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| ORIGINAL

Release characterization and biological effect of Glass Ionomer Functionalized with two different chlorohexidine derivatives: an in vitro study

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ABSTRACT

Objective: To evaluate the effect of adding two different chlorohexidine derivatives; chlorhexidine hexametaphosphate and chlorhexidine digluconate to glass ionomer at three different concentrations (0.25%, 0.75%and 1.5%) regarding the antibacterial effect, chlorhexidine release and fluoride release. **Methods**: A total of 405 specimens were prepared and tested after 7 days, 3 months and 6 months of storage in distilled water (n=5).For testing antibacterial effect, chlorhexidine release and fluoride release, the mix was packed in a ready-made Split Teflon molds to obtain disc-shaped specimen with dimensions 10 mm in diameter and 2 mm thickness according to ISO standardizations. One-way ANOVA and One-way repeated measure ANOVA test were used for statistical analysis of data. **Results**: The incorporation of chlorhexidine into ChemFil Superior glass ionomer cement in both derivatives has high significance ability to provide a long-term antimicrobial effect on Streptococcus mutans and Lactobacillius acidophilus. The chlorhexidine release was increased by adding chlorhexidine in both derivatives to GIC than the unmodified Glass-ionomer cement for study duration. However, the fluoride release was decreased in the modified specimens than the original one. **Conclusion**: Addition of chlorhexidine enhanced the antibacterial effect of the glass ionomer and chlorhexidine release. However, fluoride release was reduced than original

Indexing terms: Chlorhexidine. Chlorhexidine digluconate. Glass ionomer cement. Lactobacillius acidophilus. Streptococcus mutans.

INTRODUCTION

Dental caries is a slow chronic disease that affects the enamel, dentine and cementum. It is characterized by localized destruction of dental hard tissues by acidic by-products released from bacterial fermentation of dietary carbohydrates [1]. Dental caries remains the most common spreader disease worldwide [2].

In the early 1970's, McLean and Wilson developed glass polyalkenoate cement, also known as "glass-ionomer." These cement systems are based on polycarboxylate and silicate materials. The early glass-ionomers offered several advantages for use in children. They were tooth colored, chemically bonded to tooth structure, and released substantial amounts of fluoride for uptake by adjacent tooth structure [3].

Glass-ionomer cement was found to be used efficiently in atraumatic restorative treatment (ART) approach in children rather than other restoration materials because of its adhesion property and on tooth surfaces that have had

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only minimal preparation. Glass ionomers have been improved by altering particle size and particle size distribution to withstand stresses of mastication; this has improved both compressive strength and physical properties [4].

Resin-modified glass-ionomers (RMGI) were introduce to improve the properties of conventional glass ionomer. Resin modified glass ionomer contains hydrophilic monomers and polymers like HEMA and they have higher flexural strength compared to conventional GIC [5].

Therefore, different trials to modify glass ionomer materials have been introduced to incorporated antimicrobial into restorations to eradicate the bacteria effect and reduce the risk of recurrent caries without negative effects on the properties of restorative materials and the long-term success of restorations [6].

Glass ionomer cements leach fluoride into the oral environment. This caused elevation of fluoride concentrations close to the restoration and 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 [7].

Chlorhexidine (CHX) is a broad-spectrum antimicrobial with widespread use as a topical agent; because of its antibacterial effects on both gram-positive and gram-negative organisms. It antimicrobial properties cause membrane disruption and is efficacious against a wide range of microbes including those implicated in caries cause the inhibition of bacterial accumulation on tooth surfaces [8].

Glass ionomer cement restorative material offers lasting protection against caries. GICs modified with CHX diacetate and CHX digluconate have been reported, and these inhibited growth of Streptococcus mutans and Lactobacillus acidophilus, but there was 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].

A recent in vitro study found that the addition of 0.5% chlorhexidine digluconate to GIC resulted in increased antimicrobial properties with no significant effect on the mechanical properties or setting time. However, higher concentrations of chlorhexidine digluconate (1%, 2%) increased the setting time and decreased the mechanical properties of the GIC [9].

There is development of new chlorhexidine salts formulations to enhance anti-caries effects. Among these salts is sodium hexametaphosphate (NA-HMP) which has a strong affinity to the enamel surface because of multiple binding sites, resulting in a reduced mineral loss when associated with fluoride [10].

A study described the use of CHX-HMP as an antimicrobial modification for GICs with solid and viscous paste formulations. The CHX release from the modified cements was prolonged causing long-term antibacterial effect; however this modification had adverse effects on the mechanical properties as the particle size, formulations of CHX salt and concentration had an effect on the CHX release profile [11].

The aim of the study was to evaluate the antimicrobial effect of conventional glass ionomer modified by addition of two different chlorhexidine derivatives, at three different concentrations regarding the chlorhexidine release and fluoride release.

METHODS

Materials used in the study

Materials used in the study, their composition, manufacturers and lot number are shown in table 1.

Specimens preparation and material testing

Specimens were prepared according to the ISO Guidelines No. 9917-2:2007(E) [12] Materials were proportioned and mixed according to the manufacturers' instructions. The methods of specimen's preparation for each material are summarized in table 2.

Table 1. Materials used in the study, their composition, manufacturers and lot numbers.

Brand name	Description	Composition	Manufacturer	Lot no.
Glass ionomer cement GIC	ChemFil® Superior Powder/ liquid GIC	1 g powder contains: 1.(0.84 g) Aluminium-sodium-calcium-fluoro-phosphoro-silicate (18: 9: 8: 16: 3: 46) 0.84 g 2.(0.15 g) Polyacrylic acid (MW 30000-45000) 0.15 g Liquid: 10 ml demineralized water	DENTSPLY DeTrey GmbH, Konstonz, Germany	1709000428
Chlorhexidine digluconate 20% aqueous solution	Colorless or pale yellowish liquid. It is miscible with water, soluble in acetone and in alcohol. The structural formula: C22H30Cl2N10, 2C6H12O7	Aqueous sol of chlorhexidine which cannot be isolated as a solid, Soluble in water to at least 50%(W/V), with PH range of 5-8, density 1.06 to 1.07 Melting range between 132 °C TO 136 °C	BAJAJ Healthcare LTD, Gujarat, India	CS-00400216
Sodium hexametaphosphate HMP powder	White crystals, odorless, hexamer of composition $(NaPO_3)_6$. Sodium hexametaphosphate, mixture of polymeric metaphosphates, formula: $(NaPO_3)_6$	65-70% P2O5 basis Natrium hexametaphoshate, calgon, phosphate glass, water soluble, polyphosphate sodium salt, Soluble in water, Melting range 628 °C	Sigma-Aldrich Co., St. Louis, MO, USA)	BCBN0343v
Distilled water	Purified water USP35	50 ml purified water for oral use	FIPCO, Borg Elarab, Alexandria, Egypt	29050/2013

Table 2. Methods of specimens' preparation for each material.

Type of material	Method of specimen's preparation	Type of chlorhexidine	Method of chlorhexidine application
Conventional glass ionomer cement specimens	- Ratio of 1 scoop powder: 1 drop of liquid was mixed with P/L ratio (wt/wt) of 7.4: 1 - Half the powder was incorporated first into the liquid as quickly as possible (5seconds) and then the remainder was added and spatulated for 20 second to form a thick putty-like consistency. - Total working time was 2 minutes. - Total setting time was 2-3 minutes.	- no chlorhexidine added	- no application done.
Glass ionomer containing chlorhexidine digluconate specimens	Ratio of 1 scoop powder: 1 drop of liquid containing chlorhexidine digluconate was mixed with P/L ratio (wt/wt) of 7.4: 1 - Half the powder was incorporated first into the liquid as quickly as possible (5seconds) and then the remainder was added and spatulated for 20 second to form a thick putty-like consistency. - Total working time was 2 minutes. - Total setting time was 2-3 minutes.	- chlorhexidine digluconate liquid	- C hlorhexidine digluconate was added to the distilled water at concentrations 0.25 %, 0.75% and 1.5% of CHX digluconate.
- Glass ionomer containing chlorhexidine hexametaphosp-hates specimens	- Ratio of 1 scoop powder: 1 drop of liquid containing chlorhexidine hexametaphosphate was mixed with P/L ratio (wt/wt) of 7.4: 1 - Half the powder was incorporated first into the liquid as quickly as possible (5seconds) and then the remainder was added and spatulated for 20 second to form a thick putty-like consistency. - Total working time was 2 minutes. - Total setting time was 2-3 minutes.	- chlorhexidine hexametaphosphate powder	- chlorhexidine hexametaphosphate (HMP) was prepared by mixing aqueous 10 ml solutions of CHX digluconate and sodium HMP in a glass beaker and vigorous stirring for approximately 1 min, then the preparation was allowed to settle for 24 h to produce a precipitate. - The precipitate was filtered from the flask and discarded leaving a concentrated suspension. The suspension was centrifuged at 4760 g for 30 min, and then filtered again to discard the new precipitate. The remaining paste was removed from the centrifuge tubes using a spatula. The paste was added to distilled water to achieve concentrations of 0.25 %, 0.75% and 1.5% of CHX hexametaphospahte liquid.

After mixing, the material was packed in split Teflon mold to obtain disc-shaped specimen with dimensions 10 mm in diameter and 2 mm thickness. The molds were lined with a thin layer of Vaseline to aid in removal of the set cement. The mold was placed on a glass slide then packed with the glass ionomer then another glass slide was placed on top. The cement was compressed between two glass slides and checked for even distribution of the cement. The mixing was completed in 20 seconds and packing into the molds took a further 10 seconds, all manipulation of the cement was completed within 1 min.

Antibacterial effect

The specimenswere put agar petri dish inoculated with Streptococcus mutans and Lactobacillus acidophilus bacterial strains. Petri dish contained BHI agar (per liter: 37g Brain Heart Infusion) and incubated at 37 °C for 48 h under anaerobic conditions. Then compacted discs were placed on the inoculated media and keep the inoculated petri dish in the fridge for 2 hours for agar diffusion testing. After 2 hours, transfer the petri dishes to the inoculator at 37 °C for 24 hours. After the inoculation period, measure the length of inhibition zone for each disc. Specimens (n=5) were tested for time interval (7 days, 3months and 6 months) separately. These groups were prepared for each bacterial species.

Chlorohexidine release

The specimens were allowed to set for 30 min and then immersed in 1 mL of distilled water. The release profiles for a range of concentrations of CHX from the cement in the distilled water medium was determined using spectrophotometry. Adsorption of light at wavelength 255 nm was measured at regular intervals using a spectrophotometer (Hitachi U-1800, Hitachi, Japan) and calibration standards of 5–50mM CHX used as references to establish CHX release from the GICs into thedistilled water.

Readings obtained were converted to μmoles CHX released per unit surface area for each specimen and normalized by subtracting the mean reading for the 0% substitution, correcting for other eluents of the GIC such as the polyacrylic acid.

A linear relationship between absorbance peak height obtained from UV-Vis spectrophotometry and the chlorhexidine concentration in the reference solutions were stablished for each solution.

Fluoride release

Fluoride ion concentrations were determined in 1mL volumes of solution that was removed from the individual sample containers. Fluoride release of each specimen was measured after 7 days, 3months and 6 months using fluoride ion-selective electrode (Orion EA 940, Thermo-Electron Corporation, Houston, Texas, USA) attached to an ion meter.

Statistical analysis

Numerical data were explored for normality by checking the data distribution, using Kolmogorov-Smirnov and Shapiro-Wilk tests. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA), Statistics Version 26 for Windows.

Data showed parametric distribution so; it was represented by mean and standard deviation (SD) values. The significance level was set at $P \leq 0.05$ within all tests. One-way ANOVA test was used to study the effect of one tested variable and their interaction. Comparison of main and simple effects were done utilizing Bonferronicorrection.

One-way repeated measure ANOVA test was conducted to study the effect of time on different tested variables and their interaction. Comparison of main and simple effects were done utilizing Bonferroni correction.

One-way ANOVA followed by pairwise comparisons with Bonferroni correction were used to compare different glass ionomer materials at each time interval and repeated measures ANOVA was used to compare between different time intervals of each material.

RESULTS

Antibacterial effect results

For antibacterial effect test, inhibition zones against streptococcus mutans was tested for different groups after 7 days, 3 months and 6 months. The difference between all the groups and the control was significant ($p < 0.05$). The largest inhibition zones values was after 7 days, followed by 3 months then 6 months. For GICS containing chlorhexidine hexametaphoshate revealed to have statically significant values higher than GIC containing digluconate through the time interval and concentration of 1.5% showed the largest inhibition zones in all time for both derivatives (table 3, figure 1).

Table 3. Summary table for Mean ± standard deviation (SD) of antibacterial effect (mm) of inhibition zones of streptococcus mutans for different measurement times and concentrations of different chlorohexidine derivatives.

Concentration of different chlorohexidine derivatives	Time of measurement (mean±SD)			p-value
	7 days	3 months	6 months	
(GIC)	24.00±5.48^{Ba}	4.40±6.02^{Db}	0.00±0.00^{Db}	**<0.001***
(GIC HMP0.25%)	46.00±4.18^{Aa}	24.00±4.18^{Bb}	13.20±1.30^{Cc}	**<0.001***
(GIC HMP0.75%)	47.00±4.47^{Aa}	28.00±2.74^{Bb}	16.20±0.84^{Bc}	**<0.001***
(GIC HMP 1.5%)	54.00±5.48^{Aa}	38.00±2.74^{Ab}	17.60±0.55^{Ac}	**<0.001***
(GIC DI 0.25%)	28.00±2.74^{Ba}	6.60±6.02^{Db}	0.00±0.00^{Db}	**<0.001***
(GIC DI 0.75%)	46.00±4.18^{Aa}	14.60±0.55^{Cb}	0.00±0.00^{Dc}	**<0.001***
(GIC DI 1.5%)	50.00±6.12^{Aa}	14.60±0.55^{Cb}	0.00±0.00^{Dc}	**<0.001***
p-value	<0.001*	<0.001*	<0.001*	

Note: Different upper and lowercase superscript letters indicate a statistically significant difference within the same vertical column or horizontal row respectively*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).

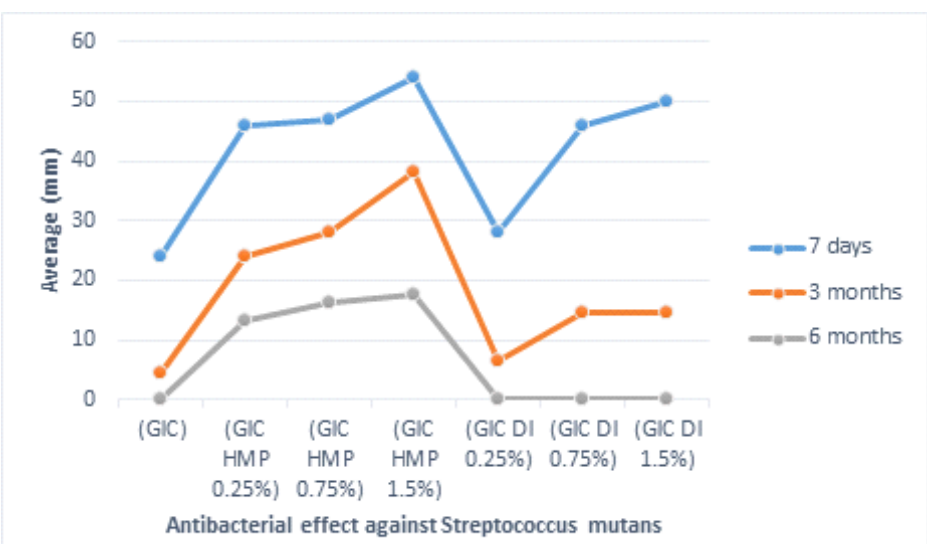

Figure 1. Line chart showing the mean average antibacterial effect (mm)of inhibition zones of streptococcus mutuans for different measurement times and concentrations of different chlorohexidine derivative with time.

For lactobacillus acidophilus test, the difference between the groups was also significant while compared with the control ($p < 0.05$). Inhibition zones against was tested for different groups after 7 days, 3 months and 6 months. The largest inhibition zones values were after 7days, followed by 3months than 6 months. For GICS containing chlorhexidine hexametaphoshate were revealed to have statically significant values higher than GIC containing digluconate through the time interval and concentration of 1.5% showed the largest inhibition zones (mm) in all time for both derivatives (table 4, figure 2).

Table 4. Summary table: Mean ± standard deviation (SD) of antibacterial effect (mm) of inhibition zones of *lactobacillus acidophilus* for different measurement times and concentrations of different chlorohexidine derivatives.

Concentration of different chlorohexidine derivatives	Time of measurement (mean±SD)			p-value
	7 days	3 months	6 months	
(GIC)	25.00±7.07^{Da}	4.40±6.02^{Cb}	0.00±0.00^{Db}	<0.001*
(GIC HMP 0.25%)	39.00±7.42^{BCa}	22.00±4.47^{Bb}	11.40±0.55^{Cc}	<0.001*
(GIC HMP 0.75%)	44.00±5.48^{Aba}	25.00±3.54^{ABb}	14.40±0.55^{Bc}	<0.001*
(GIC HMP 1.5%)	54.00±5.48^{Aa}	35.00±5.00^{Ab}	16.40±0.55^{Ac}	<0.001*
(GIC DI 0.25%)	28.00±2.74^{CDa}	6.60±6.02^{Cb}	0.00±0.00^{Db}	<0.001*
(GIC DI 0.75%)	46.00±4.18^{Aba}	7.00±6.40^{Cb}	0.00±0.00^{Db}	<0.001*
(GIC DI 1.5%)	52.00±7.58^{Aa}	7.00±6.40^{Cb}	0.00±0.00^{Db}	<0.001*
p-value	<0.001*	<0.001*	<0.001*	

Note: Different upper and lowercase superscript letters indicate a statistically significant difference within the same vertical column or horizontal row respectively*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).

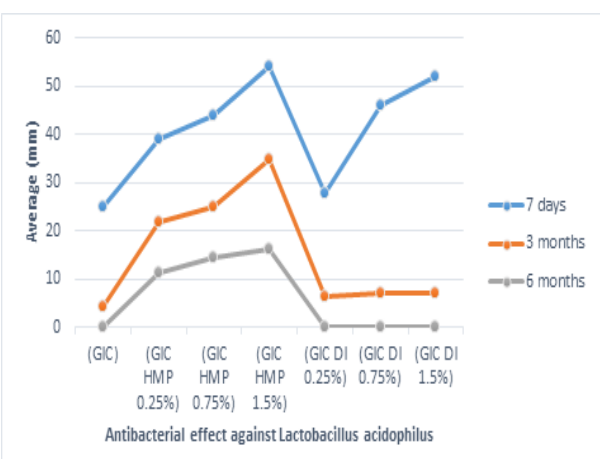

Figure 2. Line chart showing average antibacterial effect (mm) of inhibition zones of *lactobacillus acidophilus* for different measurement times and concentrations of different chlorohexidine derivatives.

Chlorhexidine release results

For 7 days' groups, GIC containing chlorhexidine derivatives were revealed to have statically significant higher values of release than unmodified GIC. After 3 months, GICS containing chlorhexidine hexametaphoshate revealed to have statically significant values higher than GIC containing digluconate and unmodified GIC. After 6 months, GIC containing chlorhexidine hexametaphoshate revealed to have statically significant values higher than GIC containing digluconate and unmodified GIC, concentration of 1.5% showed thehighest chlorhexidine release. For the Means and

standard deviations (SD) for chlorhexidine release (µg/ml) of the different tested materials with time are presented in table 5 and figure 3.

Table 5. Summary table: Mean ± standard deviation (SD) of chlorohexidine release (µg/ml) for different measurement times and concentrations of different chlorohexidine derivatives.

Concentration of different chlorohexidine derivatives	Time of measurement (mean±SD)			p-value
	7 days	3 months	6 months	
(GIC)	0.06±0.03^{Da}	0.01±0.00^{Ea}	0.01±0.00^{Da}	**0.156ns**
(GIC HMP 0.25%)	2.85±0.47^{Ca}	1.87±0.58^{BCab}	0.83±0.09^{Cb}	**<0.001***
(GIC HMP 0.75%)	4.09±0.59^{Ba}	2.50±0.36^{Bb}	1.25±0.21^{Bc}	**<0.001***
(GIC HMP 1.5%)	5.91±0.34^{Aa}	3.41±0.43^{Ab}	1.84±0.41^{Ac}	**<0.001***
(GIC DI 0.25%)	3.14±0.36^{Ca}	0.89±0.08^{Db}	0.01±0.01^{Dc}	**<0.001***
(GIC DI 0.75%)	4.07±0.64^{Ba}	1.90±0.61^{BCb}	0.01±0.01^{Dc}	**<0.001***
(GIC DI 1.5%)	4.68±0.38^{Ba}	1.25±0.23^{CDb}	0.04±0.03^{Dc}	**<0.001***
p-value	<0.001*	<0.001*	<0.001*	

Note: Different upper and lowercase superscript letters indicate a statistically significant difference within the same vertical column or horizontal row respectively*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).

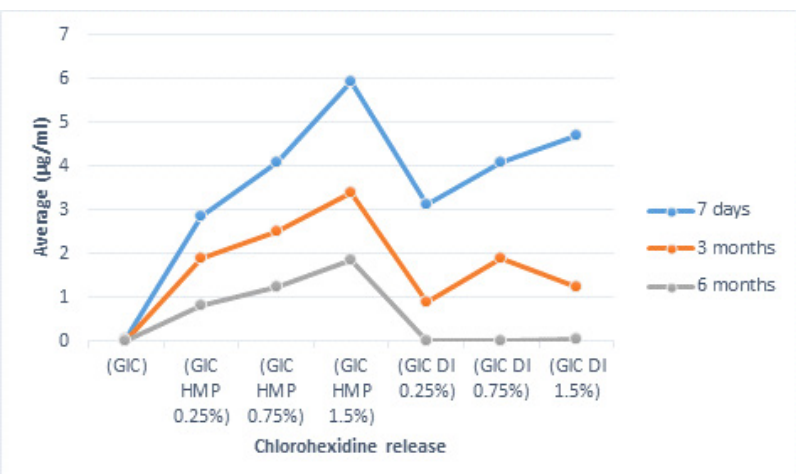

Figure 3. Line chart showing average chlorohexidine release (µg/ml) for different measurement times and concentrations of different chlorohexidine derivatives.

Fluoride release results

Unmodified GICs showed highest value of fluoride release than other concentration showed lower values with significant differences between them and the control GICs time interval of the study (7 days, 3 months and 6 months) for the Means and standard deviations (SD) for fluoride release (ppm) of the different tested materials with time are presented in table 6 and figure 4.

DISCUSSION

The concept of controlled-release therapeutic systems as in GIC-CHX to deliver a predetermined amount of CHX for a specific period is of concern to improve their clinical efficacy and increase their antimicrobial efficacy [13].

Table 6. Summary table Mean ± standard deviation (SD) of fluoride release (ppm) for different measurement times and concentrations of different chlorohexidine derivatives.

Concentration of different chlorohexidine derivatives	Time of measurement (mean±SD)			p-value
	7 days	3 months	6 months	
(GIC)	7.90±0.72^{Aa}	1.96±0.45^{Ab}	0.66±0.35^{Ac}	**<0.001***
(GIC HMP 0.25%)	4.32±1.42^{CDa}	1.31±0.18^{Bb}	0.45±0.27^{ABc}	**<0.001***
(GIC HMP 0.75%)	3.09±0.62^{Da}	1.06±0.05^{Bb}	0.18±0.11^{Bc}	**<0.001***
(GIC HMP 1.5%)	4.11±0.56^{CDa}	1.08±0.07^{Bb}	0.17±0.05^{Bc}	**<0.001***
(GIC DI 0.25%)	6.35±0.80^{ABa}	1.91±0.37^{Ab}	0.56±0.23^{ABc}	**<0.001***
(GIC DI 0.75%)	4.93±1.11^{BCa}	1.22±0.12^{Bb}	0.44±0.08^{ABc}	**<0.001***
(GIC DI 1.5%)	4.20±0.76^{CDa}	1.10±0.09^{Bb}	0.18±0.13^{Bc}	**<0.001***
p-value	<0.001*	<0.001*	<0.001*	

Note: Different upper and lowercase superscript letters indicate a statistically significant difference within the same vertical column or horizontal row respectively*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).

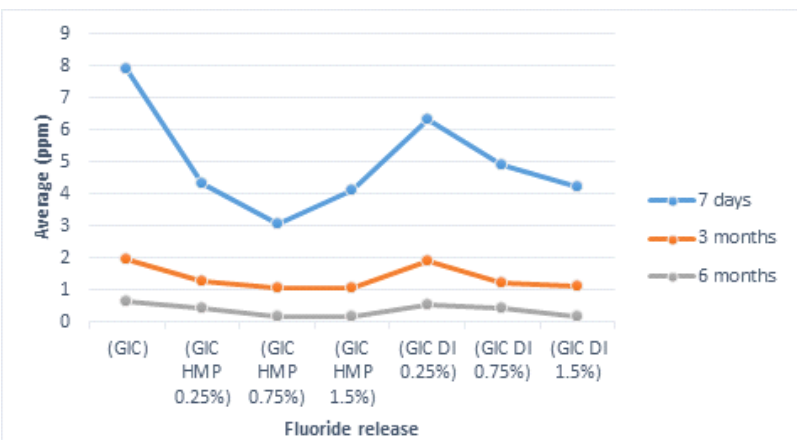

Figure 4. Line chart showing average fluoride release (ppm) for different measurement times and concentrations of different chlorohexidine derivatives.

Results of agar diffusion test showed that the sizes of inhibition zones produced against *S. mutans* and *L. acidophilus* were dependent upon the concentration of the CHX incorporated to the GIC in all tested groups. This is due to higher concentrations are direct proportion to the presence of large amount of soluble CHX in the elution medium that cause antibacterial effect. The highest results were recorded in CHX–HMP GIC 1.5% conc. antibacterial effect in all specimens [14].

This is in agreement with Botelho et al. [15] studies showed that the antibacterial-GIC combination specimens showed significant inhibition zones, which increased with the CHX concentrations.

It was found that the inhibition zone decrease in size during the duration of the study in all the specimens. The inhibition zone was the largest in agar plates after 7 days in all specimens then fades out by the time of the study. CHX digluconate specimens showed decrease in size of inhibition zone after 3 months while the CHX-HMP effect was noticed for longer duration 6 months.

This is in agreement with Bellis et al. [11] who found that CHX-HMP cements exhibited a sustained release of soluble CHX over one year and was capable of inhibiting the growth of oral pathogens in vitro [11].

This is also in agreement with Hook et al. [16] who found that the CHX release from digluconate derivatives shown that an antimicrobial effect persisted for between 40 and 90 days.

In literature, Chlorhexidine was added to different salts like diacetate, digluconate, dichloride and hexametaphosphate as a releasing molecule. In this study, CHX was added to GIC using digluconate and hexametaphosphate to compare the substantivity duration.

CHX digluconate specimens showed decrease in size of inhibition zone after 3 months while the CHX-HMP effect was noticedfor longer duration 6 months

Recent studies showed that hexametaphosphate as a large molecule can cause slow release of CHX than other salts. Large clusters of CHX–HMP particles, which were formed during the production process, could cause CHX release for longer duration. CHX was probed over a clinically relevant timescale of over one year [11].

Results of agar diffusion test showed that the sizes of inhibition zones produced against S. mutans and L. acidophilus were dependent upon the concentration of the CHX incorporated to the GIC in all tested groups. This could be attributed to the fact that higher concentrations are direct proportion to the presence of large amount of soluble CHX in the elution medium, that cause an antibacterial effect. The highest results were recorded in CHX–HMP GIC 1.5% conc. antibacterial effect in all specimen

This is in agreement with De Castilho et al. [17] who found that the antibacterial effect was concentration-dependent,since higher concentrations produced larger inhibition zones.

Also, Ribeiro et al. [18] and Botelho et al. [15] studies' showed that the antibacterial-GIC combination specimens showed significant inhibition zones which increased with the CHX concentrations.

For all concentration in both derivatives, the highest CHX release was after 7 days, then there was a decrease in the CHX release after 3 months. The lowest CHX release values was recorded after 6 months. GIC containing digluconate did not release any chlorhexidine after 6 months. This may be due formation of large clusters of CHX–HMP particles, which were formed during the incorporation of HMP in CHX. The hexametaphosphate salt, which is a large molecule, has low solubility compared to the digluconate compound, thus allow slow and sustained release of CHX than the digluconate salt

This is in agreement with Bellis et al. [11] who stated that the release pattern of chlorhexidine depends on the concentrations and the chemistry of different CHX compounds. The higher conc. of CHX–HMP, disrupt the setting process of the GIC, cause the GIC to become more porous and release more.

This is also in agreement with Hook et al. [16] who found that the CHX release from digluconate derivatives shown that an antimicrobial effect persisted for between 40 and 90 days.

For fluoride release testing, results showed that the unmodified GIC's release more fluoride than the GIC containing CHX derivatives. Thismight 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 in GIC containing CHX derivatives [19].

Also fluoride released from CHX-digluconate is more than that released from CHX-HMP. This might be due to the hexametaphosphate molecule, which is a complex molecule capable of bonding with fluoride. This makes its release to be lower than other derivatives and also lower than the unmodified specimens [16].

The initial fluoride release rate from glass ionomer was rapid initially and then gradually slowed over the experimental period.Fluoride release decrease with time but remain measurable after 60 days [20]. This may be due to high instability and erosion of GICs during the early setting period, followed by a rapid decrease in the rate of release [21].

This is in agreement with Kucukyilmaz et al. [21] who concluded that GICs had the greatest amount of fluoride ions on the 1st day. Fluoride continued to be released in relatively low amounts from day 2 until day 49. The high level of fluoride release from GIC materials on the first day was due to an initial "burst" of fluoride release from the glass particles during the setting reaction and the rapid dissolution of fluoride from the outer surface into the solution. The slower release of fluoride during next days happened due to the slower dissolution of glass particles through cement pores [21].

CONCLUSION

1. The incorporation of CHX into ChemFil Superior glass ionomer cement in both derivatives has the ability to provide a long-term antimicrobial effect on *S. mutans* and *L. acidophilus*.

2. The substantivity of CHX released was dependent on the molecule attached to in the cement either digluconate or hexametaphosphate in duration of release.

3. Fluoride release of GIC decrease by adding CHX in both derivatives and with the increase of concentrations

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