



RESEARCH ARTICLE

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY, COMPRESSIVE STRENGTH AND DIAMETRAL TENSILE STRENGTH OF GIC IX, GIC IX WITH 1% CHLORHEXIDINE AND GIC IX WITH 1% CETRIMIDE: AN IN VITRO STUDY

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ABSTRACT

Aim: To determine the antimicrobial efficacy, compressive strength and diametral tensile strength of GIC IX, GIC IX with 1% chlorhexidine and GIC IX with 1% cetrinide

Materials and Method: GIC IX was mixed with chlorhexidine and cetrinide powder to produce experimental GIC's. 10 samples of each of the three groups were prepared for each parameter. Antimicrobial efficacy was evaluated against *S.mutans* by measuring the zone of inhibition on day 0, 7 and 30 days on blood agar. Compressive and diametral tensile strength were calculated using the universal testing machine after 1hr of setting.

Results: Experimental GIC's had reduced physical properties when compared to GIC. The antimicrobial efficacy was highly improved with the addition of antimicrobials. 1% chlorhexidine produced the best results out of the two experimental GIC's.

Conclusion: GIC containing 1% Chlorhexidine can be alternatively used for pediatric restorations to provide enhanced anticariogenicity.

INTRODUCTION

The prevalence of dental caries can be considered as one of the most important pathological process in humans and bacteria play a key role in their development. Restoring the carious lesion at an early stage is an ideal treatment option (Herrera *et al.*, 1999). Atraumatic Restorative Technique comes out to be a suitable option for restoration in pediatric populations as it is associated with minimal discomfort. The procedures used in this treatment of caries do not always eliminate all the microorganisms in the residual tissues and streptococcus mutans have been a commonly isolated species. The persisting cariogenic bacteria, with the lack of hermetic seal, can cause recurrent caries, leading to failure of restoration. One possible solution to overcome this problem is to use adhesive dental materials with antimicrobial activity (Herrera *et al.*, 1999). Conventional Glass ionomer cements (GIC) were first introduced in 1972 by Wilson and Kent as a tooth coloured and chemically adhesive anticariogenic material.

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It possess unique properties that include adhesion to moist tooth structure and base metals, anticariogenic properties due to release of fluoride, thermal compatibility with tooth enamel because of low coefficients of thermal expansion similar to those of tooth structure, biocompatibility and low cytotoxicity. The limitations include the brittleness and poor fracture toughness. GIC has been modified time and again to enhance it's mechanical and biological properties. The idea of enhancing the spectrum of anticariogenicity of GIC by using antibacterial agents originated from the concept of Miller. The most appropriate choice of antibacterial agents to combine with GIC would be those antiseptic agents that have proven to be useful in clinical dentistry, and are the ones that do not disturb the physical properties (Kidd, 1991). Chlorhexidine is one of the most widely used antimicrobial agent in dentistry and has been widely used for disinfection before placement of restorations (Ahluwalia *et al.*, 2012). Cetrinide is a cationic surfactant, a quaternary ammonium compound, which has demonstrated it's effectiveness against both gram positive and negative bacteria. It is commonly used as a topical antiseptic and is non-toxic at various clinical concentrations (Matilde Ruiz-Linares *et al.*, 2014). This study aims to evaluate the

physical and antibacterial properties of glass ionomer cements containing chlorhexidine diacetate and cetrимide in a 1% concentration.

MATERIALS AND METHODS

Experimental GIC's were prepared by incorporating chlorhexidine diacetate powder in 1% W/W (1g of Chlorhexidine diacetate in 100g of powder) and Cetrимide in 1% W/W (1g of Cetrимide powder in 100g of powder) into GIC powder with powder liquid ratio of 3.6:1 (1 scoop of powder to 1 drop of liquid) (Mohanavelu Deepalakshmi *et al.*, 2010).

Group A: (Control): GIC IX (GC Gold Label IX HS Posterior Extra)

Group B: GIC IX (GC Gold Label IX HS Posterior Extra) + 1% Chlorhexidine

Group C: GIC IX (GC Gold Label IX HS Posterior Extra) + 1% Cetrимide

The powder for each of the groups was dispensed on the mixing pad in the recommended ratio of 3.6:1. It was then manipulated using an agate spatula by the folding method. A sterile cement carrier was then used to carry the cement to the desired mould that was pre-coated with petroleum jelly to facilitate removal. This cement was then allowed to set and was retrieved in 10 minutes from the mould. (Figure 1) The excess material if any was removed using a wet sandpaper. Any samples with gross deformities and voids were discarded. These specimens were then stored in airtight containers for 24 hrs and were then tested for compressive and diametral tensile strength testing. However, the storage time was 1 hr in case of antimicrobial efficacy tests.



Figure 1. Steel moulds and representative discs for each test

A) Antimicrobial efficacy

The antibacterial activity of the set materials against *Streptococcus mutans* (MTCC 497) was assessed using the agar diffusion test. *Streptococcus mutans* strain (MTCC No. 497) was procured from Microbial type culture collection and Gene bank, Chandigarh. The ampule was then open under sterile conditions and the lyophilized strain was then processed

in sterile nutrient broth to create a suspension. 10 specimens for each of the group were prepared by a single operator using standardized moulds of inner dimensions of 10mm diameter and 2.5mm thickness and were then stored in a sterile container for an hour before testing. This suspension was then inoculated on blood agar plates and the control disc along with the two experimental discs were placed on the agar. These plates were then processed anaerobically for 48hrs. Zone of inhibition were measured in millimetres using a digital calliper at three different points. Sizes of the inhibition zones were calculated by subtracting the diameter of specimen, 10mm from the average of the three measurements of the halo. (Figure 2). The above procedure was repeated at the 7th day and the 30th day using the same specimen. In between the readings the samples were placed in labelled sterile containers in distilled water at a temperature of 37°C.

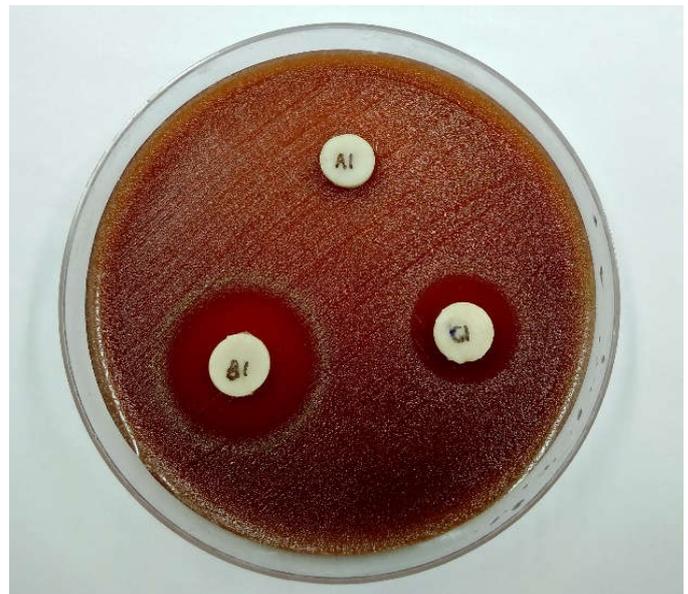


Figure 2. *Streptococcus mutans* on blood agar

B) Compressive Strength

Ten specimens for Compressive Strength testing for each of the groups were prepared by a single operator using standardized moulds with inner dimensions of 5 mm thickness and 5 mm diameter. These samples were then stored in airtight containers for 24 hrs prior to testing. Prior to testing, the diameter of each specimen was determined using a metal scale and specimens were placed with their flat ends up between the plates of universal testing machine. (Figure 3) A compressive load was applied along the long axis at a crosshead speed of 1 mm/min. The maximum force applied when the specimen fractured was recorded, and the CS was calculated by the following equation:

$$\text{Compressive Strength} = \frac{4F}{\Pi d^2},$$

Where F=force resulting in failure of specimen and d=diameter of the specimen (Ahluwalia *et al.*, 2012).

$$\Pi = 3.1416$$

C) Diametral Tensile Strength

Ten specimens for Diametral tensile strength testing for each of the groups were prepared with inner dimensions of 3 mm

thickness and 6 mm diameter. These samples were then stored in airtight containers for 24 hrs prior to testing. Prior to testing, the diameter of each specimen was determined using a metal scale and specimens were placed with their flat ends perpendicular to the plates of universal testing machine. (Figure 4) A compressive load was applied at a crosshead speed of 1mm/min. The maximum force applied when the specimen fractured was recorded, and the Diametral tensile strength was calculated by the following equation:



Figure 3. Compression strength testing



Figure 4. Diametral tensile strength testing

$$\text{Diametral Tensile Strength} = 2P/\Pi DT,$$

Where P=load applied, D=diameter of the specimen, and T=thickness of the specimen (Ahluwalia *et al.*, 2012).

$$\Pi = 3.1416$$

RESULTS

The antimicrobial efficacy was recorded at Day 0, 7 and 30. No zone of inhibition was seen in any of the samples of GIC at Day 0 and thus was not considered for further statistical analysis. A mean zone of inhibition for Group B was recorded as mentioned in Table 1 and of Group C as mentioned in Table

2. The results were then evaluated for each group using ANOVA and Post Hoc Tukeys analysis. There was a statistically significant difference among means of zone of inhibition of group B between Day 7 and 30 and Day 0 and 30. However no significant difference was seen between Day 0 and 7. Similar results were obtained for Group C. Group B and C were then compared using Unpaired 't' test (Table 3) and a highly significant difference was seen in the mean zone of inhibition for Day 0, 7 and 30 with a higher mean for GIC with 1% chlorhexidine. The compressive strength and Diametral tensile strength was evaluated for all the three groups and the means were compared using ANOVA and Post Hoc Tukey's analysis. On statistical analysis there was a significant difference between the compressive strength of control and Experimental GIC's (Table 4).

Table 1. Comparison of antimicrobial efficacy in terms of mean (SD) at different time intervals in group b using ANOVA test

Time interval	No of samples	Mean (SD)
Day 0	10	13.80 (0.6)
Day 7	10	13.70 (0.8)
Day 30	10	10.60 (0.8)
F value	-	55.509
P value	-	<0.001**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 2. Comparison of antimicrobial efficacy in terms of mean (SD) at different time intervals in group c using ANOVA test

Time interval	No of samples	Mean (SD)
Day 0	10	7.90 (0.3)
Day 7	10	7.70 (0.6)
Day 30	10	4.20 (1.0)
F value	-	80.075
P value	-	<0.001**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 3. Comparison of antimicrobial efficacy in terms of mean (SD) at day 0, 7 & 30 among both the groups using unpaired 't' test

Time interval	Group B Mean (SD)	Group C Mean (SD)	t value	p value
Day 0	13.80 (0.6)	7.90 (0.3)	26.386	<0.001**
Day 7	13.70 (0.8)	7.70 (0.6)	17.823	<0.001**
Day 30	10.60 (0.8)	4.20 (1.0)	15.179	<0.001**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 4. Comparison of compressive strength (MPa) in terms of mean (SD) among all the 3 groups using ANOVA test

Group	No of samples	Mean (SD)
GIC IX	10	69.03 (4.2)
GIC IX With 1% Chlorhexidine	10	55.55 (4.2)
GIC IX With 1% Cetrimide	10	58.83 (6.2)
F value	-	20.117
P value	-	<0.001**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 5. Comparison of diametral tensile strength (MPa) in terms of mean (SD) among all the 3 groups using anova Test

Group	No of samples	Mean (SD)
GIC IX	10	11.18 (1.2)
GIC IX With 1% Chlorhexidine	10	8.62 (1.0)
GIC IX With 1% Cetrimide	10	8.44 (1.3)
F value	-	16.794
P value	-	<0.001**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)

However no significance difference was seen between the two experimental groups. Similar results were obtained for Diametral tensile strength. (Table 5)

DISCUSSION

Dental caries is a global epidemic with severe physical, psychological as well as social consequences. The therapeutic procedures used for its treatment aim at reducing or eliminating the microorganisms. Streptococcus mutans are considered to be the most important group of bacteria initiating caries lesions. The number of salivary Streptococcus mutans in the oral cavity is correlated to the formation of new carious lesions, and it is generally accepted that reducing their numbers also reduces caries activity (Krasse, 1989; Edward *et al.*, 1999). Restorative dentistry is now moving away from complete caries removal to an ultraconservative approach, preserving tooth structure and preventing pulpal injury. Clinical procedures now involve incomplete caries removal of only the infected dentin thereby leaving a few microorganisms behind (Kidd, 1991). According to a study done by Texiera et al Streptococcus mutans, is also found in sealed carious dentin (Damé-Teixeira *et al.*, 2014). It has recently been reported that the genotypic diversity of Streptococcus mutans decreased after partial dentin removal and sealing, whereas the virulence traits of Streptococcus mutans were unchanged, maintaining the same cariogenic potential. It was also seen that streptococcus mutans adhered to the surface of restorations and thereby can result in formation of secondary caries (Eick *et al.*, 2004).

Due to the persistence of cariogenic bacteria after superficial caries removal, efforts to eliminate or reduce residual bacteria in affected dentin aiming to decrease the risk of caries progression persist. Among possible strategies, use of materials with bacteriostatic potential and incorporation of antimicrobials into the same have been recommended. Glass Ionomer Cement is one such material that is commonly used for restoring deciduous teeth with bioadhesive properties and a known anticariogenic effect due to fluoride release. However Eick et al observed that S. mutans were found on the surface of GIC despite the fluoride releasing ability. Also high amounts of plaque were noted that could be attributed to the surface roughness of glass ionomer restorations (Eick *et al.*, 2004). Another study by Yap et al failed to confirm a correlation between fluoride release and antibacterial property of GIC (Yap and Khor, 1999). Thus a need to reduce or eliminate microorganisms underneath the restorations was felt. Among the different approaches that have been described for the aforesaid purpose, addition of antimicrobials to GIC is the most viable option. Chlorhexidine and Cetrimide are antiseptics with a wide range of activity. Chlorhexidine is commonly available as chlorhexidine digluconate or chlorhexidine diacetate of which the latter is more stable, is not prone to decomposition, and can be easily blended into the GIC and is thus used in this study. In this study the antimicrobial efficacy was evaluated for GIC, GIC with 1% chlorhexidine and GIC with 1% cetrimide against S. mutans (MTCC 497). No zone of inhibitions were seen in relation to GIC. This finding is consistent with that of Sudhir et al (2015). (Mittal *et al.*, 2015) A mean zone of inhibition of 13.8mm and 7.90mm was seen with GIC containing 1% Chlorhexidine and GIC with 1% Cetrimide respectively on Day 0. Day 7 and Day 30 values were obtained by the above method. A progressive decline was seen in the mean values of both Group B and C

over a period of time (Table 1 & 2). The mean at Day 7 was 13.7mm and 7.7mm for Group B and C respectively. These values dropped down to 10.6mm for Group B and 4.2mm for Group C at Day 30. Statistical analysis was done using ANOVA and Post Hoc analysis. No significant difference was seen in between Day 0 and Day 7 ($p < 0.05$) readings for both the groups. However, a highly significant decrease was seen in the antimicrobial activity of Group B and Group C after 30 days (Table 1 and 2) ($p < 0.001$). Both Chlorhexidine and Cetrimide had a significant decline in the zone of inhibition against S. mutans at 30 days but still both the GIC's had retained their antibacterial potential. When the means of Chlorhexidine containing GIC and Cetrimide containing GIC were compared at Day 0, Day 7 and Day 30 using unpaired 't' test a highly significant difference ($p < 0.001$) was seen between the two with chlorhexidine containing GIC having a better antimicrobial efficacy. (Table 3) These results show a better antimicrobial efficacy against S. mutans with GIC containing 1% Chlorhexidine followed by GIC containing 1% Cetrimide. No zone of inhibition was seen in Group A, thus establishing the ineffectiveness of the fluoride released by GIC in inhibiting the growth of S. mutans.

The results in our present study are consistent with the findings by Sanders et al., 2002; Takshi et al., 2006; Deepalakshmi et al., 2010; Prabhakar et al., 2002 and Mittal et al., 2015 all of which stated a better activity of chlorhexidine containing GIC as compared to normal GIC. In the study by Botelho et al., 2003 and Deepalakshmi et al., 2010 Chlorhexidine and Cetrimide containing GIC had similar zone of inhibitions against S. mutans which is not similar to the findings that are observed in the present study. One of the main concerns of our study is the inability of the agar diffusion method to distinguish between the bactericidal and bacteriostatic effects of the introduced antimicrobials. Another drawback of this test is its inability to provide any information regarding the viability of the test organisms within the inhibition zone (Turkun *et al.*, 2008). The resistance to fracture within a restorative material is specified by a fracture stress, which is often referred to as the strength of the material (Yap *et al.*, 2003). Two mechanical strength tests that is the Compressive and Diametral Tensile test were used in our study. Mean values of compressive strength were recorded as 69.03, 55.55 and 58.84Mpa for GIC, GIC with 1% Chlorhexidine and GIC with 1% Cetrimide respectively (Table 4). These values were then subjected to ANOVA and Post Hoc analysis. A significant difference was seen between GIC and both the other experimental GIC's. However no significant difference was seen between the two experimental GIC's (Table 4). The addition of 1% Chlorhexidine and Cetrimide produced a significant decrease in the compressive strength of GIC. Similar results were also obtained in a study done by Sudhir Mittal (Mittal *et al.*, 2011).

In our study the values of the compressive strength were lower as compared to those observed by Takashi et al., 2006; Bresicani et al., 2008; Deepalakshmi et al., 2010 and Shalini et al., 2014. In these studies a mean range of 90 – 150 Mpa was observed. This could be due to the difference in the method of casting the specimens and the mechanical testing procedure. Since the detection of internal defects was beyond the confines of our study, their effect on the compressive strength of the material cannot be ignored. The compressive strength of experimental groups containing CHX and antibiotics decreased in a concentration-dependant manner.

The cross-linking in GIC is because of the coordination of Al^{3+} and Ca^{2+} with the COOH groups on the acidic polymers. Due to vitrification of GIC with antimicrobials, many of these COOH groups are prevented from participating in these coordination complexes leading to a lower compressive strength (Moshaverinia *et al.*, 2010). In addition, variation in the P/L ratio by addition of antimicrobials may also have attributed to the decrease observed in compressive strength (Wilder *et al.*, 1998). Also, the powdered antibiotic particles which are added to GIC easily absorb water. The absorption of water can also decrease the compressive strength of the GIC. The diametral tensile strength (DTS) is a critical requirement of any restorative material, because many clinical failures are due to tensile stresses. The mean of the diametral tensile strength for group A was 11.18MPa, Group B was 8.63Mpa and Group C was 8.44Mpa (Table 5). These were then statistically analysed using ANOVA and Post Hoc analysis. Group B and Group C showed no significant difference in DTS whereas a highly significant difference was seen when Group A was compared to Group B or Group C. GIC had a higher strength when compared to both the experimental groups. These findings were in conjunction with those stated in the literature by Bresicani *et al.*, 2008; Ahluwalia *et al.*, 2012; Mittal *et al.*, 2015; Turkun *et al.*, 2008 and Yap *et al.*, 2003. Considering the various above stated parameters the addition of 1% Chlorhexidine to GIC can be considered as an effective alternative to GIC in cases of high caries activity when compared to GIC and GIC with 1% cetrimide.

Conclusion

There is a strong demand of a restorative material in pediatric dentistry with high antimicrobial efficacy along with good physical characteristics. All efforts should be thus directed towards such a material with bioadhesive and biocompatible properties. Various methods should also be introduced to incorporate these antimicrobials chemically into GIC rather than simple mechanical mixing of the same. In vivo studies are required in the near future to provide sufficient data for introduction of modified GIC's into current clinical practice. Nano particles of these antimicrobials can also be effectively used to enhance the antimicrobial efficacy of GIC without altering the physical properties of the restorative cements. The future of dentistry lies in the effective utilization of current resources with advanced technological expertise to deliver optimum and enhanced dental care to our paediatric population.

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