Antibacterial Activity of Triclosan Incorporated Glass Ionomer Cements – An *in vitro* Pilot Study

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***Objectives**: The aim of this pilot study was to evaluate the antibacterial activity of glass ionomer cement impregnated with different concentrations (0.5%, 1.25% and 2.5%) of a non releasing bactericide – Triclosan (TC) against two common cariogenic bacteria – Lactobacillus acidophilus and Streptococcus mutans; and to compare Triclosan incorporated GIC with chlorhexidine (CHX) incorporated GIC (2.5%) in terms of antibacterial activity. **Methods:** Chlorhexidine or Triclosan were added to glass ionomer cement powder to achieve 2.5% CHX – GIC (positive control – Group II), 0.5%, 1.25% and 2.5% TC-GIC (experimental groups III, IV and V respectively) formulations. Restorative glass ionomer cement (Fuji IX GC – Group I) served as negative control. The powder and liquid were mixed and inserted into the wells punched in agar plates (10mm x 4mm). The agar diffusion method was used to determine the antibacterial activity of the cements after 1, 7 and 30 days. Mean values were compared between different study groups using One-way ANOVA and Tukey's HSD procedure at a significance level of 5% .**Results:** Triclosan incorporated GIC was more effective against L.acidophilus and S.mutans than Chlorhexidine incorporated GIC. Triclosan at a concentration of 2.5% was more effective than at lower concentrations. At all time periods studied, the maximum zone of inhibition against L.acidophilus was produced by Group V. Against S.mutans, on days 1, 7 and 30, there was no significant difference between Groups II and IV ($p>0.05$), while the other groups showed significant differences. **Conclusion:** The use of triclosan as an antibacterial additive in GIC holds promise and further clinical research is needed in this direction.*

***Keywords:** Triclosan, glass ionomer cement, chlorhexidene, antibacterial, Atraumatic restorative treatment, agar diffusion*

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INTRODUCTION

The World Health Organisation (WHO) oral health care program recommends Atraumatic Restorative Treatment (ART) (currently termed Interim Therapeutic Restoration ITR) ,as one of the most suitable caries controlling approaches in primary and health programmes. The global promotion of ITR is one of its major objectives. This procedure has been found to be very useful in young children who suffer from rampant caries.[1]

Glass ionomer cement (GIC) is the material of choice in ITR approach owing to its adhesive property, biocompatibility and caries protective effect due to fluoride release. The presence of fluoride is considered to promote remineralization of calcified tissues. Further, fluoride present in glass ionomer cement is responsible for formation of fluorapatite crystals which are resistant to caries.[2]

It has been reported that *Streptococcus mutans* is the main bacteria responsible for caries initiation whereas *Lactobacillus acidophilus* is the principal bacteria related to caries progression.[3,4] Studies have revealed that Lactobacillus species are predominant in deep carious lesions.[3] *S. mutans* and *L. acidophilus* can produce great amounts of acids and are tolerant to acidic environments.[5]

During the process of removal of the carious lesions solely with hand instruments, there is possibility of removal of insufficient caries and the microorganisms are likely to be viable for a period of atleast two years under the glass ionomer cement restoration.[2,6] Therefore, reinforcing the conventional glass ionomer cement with additional antibacterial agents may be effective in management of carious

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lesions.[7,8,9] Among the antiseptics, Chlorhexidine (CHX) has proven to be safe and effective. Studies have shown that incorporation of chlorhexidine or its derivatives into glass ionomer cements improves the antimicrobial effect of the glass ionomer cements on cariogenic microorganisms.[5,7,8,10] However, the addition of chlorhexidene has been claimed to interfere with the acid base setting reaction of GIC, resulting in breakdown of the structure. The compressive strength of GIC decreases with increasing concentration of CHX.[5] This necessitates the use of additives which do not get released from the cement, yet show antibacterial activity.

An antibacterial agent of interest is Triclosan, a broad spectrum antimicrobial agent which has been extensively used in mouth washes and dentifrices.[11] Triclosan has been shown to be safe and effective as an antimicrobial agent in oral health care products. There is no documented evidence on the use of Triclosan incorporated glass ionomer cements.

This study compares and evaluates the antibacterial effect of triclosan incorporated glass ionomer cement (TC–GIC) against *Streptococcus mutans* and *Lactobacillus acidophilus* using an agar diffusion model.

MATERIALS AND METHOD

Restorative glass ionomer cement (Fuji IX, GC, Tokyo, Japan; Lot number 0811011) was used as the negative control (Group I).

CHX-GIC (Positive control): 0.075g of Chlorhexidine diacetate (HPLC Purified; Sigma Aldrich, Steinheim, Germany; Batch number: 08BPLS/CHA002) was added to 2.925g of glass ionomer powder to obtain a 2.5% formulation (Group II).

TC-GIC: Three different formulations of TC – GIC were prepared based on the concentration of triclosan (HPLC Purified; Sigma Aldrich, Steinheim, Germany; Lot number 0000043758). Group III – 0.5%TC-GIC was prepared by adding 0.015g of Triclosan to 2.985g of glass ionomer powder; Group IV – 1.25%TC-GIC by adding 0.037g of Triclosan to 2.96g of glass ionomer powder and Group V-2.5%TC-GIC by adding 0.075g of Triclosan to 2.925g of glass ionomer powder.

Agar diffusion test:

Antimicrobial activity of these materials against *Lactobacillus acidophilus* (ATCC 43121) and *Streptococcus mutans* (ATCC 55221) was tested after a period of 1, 7 and 30 days using the agar diffusion model. The microorganisms were sub cultured in appropriate culture media and under gaseous conditions to confirm their purity. The microbes were individually inoculated into tubes containing 5 mL of sterile 0.9 % saline solution. The suspension was adjusted spectrophotometrically at 800 nm (Optical Density 800), which was used to match the turbidity of 1.5×10^8 CFU mL^{-1} (equivalent to 0.5 McFarland standard). Five hundred µL of *L.acidophilus* suspension was used to inoculate glass bottles containing 50 mL of Lactobacillus MRS agar (Hi Media Labs, Bangalore, India) at 46º C mixed and poured onto 130 mm plates containing a previously set layer of Mueller Hinton agar (Hi Media Labs). Five hundred µL of *S.mutans* suspension was used to inoculate glass bottles containing 50 mL of Tryptic soy agar (Hi Media Labs, Bangalore, India) at 37º C mixed and poured onto 130 mm plates containing a previously set layer of Mueller Hinton agar (Hi Media Labs). 50 specimens were prepared with 10 specimens in each group respectively.

For each culture plate, five standardized wells with a diameter of 10mm and height 4 mm were punched into the agar with a sterile metal ring. The powder and liquid of the agents under investigation were mixed according to manufacturer's specification for 30 seconds with sterile agate spatula on mixing pad and inserted into the wells within one minute using a centrix syringe. The plates were then incubated at 37ºC for 24hours following which the diameters of the circular inhibition zones produced around the specimens were measured in millimeters with a metallic scale at three different points and the mean was recorded as day 1 value.

After measurement of the initial inhibition zone, all samples were removed aseptically from the bacterial plates and rinsed with sterilized deionized water to remove any attached bacteria. Each sample was then stored in sterilized deionized water until day 6. On the 6th day, new culture media were prepared with fresh agar and placed in Petri dishes. Five standardized wells were punched into this new agar plate and bacterial inoculation was made over the agar surfaces with 0.5mL of the bacterial suspension. The specimens were taken out from the deionized water and placed into the new wells. The plates were then incubated at 37ºC for 24 hours, and the inhibition zones around the specimens were measured and recorded in millimeters as day 7 value. After performing the measurements, each sample was removed and stored in sterilized deionized water until day 29.The procedure was repeated with fresh agar plates inoculated with microorganisms on the 29th day for obtaining inhibition zones for day 30. All measurements were performed by the second author and the microbiologist who were blinded to the experimental groups.

Mean and standard deviation were estimated for each study group. Mean values were compared between different study groups using One-way ANOVA with $p < 0.05$ as significance level. Multiple range test by Tukey's HSD procedure was employed to identify the significant groups at 5% level.

RESULTS

The comparison of mean values of the zone of inhibition against *Lactobacillus acidophilus* and *Streptococcus mutans* among different study groups are presented (Figures 1 and 2). Group V (2.5%TC-GIC) showed the maximum zone of inhibition against *L.acidophilus* and the negative control (Group I) showed no zone of inhibition at all time periods studied.

On days 1, 7 and 30, there was significant difference in the mean zone of inhibition against *L.acidophilus* between all groups ($p < 0.05$) except between Groups III (0.5% TC-GIC) and IV (1.25%TC-GIC) ($p>0.05$). Against *S.mutans*,

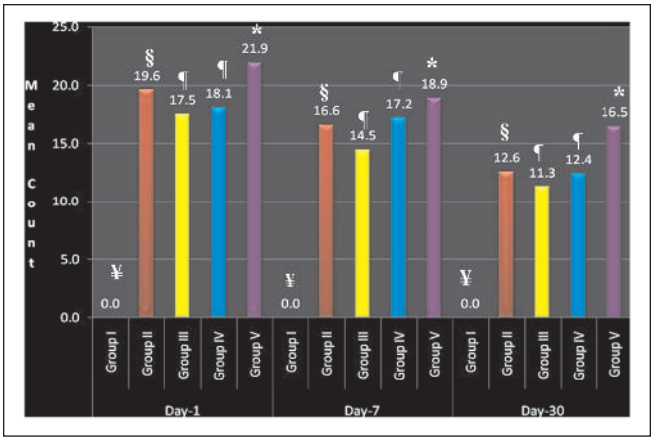

Figure 1. Mean values of the zone of inhibition (in mm) against Lactobacillus acidophilus

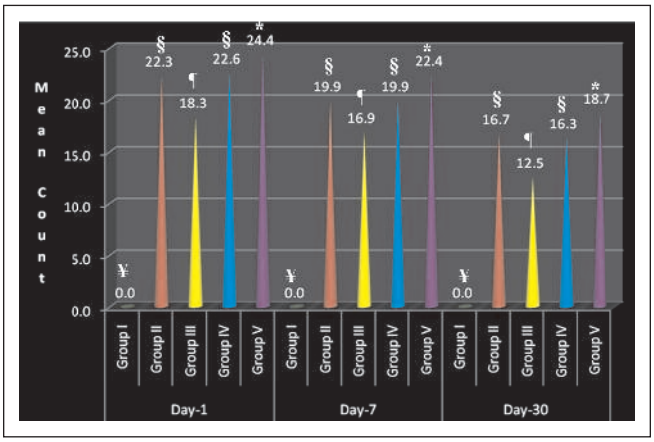

Figure 2. Mean values of the zone of inhibition (in mm) against Streptococcus mutans.
Same symbols indicate no significant differences between groups ($p > 0.05$)
Groups labelled with * indicate those with highest mean value of zones of inhibition

on days 1, 7 and 30, there was no significant difference between Groups II and IV ($p>0.05$), while the other groups showed significant differences.

From day 1 to day 7, days 1 to 30 and 7 to 30, there was no significant difference between groups II and III against *L.acidophilus* Against *S.mutans*, group IV showed significantly higher zones of inhibition (2.7 ± 0.8 and 6.3 ± 0.9mm respectively) except from day 7 to 30, where group III showed higher zones of inhibition (4.4 ± 0.8mm). Since there was 100% interrater and intrarater agreement, a test to evaluate examiner reliability was deemed unnecessary.

DISCUSSION

The potential limitation of ITR is difficulty in removal of the entire carious lesion using only hand instruments with the likelihood of residual caries in the cavity.[1,2,6] This necessitates complete removal of the cariogenic lesion as a prerequisite for control of caries progression. High percentages of *S.mutans* and *L.acidophilus* have been isolated from recurrent caries lesions.[3,10]

Though it is widely considered that fluoride released from restorative materials, glass ionomer in particular, has a caries preventive effect, many studies have confirmed the contrary. It has been established that secondary caries is one of the most common reason for failure of glass ionomer cements.[7,12,13,14] A landmark systematic review showed no conclusive evidence for or against inhibition of secondary caries by GIC was obtained.[15]

Recently, several faster setting, high viscosity glass ionomer cements have been made available. These materials set faster and are of higher viscosity because of finer glass particles, anhydrous polyacrylic acids of high molecular weight and a high powder-to-liquid mixing ratio. GIC may not be able to completely prevent the formation of recurrent caries adjacent to the restoration, despite evidence of fluoride release and dentin fluoride uptake. Therefore, it has to be concluded that fluoride release and uptake do not guarantee anticariogenicity.[15,16]

The addition of antibacterial agents to restorative materials is gaining popularity with the aim of suppressing of growth of bacteria under restorations to minimize the risk of recurrent caries.[4,5,7,8,9,10] Chlorhexidine incorporated glass ionomer cement has been reported to be effective against Streptococcus species. Chlorhexidine diacetate at a concentration of 2.5% has been established to be very effective for a long duration of time against *L. acidophilus* (60 days) and *S. mutans* (90 days).[8] However the reports on the effect of chlorhexidene on the physical and mechanical properties of glass ionomer cements are not conclusive. However, the incorporation of a soluble antimicrobial like CHX acetate and gluconate to GIC may result in a dramatic decrease in concentration of the additive and compromise the physicomechanical properties of the cement.[5,17,18,19] In lieu of these technical shortcomings, it appears prudent to incorporate sparingly releasing, yet effective antibacterial agents into the cement.

In the present study the antimicrobial action of Triclosan incorporated GIC was compared with Chlorhexidine diacetate incorporated GIC. It was also directed towards identifying the optimal concentration of triclosan to be added to GIC, so that the cement will exhibit antibacterial action for atleast 30 days. Triclosan [5-chloro-2-(-2, 4-dichlorophenoxyl) phenol] is a synthetic, nonionic, broad spectrum antimicrobial agent.[20,21] It has been suggested that Triclosan incorporated composite resins show better antimicrobial properties than cholorhexidene incorporated composite resins. The proposed mechanism of action of triclosan suggests the material to be an immobilized bactericide which does not leach out of the carrier material, thereby favouring long term anticariogenic activity.[19]

The antibacterial activity was evaluated using agar diffusion test. The agar diffusion test is an accepted method to initially differentiate antibacterial activity between materials.[22,23,24] The process is relatively inexpensive and can be performed rapidly. However, there are also some limitations with this test method. One of the main concerns is the inability of the method to distinguish between bacteriostatic and

bactericidal effects, so the test does not provide any information about the viability of the test microorganisms within the inhibition zone. Moreover, the test does not simulate the clinical condition where multiple species of bacteria grow in complex biofilms.[22]

In our study it was observed that the materials had more antibacterial effect during the process of setting than when completely set. This bactericidal effect could be partially because of the low pH during the setting reaction of dental cements.[22] The use of deionised water for experimental purpose to store GIC has been recommended by various investigators.[22,25] This simulates the clinical scenario where the restoration is continually bathed by oral fluids. Maintaining the specimens in the media for all the time durations evaluated will not provide a true indication of how long the additives will have the antimicrobial effect. Theoretically, saliva like media is preferable to water. However artificial saliva has two main pitfalls –it is acidic and hence causes leaching of components of glass ionomer cement; it forms insoluble calcium fluoride coating on the sample surface.[25]

Our study showed that group I - Glass ionomer cement did not show any antimicrobial activity against *Lactobacillus acidophilus* and *Streptococcus mutans*. The mean zone of inhibition against both *L.acidophilus* and *S.mutans* was significantly ($p<0.05$) lower on day 1, day 7 and day 30 when compared to the other groups tested. This may be due to the reason that Type IX GIC was used as control. Conventional glass ionomers have been claimed to exhibit antibacterial activity primarily by fluoride release. However, it has been elucidated that fluoride release may increase the resistance of dentin to demineralization, but does not inhibit acid production by bacteria.[26]

Group II (GIC containing 2.5% Chlorhexidine diacetate) had superior antimicrobial activity against *L.acidophilus* on day 1, day 7 and day 30, when compared to Group I, Group III and Group IV ($p<0.05$) and significantly lesser compared to Group V. Similar values were obtained for *S.mutans* except for Group II and Group IV which were not statistically significant ($p>0.05$). From the results of our study, we speculate that *S.mutans* is more susceptible to the action of Triclosan when compared to *L.acidophilus*.

Group III (0.5 % TC-GIC), Group IV (1.25% TC-GIC) and Group V (2.5% TC-GIC) were found to have antibacterial activity against both *L.acidophilus* and *S.mutans* on days 1, 7 and 30. The sizes of inhibition zones produced by Group III, Group IV and Group V were clearly dependent upon the concentration of Triclosan incorporated into glass ionomer cement. The higher the concentration, larger was the zone of inhibition. Against *S.mutans*, the Group IV and II were not significantly different, which shows that both 1.25% Triclosan and 2.5% Chlorhexidine diacetate have the same antibacterial action against *S.mutans*.

In this study, Group V (2.5% TC-GIC) produced larger zones of inhibition compared to the other groups, demonstrating that Triclosan is more effective in destroying both the microorganisms. The primary site of action is the cytoplasmic membrane and uptake of Triclosan by the cell wall which is speculated to be by diffusion. It has been reported that the primary antimicrobial action of Triclosan is directed toward RNA and protein synthesis in bacteria and not against DNA.[20]

Triclosan is suggested to act on these microorganisms, especially *L.acidophilus* by increasing the permeability of the bacterial cell wall.[19] Experiments carried out in the pH stat assay system using *S.mutans* as test organism suggested that Triclosan may also play a role in inhibiting glucose metabolism in *S.mutans*.[20,21,27] A decrease in the size of zone of inhibition was seen among groups II, III, IV and V during the 30 days which correspond to the decrease in available Triclosan. This may be a result of the loss of material by elution from the glass ionomer cement. Under the conditions of this study, loss of material is a surface characteristic. In the clinical setup, such loss of material by elution may occur due to entry of fluids via any pathway of leakage.[15,24]

This is only a preliminary study showing the superior antibacterial activity of triclosan (a non-releasing bactericide) incorporated GIC as compared to chlorhexidine incorporated GIC. However, this study was carried out under experimental conditions and direct correlations cannot be extrapolated to the clinical scenario. In the clinical situation numerous variables may influence the antibacterial action of these additives – flow of saliva, temperature variations, effective surface area of the restoration and interactions between bacterial plaque and the tooth. Nevertheless, this study serves to identify a new and effective antibacterial incorporation in glass ionomer cements, owing to the current knowledge that the presence of fluoride in glass ionomers does not guarantee antibacterial activity. Future research should be directed towards the long term antibacterial activity of triclosan incorporated GIC, influence of triclosan on the chemistry and physico-mechanical properties of GIC and its release profile in the clinical scenario.

CONCLUSIONS

Under the conditions of this in vitro study, it may be concluded that :

- Chlorhexidine diacetate incorporated GIC and Triclosan incorporated GIC are active against *Lactobacillus acidophilus* and *Streptococcus mutans*, the antibacterial activity lasting for a period of 30 days.
- 2.5% Triclosan added to glass ionomer cement showed more antimicrobial activity than 2.5% CHX incorporated GIC against *Lactobacillus acidophilus* and *Streptococcus mutans*.
- Triclosan being a non releasing bactericide may prove to be more advantageous than chlorhexidene as an antibacterial additive in glass ionomer cements.

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