconate or diacetate into a glass-ionomer cement: porosity, surface roughness, and anti-biofilm activity. *American Journal of Dentistry* 2014; **27**: 318-22.
12. Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. *Dental Materials* 2006; **22**: 647-52.
13. Cheng L, Weir MD, Xu HH, Kraigsley AM, Lin NJ, Lin-Gibson S, Zhou X. Antibacterial and physical properties of calcium-phosphate and calcium-fluoride nanocomposites with chlorhexidine. *Dental Materials* 2012; **28**: 573-83.
14. Iz SG, Ertugrul F, Eden E, Gurhan SI. Biocompatibility of glass ionomer cements with and without chlorhexidine. *European Journal of Dentistry* 2013; **7**: S89-S93.
15. de Castilho AR, Duque C, Negrini Tde C, Sacono NT, de Paula AB, de Souza Costa CA, Spolidorio DM, Puppim-Rontani RM. In vitro and in vivo investigation of the biological and mechanical behaviour of resin-modified glass-ionomer cement containing chlorhexidine. *Journal of Dentistry* 2013; **41**: 155-63.

16. Barbour ME, Maddocks SE, Wood NJ, Collins AM. Synthesis, characterization, and efficacy of antimicrobial chlorhexidine hexametaphosphate nanoparticles for applications in biomedical materials and consumer products. *International Journal of Nanomedicine* 2013; **8**: 3507-19.
17. Wood NJ, Jenkinson HF, Davis SA, Mann S, O'Sullivan DJ, Barbour ME. Chlorhexidine hexametaphosphate nanoparticles as a novel antimicrobial coating for dental implants. *Journal of Materials Science: Materials in Medicine* 2015; **26**: 201.
18. Hook ER, Owen OJ, Bellis CA, Holder JA, O'Sullivan DJ, Barbour ME. Development of a novel antimicrobial-releasing glass ionomer cement functionalized with chlorhexidine hexametaphosphate nanoparticles. *Journal of Nanobiotechnology* 2014; **12**: 3.
19. Barbour ME, O'Sullivan DJ, Jagger DC. Chlorhexidine adsorption to anatase and rutile titanium dioxide. *Colloids and Surfaces A* 2007; **307**: 116-20.
20. Bapna MS, Gadia CM, Drummond JL. Effects of aging and cyclic loading on the mechanical properties of glass ionomer cements. *European Journal of Oral Sciences* 2002; **110**: 330-34.
21. Mitra SB, Kedrowski BL. Long-term mechanical properties of glass ionomers. *Dental Materials* 1994; **10**: 78-82.
22. Baig MS, Dowling AH, Cao X, Fleming GJ. A discriminatory mechanical testing performance indicator protocol for hand-mixed glass-ionomer restoratives. *Dental Materials* 2015; **31**: 273-83.