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EVALUATION OF ANTIBACTERIAL EFFECT OF GLASS IONOMER CEMENT INCORPORATED WITH DIFFERENT ANTIBACTERIAL AGENTS: AN IN-VITRO STUDY

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Abstract

Background: The atraumatic restorative treatment (ART) was developed with an intention of providing treatment in regions where routine treatment procedures were not accessible. However, the removal of carious dentin by this technique may not be complete and the use of GIC has the possibility of microleakage. So therapeutic benefit may be gained by combining antibacterial agents with GIC materials. The most appropriate choice of antimicrobial agents for combining with GIC would be chlorhexidine, cetrimide, cetylpyridinium chloride. Hence the use of a dental material with fluoride releasing ability with an additional antibacterial effect would definitely be an added advantage.

Objectives: To evaluate and compare the antibacterial activity of glass ionomer cement containing different antimicrobial agents with that of conventional glass ionomer cement against.

Material and Methods: An antibacterial agents Chlorhexidine gluconate (1%), cetrimide (1%) and cetylpyridinium chloride (1%) will be added to the GIC powder and the mixing will be done according to the manufacturer's instructions. Conventional GIC will be used as the control. Disc shaped cement specimens using standardized brass moulds will be made. Prepared specimens will be used to check the antimicrobial activity at different time intervals.

Results: All the test groups had significantly higher amount of antibacterial activity compared to the control.

Conclusion: Hence, we can incorporate the different antibacterial agents in the conventional GIC for improved antibacterial activity.

Key words:antibacterial activity, Chlorhexidine gluconate, cetylpyridinium chloride, GIC

Introduction

Nowadays concept of 'extension for prevention' has changed to preservation of as much sound tooth structure as possible i.e prevention of extension as compared to the surgical model of drilling the cavity to make it more geometrically perfect and filling it with the most compatible artificial materials.[1]which lead to a continuous process of replacement dentistry wherein the cavity just got larger and the tooth weaker.This called for a technique with minimal invasion at a relatively low cost. The atraumatic restorative treatment (ART) was thus developed with an intention of providing treatment in regions where routine treatment procedures were not accessible.

The choice for glass ionomer cement in ART is based on its self-curing and potential caries preventive properties. The advantages of GIC includelong-term and slow release of fluoride into enamel, dentin, saliva and plaque, biocompatibility, it does not require mixing machines and curing lights, its ability to chemically bond to enamel and dentin, reduced caries progression in tooth tissues that are in contact with the material.[2]

In this aspect therapeutic benefit may be gained by combining antibacterial agents with GIC materials. From the various literature searches in the field of dentistry we found that only chlorhexidine has been used extensively with the GIC to give an increased antibacterial effect in vitro as well as up to some extent in vivo also. Although numerous efforts have been made on improving antibacterial activities of dental restoratives, most of them have been focused on release or slow-release of various incorporated low molecular weight antibacterial agents such as antibiotics, zinc ions, silver ions, iodine and chlorhexidine. However, release or slow-release can lead or has led to a reduction of mechanical properties of the restoratives over time, short-term effectiveness, and possible toxicity to surrounding tissues if the dose or release is not properly controlled.[4]

Polymers containing quaternary ammonium (QAS) or phosphonium salt (QPS) groups have been studied extensively as an important antibacterial material and used for a variety of applications due to their potent antibacterial activities. These polymers are found to be capable of killing bacteria that are resistant to other types of cationic antibacterials.

The most appropriate choice of antibacterial agents for combining with GIC would be antiseptic agents that have been proven useful in clinical dentistry such as chlorhexidine, cetrimide, cetylpyridinium chloride.[4]

Hence, this study intends to evaluate the antibacterial activity of conventional glass ionomer cement used for ART with different antibacterial agents.

Objectives

To evaluate and compare the antibacterial activity of glass ionomer cement containing different antibacterial agents with that of conventional glass ionomer cement against.

Material and Methods

Present study was conducted in Department of Preventive and Community Dentistry, College of Dental Sciences, Davangere, Karnataka for the duration of 60 days. Antibacterial effect of the specimens was analyzed in Department of Oral Pathology, College of Dental Sciences, Davangere, and Karnataka. Fluoride release from the specimens was analyzed at Department of Environmental Engineering, Bapuji Institute of Engineering and Technology, Davangere.

A double blinded in-vitro study was designed. In this study, a total of 4 cements- one conventional GIC and three GICs containing the three different antibacterial agents were analysed for antibacterial effect at different intervals over a period of 60 days.

Sample size was determined using the formula:

$$n = 2\left[\frac{z\alpha\,(\sigma)}{dd = X_1 - X_2}\right]^2$$

where Zα = Table value

σ = SD

$$= 2\left[\frac{2(1.5)}{2.2n}\right]^2$$ = Sample size

$$= 7.66 - 5.46$$

$$= 3.71$$

$$\approx 4$$

Hence using the above formula the sample size was doubled and was taken as 8.

Materials used:

1) Fuji IX GIC (High Strength Posterior Restorative) was obtained from our department store.
2) Antibacterial agents**:** were obtained from the HIMEDIA laboratory

a) Chlorhexidine gluconate
b) Cetrimide
c) Cetylpyridinium chloride

3) Microbial strain used:

Streptococcus mutans was obtained from Microbial Type Culture Collection (MTCC).

Methodology

A total of 128 disc shaped cement specimens were made and they were distributed among the four groups: 1 control and three test groups.

Groups	**Composition of GIC**
Control	Conventional GIC
Group I	GIC with 1% chlorhexidine gluconate
Group II	GIC with 1% cetrimide
Group III	GIC with 1% cetylpyridinium chloride

CONTROL GROUP: Conventional glass ionomer cement (GC Fuji IX gold label, high strength posterior restorative) was used as the control. The quantity of powder used was 10g.

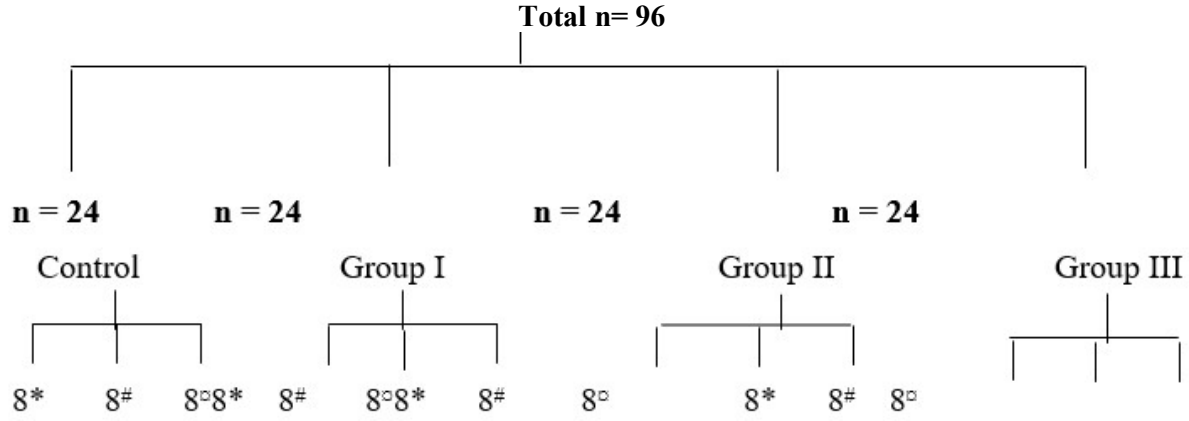
TEST GROUPS: An antibacterial agents containing Chlorhexidine gluconate (1%), cetrimide (1%) and cetylpyridinium chloride (1%) was added to the conventional glass ionomer cement to obtain the test groups.

GROUP I: To obtain a concentration of 1% w/w of the chlorhexidine gluconate, 0.1g of chlorhexidine gluconate was added to 9.9g of conventional glass ionomer cement powder, making up the total to 10g of powder.

GROUP II: To obtain a concentration of 1% w/w of the cetrimide, 0.1g of cetrimide was added to 9.9g of conventional glass ionomer cement powder, making up the total to 10g of powder.

GROUP III: To obtain a concentration of 1% w/w of the cetylpyridinium chloride, 0.1g of cetylpyridinium chloride was added to 9.9g of conventional glass ionomer cement powder, making up the total to 10g of powder.The resultant mixture was thoroughly triturated in a mortar and pestle to obtain the powder for each of the test groups.

Distribution of the sample for antibacterial activity:



* = For immediately after setting
# = For 2hrs after placing in the distilled water
¤ = For 60 days after placing in the distilled water

Preparation of tests specimens:

A total of 96 disc shaped cement specimens using standardized brass mouldsof inner diameter 10 mm and height 2 mm were made and used to test the antibacterial activity.

For the test groups, the powder containing pre weighed glass ionomer cement and pre weighed antibacterial agent was mixed with the liquid of glass ionomer cement (powder/liquid ratio = 3:1) at room temperature on a mixing pad with a plastic spatula and then placed in the brass molds. For the control group, conventional glass ionomer powder and liquid were mixed according to the manufacturer's instructions.[4]

A matrix mylar strip was first secured on a glass plate to form the base of the mold. The restorative material was then mixed and placed in the mold. The mold was then covered with a second mylar strip. A glass plate was then placed over the mold and pressure was applied to extrude excess material. After setting, the pellets were removed from the mold and the excess was trimmed using a Bard Parker blade. All specimens were made in the same mold to guarantee the same size, shape and total area.[6]The study was carried out to Evaluate the antibacterial activity of the cement specimens against *Streptococcus mutans.*

Bacterial Inhibition Test

The bacterial inhibition test of the specimens was evaluated against *Streptococcus mutans* (MTCC 497) cultured on Mitis Salivarius Bacitracin (MSB) agar using agar diffusion method. The zone of inhibition around the specimens was then measured from the mean diameter of two perpendicular diameters of the zone of inhibition minus the area of the test specimen. The prepared specimens were stored in deionized distilled water and the specimens were subjected to agar diffusion technique immediately after setting of the specimens. Second and third time agar diffusion technique was performed after storing the specimens in the deionized water at 37ºC for two hours and 60 days respectively and then carefully drying the specimens using filter paper and then placed on agar as above.[5] The antibacterial activity of the cement specimens were evaluated using the agar inhibition test in a laminar airflow unit.

Agar Diffusion Testing

Suspension of the *Streptococcus mutans* (MTCC 497) strains prepared in Phosphate Buffered Saline at contains approximately $1.5x10^8$ organisms/ml by using the McFarland 0.5 turbidity tube were flood-inoculated onto the surface of 10 ml of Mitis Salivarius Bacitracin (MSB) agar.[5]

Powder and liquid of each experimental group were mixed, then placed into the mold as described previously and allowed to set for 10 minutes at room temperature.[4] The set specimens were placed onto the MSB agar plate inoculated with the bacterial strains such that two specimens of a material

were applied to each plate. The plates were then incubated at 37± 0.5ºC for 48 hours and the diameters of zones of inhibition produced around the specimens were measured at three different points. The sizes of inhibition zones were calculated by subtracting the diameter of the specimen (10mm) from the average of the three measurements of the halo.[7]The observations were made separately for each of the groups, against for *Streptococcus mutans* (MTCC 497) at immediately after setting, 2 hours and 60 days.

Statistical Analysis

Data collected by experiments was computerized and analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0. Data comparison was done by applying specific statistical tests to find out the statistical significance of the results. The mean and standard deviation of the zone of inhibition at different time intervals was calculated. The variation in zone of inhibition between different specimen groups at same time interval and within the group at different time intervals was analyzed using One way Analysis of variance (ANOVA) followed by Tukey's post hoc test for pair-wise comparison. p value less than 0.05 was considered statistically significant.

Results

The present in-vitro study was conducted to evaluate antibacterial effect of glass ionomer cement incorporated with chlorhexidine gluconate (1%), cetrimide (1%) and cetylpyridiniumchloride (1%), GIC (FUJI IX) was used as a control. The study result shows that there is a statistically significant antibacterial effect from the GIC incorporated with the chlorhexidine gluconate (1%), cetrimide (1%) and cetylpyridinium chloride (1%) compared to the control group. Highest antibacterial activity was reported with Cetrimide + GIC group and highest fluoride release was reported with control group. The observations for antibacterial effect were made separately for each of the groups, against *Streptococcus mutans* (MTCC 497) at immediately after setting of the cement, 2 hours and 60 days. The results are presented in the form of tables and figures. The results showed the following observations:

TABLE I: Inter group comparison of the inhibition zones (mm) produced by the control and the test groups against *Streptococcus mutans* at immediately after setting, after 2 hours and after 60 days.

Time Interval	Groups				ANOVA		Tukey' post hoc
	Control	Group I	Group II	Group III	F value	p value	
Immediately after setting	2.13 ± 0.21	15.84 ± 1.27	23.72 ± 0.95	9.55 ± 0.60	2784.86	0.001 (HS)	3>2>4>1
After 2 hours	0.68 ± 0 .24	15.78 ± 1.73	23.32 ± 1.25	8.95 ± 0.68	1754.43	0.001 (HS)	3>2>4>1
After 60 days	0	6.70 ± 1.31	13.53 ± 1.10	4.17 ± 0 .36	1005.43	0.001 (HS)	3>2>4>1

1 = control group, 2= group 1, 3 = group 2, 4 = group 3, HS = Highly Significant.

Zone of inhibition produced by the control group was minimum compared to the test groups. The mean zone of inhibition produced by the control group immediately after the setting of the cement specimens was 2.13 ± 0.21 mm, after 2 hrs keeping the specimens in distilled water the zone of inhibition produced was 0.68 ± 0 .24 and after 60 days control group had no any antibacterial activity. Maximum zone of inhibition was produced by the Cetrimide + GIC group followed CHX

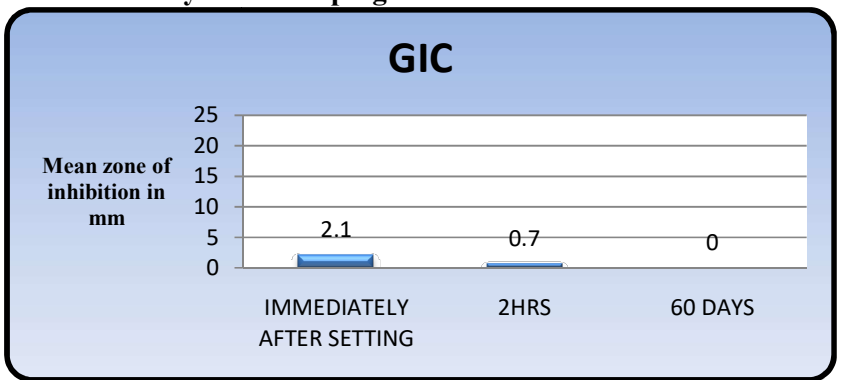
gluconate + GIC group and least by the Cetylpyridinium chloride + GIC among the test groups. The mean zone of inhibition produced by the Cetrimide + GIC group immediately after the setting of the cement specimens was 23.72 ± 0.95mm, after 2 hrs keeping the specimens in distilled water the zone of inhibition produced was 23.32 ± 1.25 mm and after 60 days 13.53 ± 1.1 mm. The mean zone of inhibition produced by the CHX gluconate + GIC group immediately after the setting of the cement specimens was 15.84 ± 1.27 mm, after 2 hrs keeping the specimens in distilled water the zone of inhibition produced was 15.78 ± 1.73 mm and after 60 days 6.70 ± 1.31 mm. The mean zone of inhibition produced by the Cetylpyridinium chloride + GIC group immediately after the setting of the cement specimens was 9.55 ± 0.60 mm, after 2 hrs keeping the specimens in distilled water the zone of inhibition produced was 8.95 ± 0.68 mm and after 60 days 4.17 ± .36 mm.The difference of zone of inhibition produced by test groups was statistically highly significant (p=0.001) when compared to the control group.So, the maximum mean zone of inhibition produced by group II followed by group I, group III and least by the control group.

TABLE II: Intra group comparison of the inhibition zones (mm) produced by the control and the test groups against *Streptococcus mutans* at immediately after setting, after 2 hours and after 60 days

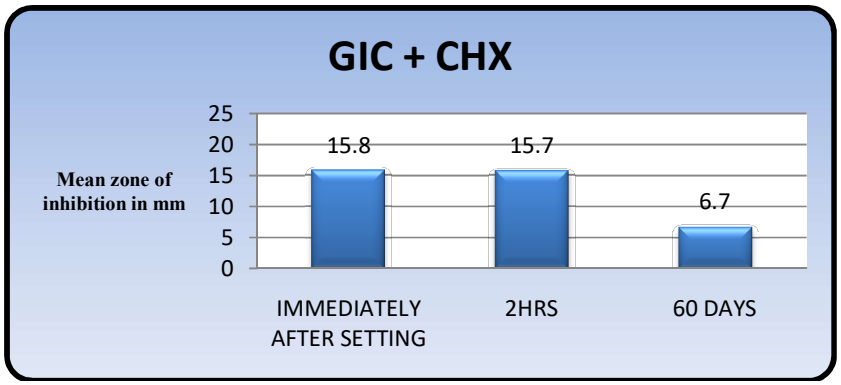
S.N.	TIME		Groups			
			Control	Group I	Group II	Group III
1	Immediately after setting		2.13 ± 0.21	15.84 ± 1.27	23.72 ± 0.95	9.55 ± 0.60
2	After 2 hrs		0.68 ± 0 .24	15.78 ± 1.73	23.32 ± 1.25	8.95 ± 0.68
3	After 60 days		0	6.70 ± 1.31	13.53 ± 1.10	4.17 ± 0 .36
ANOVA		F value	669.38	105.79	212.31	213.41
		p value	0.001*	0.001*	0.001*	0.001*
Tukey's post hoc			1,2>3	1,2>3	2,1>3	1,2>3

1 = immediately after setting, 2 = after 2 hrs, 3 = after 60 days,
*** = Highly significant**

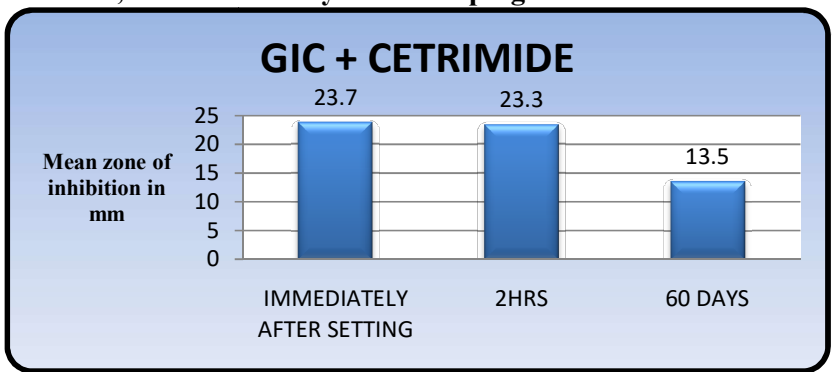
Graph I: Mean zone of inhibition of the control group immediately after setting, 2hrs and 60 days after keeping in the deionized water.



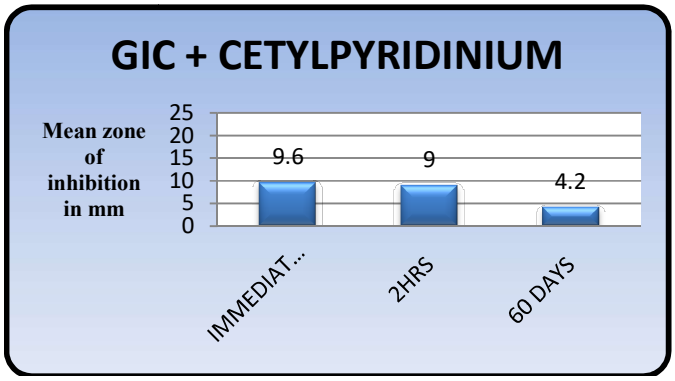
Graph II: Mean zone of inhibition of the GIC + Chlorhexidine Gluconate immediately after setting of the material, 2hrs and 60 days after keeping in the deionized water.



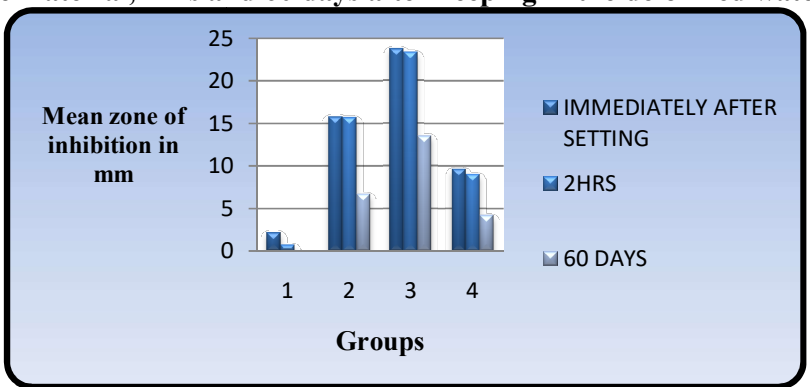
Graph III: Mean zone of inhibition of the GIC + Cetrimide immediately after setting of the material, 2hrs and 60 days after keeping in the deionized water.



Graph IV: Mean zone of inhibition of the GIC + Cetylpyridinium Chloride immediately after setting of the material, 2hrs and 60 days after keeping in the deionized water.



Graph V: Mean zone of inhibition of the control and test groups immediately after setting of the material, 2hrs and 60 days after keeping in the deionized water.



1= GIC, 2 = GIC + Chlorhexidine Gluconate, 3 = GIC + Cetrimide, 4 = GIC + Cetylpyridinium Chloride

Discussion

Atraumatic Restorative Treatment or ART is to perform minimal cavity preparation using only hand instruments followed by restoration of the cavity with an adhesive filling material, such as glass ionomer cement[8], which were a natural choice in ART procedures because they demonstrate an antibacterial effect against cariogenic organisms.[9] Clinical studies have demonstrated that one of the main factors responsible for replacement of dental restorations is the presence of secondary caries, which is true for the ART approach as well. The ability of a restorative material to resist secondary caries and microleakage at its margins will, to a great extent, determine whether a restoration will succeed or fail.[10,11] Furthermore, although it is believed that hand excavation is capable of removing most of the infected dentin, research has shown that bacteria remain even after complete hand excavation within the tubuli of affected dentin.[10]As the ART approach is being utilized by an ever-increasing number of dental professionals around the world, there seemed a need to update the data about the longevity of ART restorations.[11]

Hence various attempts have been made to improve the antibacterial properties of the existing glass ionomers. The most appropriate choice of antibacterial agents for combining with a GIC would be antiseptic agents that have been proven useful in clinical dentistry. Cationic disinfectants have been investigated both in vitro and in vivo for their antibacterial effects against micro-organisms.[4]

The rationale for using the 1% concentration of the antibacterial agent was that as shown by the various studies conducted world wide as the concentration of the antibacterial agent increases, the mechanical properties of the resulting material decreases. Studies have also shown that with 1% concentration the resulting material retains its mechanical properties with added antibacterial activity. [5,12]

The present in vitro study is a modest attempt to explore the influence of addition of three different antibacterial agents to the formulation of glass ionomer cement, keeping in mind its potential implications for use in the ART approach.

Evaluation of antibacterial activity

The first part of the study comprised the determination of the antibacterial activity of the experimental groups. The antibacterial activity of the set cement specimens of the control and the test groups were evaluated against *Streptococcus mutans* (MTCC 497) using the bacterial inhibition test.

Streptococcus mutans is established as the leading cause of dental caries worldwide and is considered to be the most cariogenic of the oral streptococci. It has been implicated most of all as the initiator of dental caries.[9,10]However recent research has shown that although *S.mutans* is one of the most researched cariogenic microorganisms, it is only one of more than 500 species found in

dental plaque. A study in contradiction to the earlier studies report that *S.mutans* is not detectable in 10 to 20 percent of people who have severe caries.[11]

Since it was impossible to replicate the oral environment with its diverse oral microbiota, the species most commonly implicated in dental caries was chosen in the present study namely *Streptococcus mutans.*

The rationale for use of set specimens in our study was mainly to offset the effect of pH on antibacterial activity of glass ionomer cement. In addition it also enabled the standardization of the size of the specimens and therefore the amount of antibacterial substance in each specimen.[4] The agar inhibition tests are in fact an extension of a procedure commonly used in medical laboratories to determine antibiotic sensitivities of bacterial isolates. The result is the identification of potentially effective therapeutic agents and the size of the inhibitory zones reveals the microbial sensitivity, agent solubility and the quantity of agent released within the first few hours after placement. This method was chosen because it can be performed rapidly and easily with a large numbers of specimens and is relatively inexpensive. However the clinical relevance of the method can be revealed only when in vitro data are extrapolated to oral bacteria colonizing around or on restorative materials.[11]

In the present study, the cements specimens were assessed for their antibacterial activity immediately after setting of the cement specimens, 2hrs after placing the specimens in deionized water and 60 days after placing the specimens in deionized water. The reason behind placing the specimens in the deionized water for 2 hrs and for 60 days was to evaluate and compare the change in antibacterial activity.

Over a period of time. So we can confirm that the incorporated antibacterial agent is leachable even after the aging in deionized water and retain its antibacterial activity.[12] Table I shows the group wise comparison of the zones of inhibition (mm) produced by the control and the test groups against *Streptococcus mutans* immediately after setting of the cement specimens, 2hrs after placing the specimens in deionized water and 60 days after placing the specimens in deionized water.

In our study, zone of inhibition produced by the control group (high strength posterior restorative GC Fuji IX) immediately after the setting of the cement specimens was 2.13 ± 0.21 mm, after 2 hrs keeping the specimens in deionized water the zone of inhibition produced was 0.68 ± 0.24 against *Streptococcus mutans* which was in accordance with study conducted by Ferreira GFS.[33]

But it was in contrast to the study conducted by Tuzuner T[14] in which control group had no any antibacterial activity. After placing the specimens in deionized water for 60 days there was no antibacterial activity which was in accordance with the study conducted by Tuzuner T.[14]

The study conducted byDavidovich E[15] also showed the inhibition of *Streptococcus mutans* by the GIC for at least one week. The study conducted by Shirani F[16] after the 5-day incubation period of test specimens of GIC against *Streptococcus Mutans*, no bacterial growth was seen in any of the specimens. However in that study the GIC material which was used was different from the material used in the present study.

Zone of inhibition produced by the group II (Cetrimide + GIC) had the maximum zone of inhibition which was in accordance with the study conducted by Tuzuner T.[14]

The reason for greater potency of the group II (Cetrimide + GIC) could be due to the high elution rates of the antibacterial agent from the GIC or due to the synergistic interactions between the antibacterial agent and the GIC. Synergism has been shown to occur between the metal ions and cationic antibacterial agents.[4]

The zone of inhibition produced by the group I (CHX gluconate + GIC) was less compared to the group II (Cetrimide + GIC) and least zone of inhibition was produced by the group III (Cetylpyridinium chloride + GIC). Numerous studies have shown that addition of chlorhexidine in various forms and at various concentrations in GIC increases the antibacterial activity of the resulting material to great extent.[14,17]

The differences in inhibition between these groups may be related to their inherent potency, to different solubilities and hence elution from the material, to binding between the antibacterial and

GIC or agar constituents, or to synergistic or antagonistic interactions between the antibacterial agents and the GIC.[4]

The zone of inhibition produced immediately after the setting of the cement specimens and after 2 hours keeping the specimens in deionized water was almost similar for all the test groups and the result was statistically not significant. The zone of inhibition produced after placing the specimens in deionized water for 60 days there is statistically highly significant (p=0.001) decrease was seen in all the test groups when compared to the zones of inhibition produced immediately after the setting of the cement specimens and keeping the specimens in deionized water for 2 hours. Thus the antibacterial activity was found to decrease over a period of time in all the test groups as well as in control group. That indicates as the time progresses there will be decrease in the antimicrobial activity of the cements.

Hence from the above study the antibacterial activity from the GIC incorporated with the different antibacterial agents we can stat that we can incorporate CHX and Cetrimide with the GIC for better antibacterial activity ability for the good prognosis of the Atraumatic Restorative Treatment as well as for other GIC restorations.

Conclusion

The following conclusions can be drawn from the study:

- Conventional glass ionomer cement incorporated with different antibacterial agents displayed antibacterial action against *Streptococcus Mutans* till the end of the observational period.
- The incorporation of antibacterial agents into glass ionomer decreased the fluoride releasing ability of the conventional glass ionomer cements.
- Among the test groups CHX gluconate + GIC had good long term fluoride release when compared to the other test groups where as Cetrimide + GIC group had initial high fluoride release and decreased fluoride release over long period.
- Cetylpyridinium chloride + GIC group had the least amount of fluoride release and antibacterial activity among all the test groups. Further research need to be conducted for the antibacterial and fluoride release activity of Cetylpyridinium chloride incorporated with the GIC.

Within the limitations of the present study, it may be concluded that the incorporation of antibacterial agents to glass ionomer at 1% w/w may be an effective tool in inhibiting the organisms that are responsible for both initiation and progression of caries. The glass ionomer cement with antibacterial agents may be seen as an alternative restorative material for use in Atraumatic Restorative procedures where it is particularly important to rule out the possibility of residual cariogenic microorganisms.

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PHOTOGRAPH I,II : MATERIALS USED AS CONTROL

PHOTOGRAPH III : ARMAMENTARIUM USED FOR MAKING CEMENT SPECIMENS

PHOTOGRAPH IV : ARMAMENTARIUM FOR BACTERIOLOGICAL STUDY

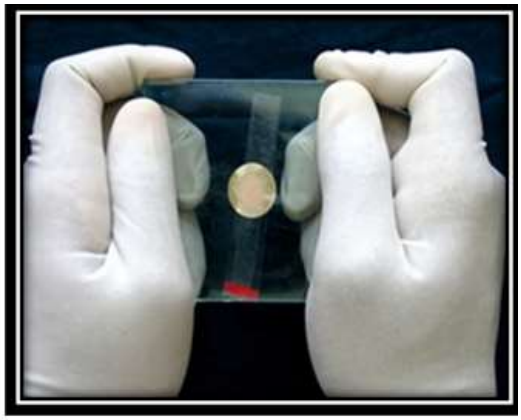
PHOTOGRAPH V : ELECTRONIC WEING MACHINE

PHOTOGRAPH VI : CULTURE MEDIA FOR STREPTOCOCCUS MUTANS

PHOTOGRAPH VII : INOCULATION OF STREPTOCOCCUS MUTANS

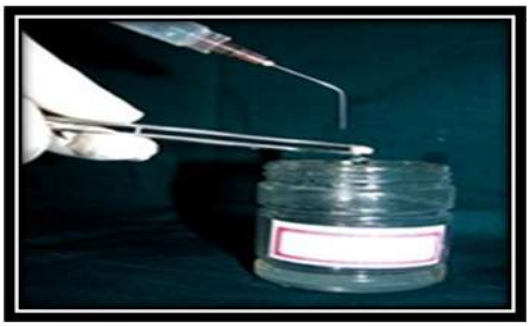
PHOTOGRAPH VIII : LAMINAR AIR FLOW UNIT

PHOTOGRAPH IX : PREPARATION OF TEST GROUP

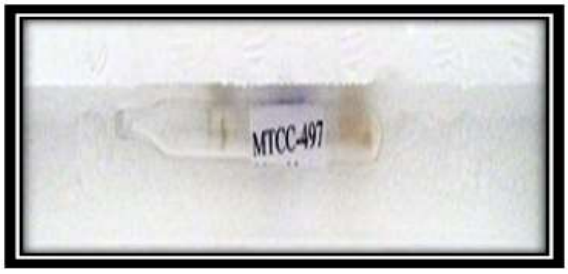


PHOTOGRAPH X : PREPARATION OF TEST SPECIMENS

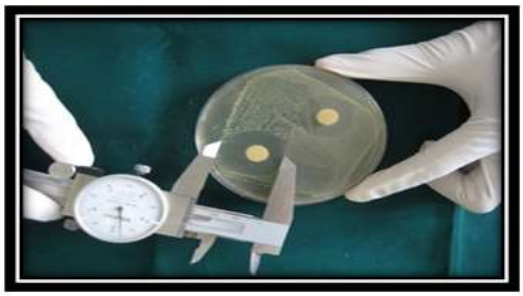
PHOTOGRAPH XI : PREPARED TEST SPECIMEN



PHOTOGRAPH XIV : RINSING THE SPECIMEN BEFORE IMMERSING IN THE FRESH SOLUTION

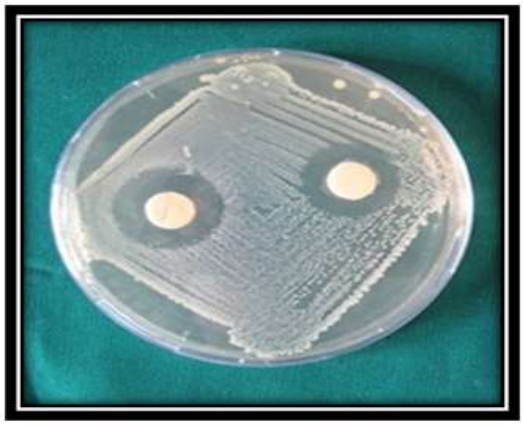


PHOTOGRAPH XV : STREPTOCOCCUS MUTANS STRAINS



PHOTOGRAPH XVI : MEASURING THE ZONE OF INHIBITION WITH DIAL CALIPER

PHOTOGRAPH XVII : ZONE OF INHIBITION AFTER 2 HRS & 60 DAYS - CONTROL GROUP



PHOTOGRAPH XVIII : ZONE OF INHIBITION AFTER 2 HRS - CHX + GIC GROUP

PHOTOGRAPH XXI : ZONE OF INHIBITION AFTER 2HRS - CETYL PYRIDINIUM + GIC GROUP

