ORIGINAL RESEARCH

To Evaluate the Antimicrobial Efficacy of Conventional Glass Ionomer Cement incorporated with Different Antibiotics: An in Vitro Study

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ABSTRACT

Introduction: Dental caries is a perennial public health problem that dates back to antiquity. ART make use of manual instruments to eliminate bacteria from the cavities which is not as effective as rotary burs. Cariogenic bacteria can survive incarceration under GIC restoration and remain viable for up to 2 years resulting in secondary caries. The GIC cement which we are using has undergone lots of modifications, and the material has many advanced forms like metal reinforced GICs, viscous esthetic conventional GICs etc. In this study, we are evaluating the efficacy of such a modified form of GIC incorporated with antibiotics which can inhibit bacteria. **Methods:** Antibiotics (ciprofloxacin, metronidazole, amoxicillin and minocycline) were incorporated into GIC Fuji IX powder at 2% w/w ratio to form individual and a combination experimental groups (n=15). GIC Fuji IX served as control. The experimental GIC specimens were placed on Mitis Salivarius Bacitracin agar plates which were previously inoculated with Mutans Streptococcus, and after 24 hours the area of inhibition was measured. **Results:** All experimental groups showed inhibition zone against S. mutans which was greater than that seen in control group ($p<0.05$). **Conclusion:** From the results obtained from the present study we concluded that experimental GICs containing antibiotics were effective in inhibiting S. mutans individually and in combination at 2% w/w concentration. Ciprofloxacin, minocycline and combination groups showed better results compared to amoxicillin and metronidazole

KEYWORDS: Antibiotic, Agar diffusion method, Atraumatic Restorative Treatment, Ciprofloxacin, Glass ionomer cement

INTRODUCTION

Atraumatic Restorative Treatment (ART) is a restorative technique in which carious dental tissues are removed using minimally invasive technique. ART uses hand instruments to remove infected dentine and stop the progression of caries.[1] This technique can be used for treating early childhood caries in developing countries where the resources are scarce.[2] The main attractive advantage of this technique is its straightforwardness and simplicity and the low cost comparing to rotary instrument treatment approach.[3] The minimally-invasive procedure is largely pain-free and readily accepted by children.[4,5] A recent study demonstrated the dramatic improvements in oral health achieved in both primary and permanent dentition of children when the ART approach replaced the use of conventional instrumentation in mobile dental clinics.[6]

In 1972 Wilson and Kent introduced glass ionomer cement, a tooth colored material which can bond chemically with the tooth structure and additionally gives an anticariogenic effect, Presently GIC is one of the widely used restorative material in dentistry. The anti cariogenic potential of glass ionomer cement is due to the release of fluoride over a long period of time and thus results in reduction in caries progression in and around the restoration.[7] However, the anticariogenic action of GIC is sufficient to arrest the caries under restoration is still doubtful.[8] Studies show that bacteria can survive under this restoration and be viable up to two years which can result in secondary caries.[9,10] Even the fluoride present in the GIC is not potent to defend the destruction caused by the bacteria over an extended period of time.[11] Moreover, cavities after ART can have infected dentin as hand instruments are not as effective as rotary instruments to remove infected dentin.[12]

Different classes of GIC are used for lining, luting, restorative, for orthodontic purpose and the material have many modifications like metal reinforced GIC and highly viscous GIC.[13] The ion can readily travel in and out of the material which offers the opportunity to dope the cement

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with other materials allowing GIC to be more amenable for modifications.[14]

In the previous studies, researchers combined a combination of antibiotics with GIC and demonstrated an antibacterial effect. The present study used ciprofloxacin, minocycline, metronidazole, amoxycillin and a combination of all these antibiotics with GIC. The majority of these studies were done against streptococcus mutans or lactobacillus, and the antibiotics used were in combination[12,15,16].

Hence, the present study aims to evaluate the antibacterial efficacy of different antibiotics individually at a concentration of 2 % against streptococcus mutans. The antibiotics used were ciprofloxacin, metronidazole, minocycline, amoxicillin, the combination of all four antibiotics and conventional GIC as the control.

The hypothesis of the present study is that there is a difference in antibacterial efficacy between different groups. The significance level was determined as $p<0.05$.

MATERIALS AND METHODS

Preparation of antibacterial cement: Commercially available antibiotic tablets were obtained and the surface sugar coating from the tablets, was scraped off and grouped. Group I GIC (Fuji IX, GC,Tokyo, Japan) Group II Ciprofloxacin (Cipla, Sikkim, India), Group III Minocycline (Rexcie Mumbai, India), Group IV Metronidazole (J B, Mumbai, India), Group V Amoxicillin (Ranbaxy,Sun Pharmaceuticals M.P,India) and combination of all four antibiotics.The tablets were then grounded into a fine powder using a mortar and pestle. The grounded antibiotics were weighed using a standardized electronic weighing machine. Each antibiotic groups were sealed in labeled containers to ensure blinding of the investigator by a third person. Each experimental groups were prepared by adding 2% w/w antibiotics to the pre-weighed GIC powder (Table 1).

Group	Composition	Concentration
Group I	5000 mg GIC	
Group II	4900 mg GIC and 100gm of ciprofloxacin	2%
Group III	4900 mg GIC and 100gm of minocycline	2%
Group IV	4900 mg GIC and 100gm of metronidazole	2%
Group V	4900 mg GIC and 100gm of amoxicillin	2%
Group VI	4900 mg GIC and 50 mg of all four group of antibiotics.	2%

Table 1: Preparation of antibacterial cement

Preparation of molds: A plastic tubing available commercially was cut with the help of a BP blade No.11 to prepare molds of 6X6mm diameter and height (figure 1). The molds were grouped as group I (control), group II (ciprofloxacin), group III (minocycline), group IV (metronidazole), group V (amoxicillin) and group VI (combination). Total 90 molds were prepared, 15 molds per group.

Preparation of samples: The powder and liquid (P/L ratio 3.6: 1, as per the manufacturer's instruction) were dispensed for each experimental group on a mixing pad and mixed using an agate spatula for 30 seconds. The

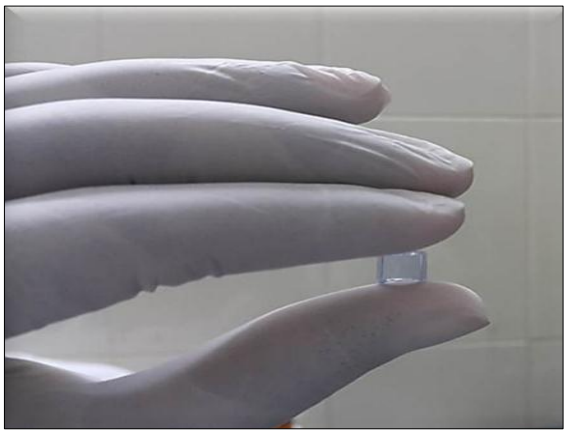


Figure 1: Prepared mold of 6 x 6 mm

material was transferred to the previously made standardized molds with a plastic filling instrument. Care was taken to uniformly fill the molds and the surplus material was removed using a glass slide from the top side, and the material was allowed to set for 30 minutes at room temperature. Then the specimen was carefully removed from the mold by applying pressure from one side using a condenser. Each mold were grouped into experimental and control group.(figure 2)

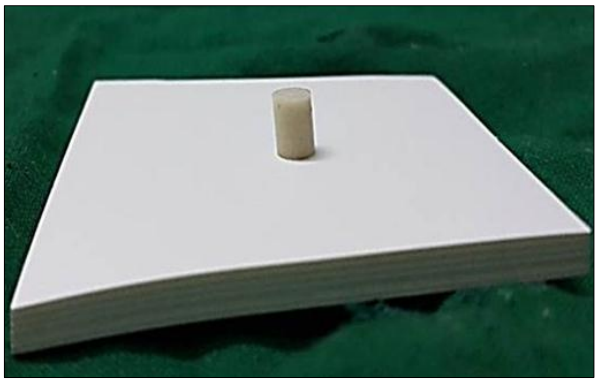


Figure 2: Set specimen before placing to agar plates

Microbial strain and growth media: S mutans was isolated from the saliva of the patient who had active carious lesions. The saliva samples were diluted in BHI broth and transferred to Mitis Salivarius Bacitracin agar (MSB Agar). The saliva samples were incubated at 37°C in candle extension jar for 24 hours. The colonies were observed for the morphology, and those resembling S mutans were confirmed by gram staining reaction and biochemical tests (sorbitol fermentation and inositol fermentation test).

S mutans was inoculated into Brain Heart Infusion broth (BHI) and incubated at 37°C for 4 hours. The turbidity of growth was adjusted to Mac Farland 0.5 opacity standard equivalent to 1.5×10^8 CFU (colony forming unit) per ml. 15µl of this adjusted culture was transferred onto a sterile MSB Agar and spread uniformly using a sterile L – spreader.(figure 3)

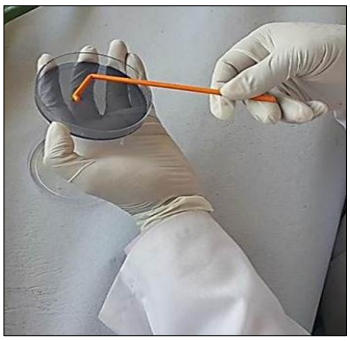


Figure 3: Bacterial culture spreading om MSB agar using L- Spreader

Wells of diameter 6mm were made in the agar medium using sterile templates. GIC blocks were placed by the microbiologist into the wells and ensured uniform contact (figure 4). Then plates were incubated in candle light jars for 24hrs.

After 24 hours, the zone of inhibition of growth of bacteria was measured using a vernier caliper (figure 5,6,7).

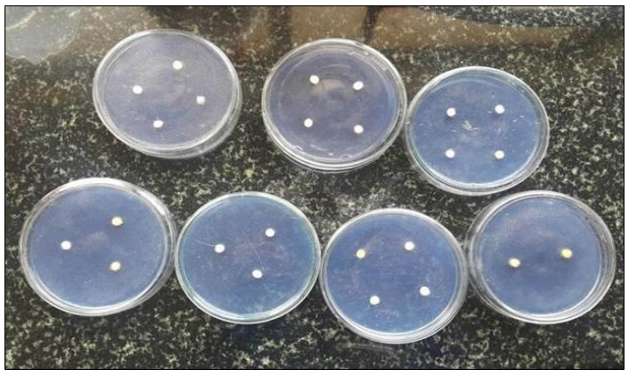


Figure 4: Set specimen placed in the agar plates

Figure 5: Zone of inhibition after incubation for 24 hours

Figure 6: Zone of inhibition after incubation for 24 hours

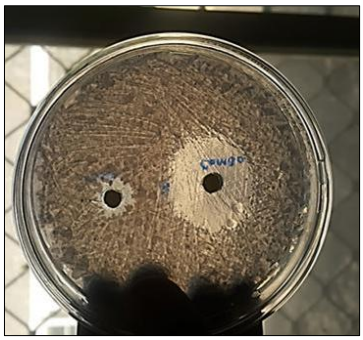


Figure 7: Zone of inhibition after incubation for 24 hours

Statistical analysis: The zone of inhibition is measured in millimeter (diameter), in all groups and was analyzed using one-way analysis of variance (ANOVA) (Table 2) followed by Tukey's Post hoc analysis (table 3).

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	7187.156	5	1437.431	784.053	0.000
Within Groups	154.000	84	1.833		
Total	7341.156	89			

Table 2: Analysis done for multiple group with one way ANOVA. ANOVA($p < 0.05$) Sig- Significance

Sample (J)	Sample (I)	Mean Difference (J-I)	Std Error	Sig
Control	Ciprofloxacin	24.60000	0.49441	0.000
Control	Minocycline	20.73333	0.49441	0.000
Control	Metronidazole	8.46667	0.49441	0.000
Control	Amoxicillin	7.33333	0.49441	0.000
Control	Combination	21.13333	0.49441	0.000

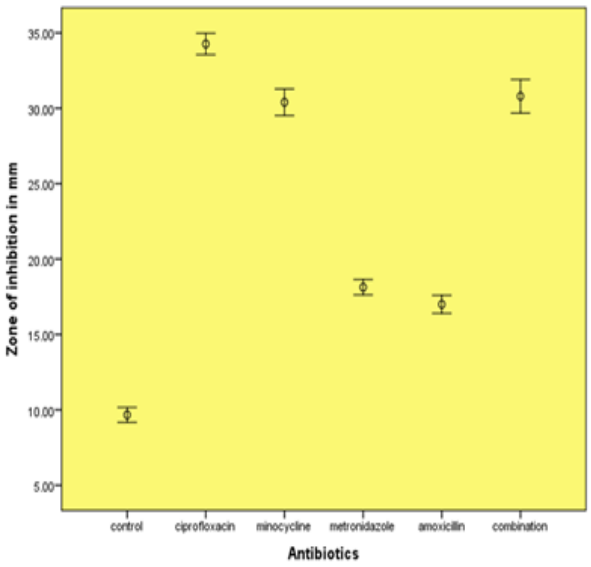
Table 3: Comparision of the means of different groups against control by Tukey's post hoc analysis ($p< 0.05$) All the experimental groups were having high statistical significance compared to the control ($p<0.00$*) Std error- standard error

RESULTS

The larger zone of inhibition was shown by ciprofloxacin (34.26 ± 1.26), followed by combination (30.80±2), minocycline (30.40±1.5), metronidazole (18.13±0.9), amoxicillin (17.00±1.06) and control (9.66±0.90)(Table 2,Graph 1). Control group exhibited the least inhibition to S mutans while ciprofloxacin gave the maximum zone of inhibition (figure 1 and 2).

DISCUSSION

Fluoride release from GIC is not sufficient to destroy the bacteria under an atraumatic restoration, even though fluoride has some antibacterial action. A study done by Vermeersch shows that the antibacterial property of GIC may be due to the low pH of the material while setting rather than the fluoride release.[17,18] In the present study, conventional GIC showed inhibition zone against S mutans. This result is consistent with Shashibhushen et al[19] and Deepalakshmi M[12] and contrary to Mittal S[16] Botelho et al[19], Yap et al[20], and Yesilyurt et al[21], in which control group, did not show any inhibition. The inhibition



Graph 1: Showing the mean and standard deviation of the control and experimental groups

of growth of mutans streptococci around the control group may be due to the release of fluoride and zinc ions into an aqueous medium. However, so many intrinsic and extrinsic factors may effect the release of the material from set specimen such as its P/L ratio, time taken for manipulation of the material, temperature, geometry of specimen, surface protection, dissolution/storage of the medium and the method used for analysis.[16]

S. mutans was the bacteria used in the study to test the efficacy of the modified cement. S. mutans bacteria can induce an acid tolerance response which make them more cariogenic and enables this pathogen to grow and survive in low pH environments.[16] We used agar diffusion method to evaluate the antibacterial efficacy, as this technique is relatively inexpensive and this test is ideal for a large number of samples which can give rapid result.[22]

The antibiotics used in the present study were ciprofloxacin, metronidazole, minocycline, amoxicillin and combination of all four antibiotics. The triple antibiotic combination (ciprofloxacin, metronidazole, and minocycline) has been used in the previous studies by Pinheiro et. al[23], Mittal S[16] and C Yesilyurt[21] In our study, antibiotics were taken individually and in combination, to assess the antibacterial effect. Amoxicillin was evaluated in this study as no literature is available on the efficacy of this antibiotic mixture; even though amoxicillin has a potent antibacterial action against streptococcus. But the results showed that the antibacterial action of the amoxicillin was the least compared to the other groups. Usually, from a set specimen antibiotic absorbs water and disseminate through agar medium. Decreased antibacterial action of amoxicillin may be due to a decrease in the absorption of material from the set specimen to the outside media.[16]

Maximum inhibition of S mutans was given by ciprofloxacin followed by the combination of all antibiotics. Minocycline showed a zone of inhibition almost similar to the combination group. But minocycline was giving a yellowish discoloration to the specimen. Ciprofloxacin alone was giving a zone of inhibition more than the combination group, against S.mutans. Metronidazole also showed a zone of inhibition comparable to other groups even though it is a narrow spectrum antibiotic and is effective against obligate anaerobes.[24] Previous studies assessed only the antibacterial efficacy of antibiotic combinations against S mutans.[16,21]

It was evident from the following studies that addition of antimicrobials into the powder of GIC decreases the compressive strength of the set specimen and there is an inverse relation between the concentration of the antimicrobials and compressive strength.[12,16, 21,22] Mittal S concluded in his study that the antibiotic GIC can be used as a base under the restorations as the mechanical properties were in the weaker side[16]. In the present study, the compressive strength of the material was not estimated and can be stated as one of the limitations.

CONCLUSION

From the present in vitro study, it can be concluded that all the GIC containing groups with 2% antibiotics showed antibacterial activity more than the conventional GIC. Ciprofloxacin has more antibacterial activity against S mutans than the antibiotic combination group. Further, clinical trials are required to back up the in vitro results. This combination of material can be a promising substitute for conventional GIC in ART treatments if all the physical properties are within limits.

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