

Evaluation of Antibacterial Property of Conventional Glass Ionomer Cement with Different Additives: An In-vitro Study

Krina Gurjar¹ Shraddha Chokshi² Zarna Sanghvi³ Pooja Joshi⁴ Setu Kalaria⁵ Aanjan Patel⁶

¹ Department of Conservative Dentistry and Endodontics, Ahmedabad Dental College and Hospital, Gujarat University, Ahmedabad, Gujarat, India,

² Department of Conservative Dentistry and Endodontics, Ahmedabad Dental College and Hospital, Gujarat University, Ahmedabad, Gujarat, India.

³ Department of Conservative Dentistry and Endodontics, Ahmedabad Dental College and Hospital, Gujarat University, Ahmedabad, Gujarat, India.

⁴ Department of Conservative Dentistry and Endodontics, Ahmedabad Dental College and Hospital, Gujarat University, Ahmedabad, Gujarat, India,

⁵ Department of Conservative Dentistry and Endodontics, Ahmedabad Dental College and Hospital, Gujarat University, Ahmedabad, Gujarat, India,

⁶ Department of Conservative Dentistry and Endodontics, Ahmedabad Dental College and Hospital, Gujarat University, Ahmedabad, Gujarat, India,

ABSTRACT

Background: This study aimed to evaluate and compare the antibacterial properties of Type IX Glass ionomer cement (GIC) incorporated with 2% chlorhexidine (CHX) and 2% cetrimide.

Methods and Material: The study comprised three test groups - Group 1 (Conventional GIC), Group 2 (GIC + 2% CHX), and Group 3 (GIC + 2% cetrimide). Each group contained 12 samples, which were evaluated for antibacterial activity using an agar diffusion test. CHX and cetrimide in powder form were added to conventional Type IX GIC at a 2% W/W ratio. The experimental GIC specimens were then placed on agar plates inoculated with *S. mutans*, and the area of inhibition was calculated. Antibacterial activity was evaluated using multiple group comparisons using a one-way analysis of variance complemented by Tukey's post-hoc test.

Results: All the experimental groups exhibited bacterial inhibition; however, Group 3 exhibited the highest antibacterial activity compared to Groups 1 and 2.

Conclusion: The present study demonstrated that experimental GICs containing CHX and cetrimide were effective in inhibiting bacteria associated with caries, and the incorporation of 2% cetrimide was optimal for giving appropriate antibacterial properties.

Keywords: Antibacterial, cetrimide, chlorhexidine, glass ionomer cement.

INTRODUCTION

The disease of dental caries has been present since ancient times and remains the most prevalent ailment affecting the human population. Bacteria is a crucial factor in the development of dental caries. If cariogenic bacteria persist and there is no proper hermetic seal, it can result in recurring decay, causing restorative treatment to fail. To address this issue, one potential solution is to use dental materials that have bactericidal or bacteriostatic properties.¹

Recent advancements in dental material have greatly enhanced the fundamental characteristics of restorative materials used in dental treatments. These innovations have enabled dental practitioners to restore the natural anatomical structure and function of teeth with minimal loss of tooth structure. This development has paved the way for aesthetic restorative treatments and minimal intervention dentistry. As a result, it is suggested that future innovations in restorative

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*Correspondence Dr. Krina Gurjar.

Department of Conservative Dentistry and Endodontics, Ahmedabad Dental College and Hospital, Gujarat University, Ahmedabad, Gujarat, India., India.

Email: krinagurjar8996@gmail.com

materials should focus on a new dimension: the development of materials that possess additional therapeutic effects.²

One proposed bio-active function for restorative materials is the ability to provide antibacterial activity, which can be advantageous in the restorative treatment of dental caries. Given that dental caries is an infectious disease, the capacity to regulate bacterial activity can help to mitigate the risk of further demineralization and cavitation, as well as control the growth of cariogenic bacteria.²

In 1972, Wilson and Kent introduced conventional glass ionomer cements (GICs) as a tooth- coloured, chemically adhesive material with the therapeutic benefit of anticariogenicity.³ As a result, it has become a widely used dental material. The ability of GICs to release fluoride over a prolonged period of time enables its potential to be used as an anticariogenic agent, rendering tooth structure more resistant to caries.⁴

Several researchers have sought to augment the antibacterial properties of GIC by introducing various synthetic and natural chemical substances such as chlorhexidine (CHX) hydrochloride, cetyl pyridinium chloride, cetrimide, benzalkonium chloride, xylitol, and triclosan, all of which possess inherent antibacterial effects.⁵ Therefore, antiseptic agents that have been shown to be effective in

MATERIALS AND METHODS

The present study was an *in vitro* design, and ethical approval for the study had been obtained from the institute where the study was conducted.

Sample collection and isolation: Samples of *Streptococcus* mutans were collected and isolated. Samples were collected using a swab stick from the tooth surface. This swab stick with the sample was allowed to mix in 1 mL sterile distilled water. Then, the suspension was created in a vortex mixer to achieve a homogeneous mixture. Thereafter, 0.5 mL of undiluted suspension was spread on a petri plate that was incubated at 37°C for 48 h, and the colonies were obtained.

Cultural and morphological characteristics: To study *Streptococcus* mutans, a bacterium that causes dental caries, the colony morphology was examined on nutrient agar. Gram staining confirmed the

clinical dentistry would be the most suitable options for combining with GIC as antibacterial agents.

Antiseptics such as CHX and cetrimide have gained widespread use over the past two decades due to their broad spectrum of action.^{1,6} However, incorporating antibacterial agents can often lead to changes in the physical properties of restorative materials. Thus, in the atraumatic restorative treatment (ART) approach, antibacterial GICs should incorporate an optimal amount of antibacterial agents that do not compromise the fundamental properties of the parent material.⁵

The cationic CHX binds to anionic components, such as salivary glycoproteins, on the tooth's surface enamel. This process reduces the adhesion of proteins to the tooth surface and delays the formation of dental pellicles. Moreover, CHX influences the colonization rate of oral bacteria on the enamel surface, resulting in prolonged antimicrobial activity. Cetrimide, a cationic surfactant, exhibits bactericidal activity and is capable of reducing the mechanical stability of biofilms. This compound has been proven effective against both gram-positive and gram-negative bacteria, exerting sustained antimicrobial activity over time. Thus, the present study aimed to investigate the antibacterial properties of conventional Type IX GIC incorporated with 2% CHX and 2% cetrimide.

gram-positive nature of the bacterium and its spherical shape. Pure colonies were obtained by subculturing the isolate on fresh media. The isolate was assessed using a bright-field microscope to see its characteristic chains of cocci. These methods helped to identify and characterize *Streptococcus* mutans from other oral bacteria.

Molecular identification of *S. mutans*: To identify the bacterial isolate, the 16S ribosomal RNA gene, a partial sequence was amplified by PCR and sequenced. The sequence was compared with the GenBank database, a public repository of nucleotide and protein sequences. An accession ID was obtained for the submitted sequence, which can be used to retrieve the record and the associated information from the database.

Sample preparation: A conventional powder and liquid FUJI IX GIC (GC Corporation, Tokyo, Japan) was used as a control. Experimental GICs were

prepared by incorporating CHX diacetate powder (Cadila Pharmaceuticals, INDIA) and cetrimide powder (Cedac Medicorp, INDIA) into the powder of Fuji IX at 2% W/W concentration. Thirty-six samples for three test groups were prepared.

Group 1: Twelve pellets of diameter 4 mm and 6 mm height were prepared by mixing Type IX GIC powder and liquid on a mixing pad according to the manufacturer's instructions.

Group 2: The mixture was prepared for 2% CHX diacetate group, for which 1 g of GIC Type IX powder was proportioned on a mixing pad and weighed to which 20 mg (2%) of CHX powder was added. The desired concentration of GIC powder and CHX powder was obtained using digital analytical scale. Twelve pellets of diameter 4 mm and 6 mm height were prepared in a plastic mould.

Group 3: The mixture was prepared for 2% cetrimide group, for which 1 g of GIC TYPE IX powder was proportioned on a mixing pad and weighed to which 20 mg (2%) of cetrimide powder was added. The desired concentration of GIC powder and cetrimide powder was obtained using digital analytical scale. Twelve pellets of diameter 4 mm and 6 mm height were prepared in a plastic mould.

Antibacterial activity of these specimens after 24 h was evaluated.

Agar diffusion method:

The antibacterial activity of the set cement specimens was evaluated using the agar diffusion method.

Media preparation: 11.40 g of Mueller-Hinton agar was added to 300 mL of distilled water and was mixed thoroughly. Then, 0.5 mL of *S. mutans* bacterial suspension from turbid nutrient broth was added to this mixture at 37°C. The media with bacterial suspension was then spread on the nutrient agar plate and left for 30 mins at room temperature. Three wells of 10 mm diameter for three different test groups were made on each nutrient agar plate with the help of a cork borer. Twelve such agar plates were prepared (12 wells for each group). Set disk-shaped specimens were placed into the wells made in the agar plates that

were inoculated with the bacterial strain and incubated at 37°C for 48 h.

Diameter of zones of inhibition produced around the specimens were measured in millimeters using HiAntibiotic Zone scale. Moreover, the size of these zones of inhibition were measured in millimeters at three different points. Sizes of inhibition zones were calculated by subtracting the diameter of the specimen, 10 mm from the average of the three measurements of the halo.

Statistical analysis:

For the evaluation of antibacterial activity, multiple group comparison was made using one-way analysis of variance (ANOVA) complemented by Tukey's post-hoc test. A P value less than 0.05 was considered statistically significant.

RESULTS

The strain of *S. mutans* was identified by examining the cultural and morphological characteristics of the colonies (Figure 1). The Gram staining of the isolated bacteria showed positive results and revealed a diploid chain pattern of the cells (Figure 2). To further confirm the identity of the strain, the partial sequence of the 16S ribosomal RNA gene was obtained and submitted to GenBank. An accession number was assigned to the strain, verifying that it belongs to *S. mutans*.



Figure 1: Isolated bacterial colonies of *S. mutans* on nutrient agar plate using four-sector streaking method.

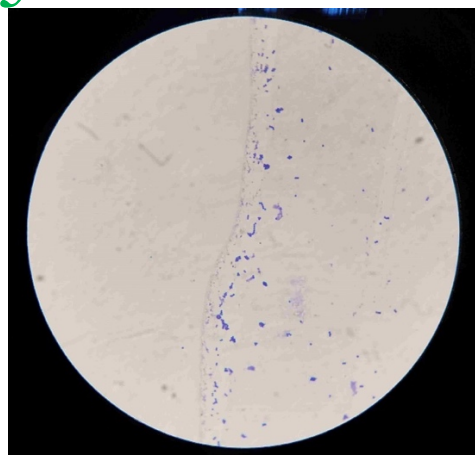


Figure 2: Microscopic image of *S. mutans* using Gram-staining which reveals a diploid chain pattern of the cells.

The data in Table 1 represents the results of the one-way ANOVA studying the effect of different GICs on the zones of inhibition against *S. mutans*. The table displays the mean, standard deviation, and standard error of the mean for each group. The total number of samples is 36. The results show that the mean zone of inhibition for GIC + 2% cetrimide is the highest (20.33 mm), followed by GIC + 2% CHX (18.75 mm), and GIC alone (14.08 mm). The one-way ANOVA test indicates that there is a significant difference between the means of the three groups ($p < .05$).

Table 2 shows the results of the Tukey post hoc test, which compares the mean difference between each pair of groups. The results indicate that there is a significant difference in the mean zone of inhibition between all groups except for GIC + 2% cetrimide and GIC + 2% CHX. GIC + 2% cetrimide produced significantly greater inhibition than GIC + 2% CHX and GIC alone. Figure 3 shows the results of agar diffusion tests against streptococcus mutans. Figure 4 shows the zones of inhibition obtained against *S. mutans*.

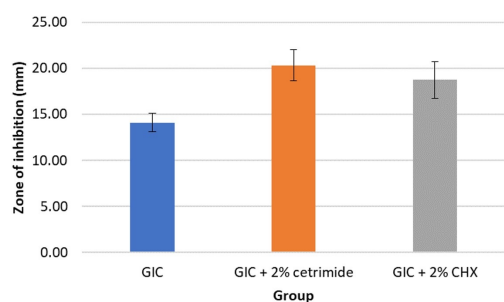


Figure 3: Mean inhibition zone of agar diffusion test against *S. mutans*. The highest mean antibacterial activity after 48 h is shown by group containing glass ionomer cement with 2% cetrimide



Figure 4: Inhibition zones obtained against *S. mutans*. CHX, chlorhexidine; CT, cetrimide; GIC, glass ionomer cement.

Table 1 One-way analysis of variance

95% Confidence Interval for Mean								
	N	Mean	SD	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
GIC	12	14.08	1.00	0.288	13.45	14.72	13.00	16.00
GIC + 2% cetrimide	12	20.33	1.67	0.482	19.27	21.39	18.00	23.00
GIC + 2% CHX	12	18.75	2.01	0.579	17.48	20.02	16.00	22.00
Tot	36	17.72	3.11	0.519	16.67	18.78	13.00	23.00

CHX, Chlorhexidine; GIC, glass ionomer cements

Table 2 Post Hoc Tests.

Dependent Variable: DATA						
Tukey HSD						
95% Confidence Interval						
GROUP	Mean Difference (I-J)		Std. Error	P-Value	Lower Bound	Upper Bound
GIC	GIC + 2% cetrimide	-6.25	.658	.000	-7.87	-4.63
	GIC + 2% CHX	-4.67	.658	.000	-6.28	-3.05
GIC + 2% cetrimide	GIC	6.25	.658	.000	4.63	7.87
	GIC + 2% CHX	1.58	.658	.056	-0.03	3.20
GIC + 2% CHX	GIC	4.67	.658	.000	3.05	6.28
	GIC + 2% cetrimide	-1.58	.658	.056	-3.20	0.03

CHX, Chlorhexidine; GIC, glass ionomer cement.

DISCUSSION

Globally, dental caries ranks among the most prevalent diseases of the human race.⁷ The realization that dental caries is reversible in the initial stages and a dynamic biochemical event at a microscopic level has changed the vision of dental professionals in recognizing the caries process.⁸

In areas with few dental facilities and dental practitioners, alternative method of treating caries is widely used. One such alternative method is Atraumatic Restorative Treatment (ART), which involves minimal cavity preparation and conservation of sound tooth structure using only hand instruments followed by restoration of the cavity with an adhesive filling material, such as GIC.⁹ Although it is believed that hand excavation is capable of removing most of the infected dentin, bacteria can still be found in the tubuli of affected dentin even after complete hand excavation. Thus, it can necessitate further investigation specifically aimed at improving the level of asepsis achieved before cavity restoration. Therefore, one possible solution is to increase the antibacterial property of the restorative cement.²

GICs release fluoride ions, which prevent dental caries by reducing enamel solubility and demineralization, and enhance remineralization of carious enamel. However, conventional GIC may not reduce bacterial counts reliably for ART restorations, so antibacterial GIC could be an effective alternative approach.¹⁰

CHX and cetrimide are effective antibacterial agents against pathogenic bacteria.¹¹ Caries inhibiting effect of CHX-containing GIC, without compromising its physical properties, was reported by Takahashi et al.¹² In current study, 2% CHX diacetate was incorporated into FUJI Type IX GIC, as it is stable and easily blended with GIC, while cetrimide is effective against both gram positive and negative bacteria, with the ability to penetrate inaccessible areas such as the inner lumen of dentinal tubules due to its surface tension-reducing characteristics. Moreover, cetrimide up to 2% concentration is non-toxic.

The modified cement was effective against *S. mutans*, which is a highly cariogenic pathogen that

thrives in low pH conditions. *S. mutans* was chosen as the test organism due to its significant role in initiating dental caries.¹³ The amount of fluoride released by FUJI IX is insufficient to provide antibacterial action and can lead to secondary caries and failure of ARTs.

Agar plate diffusion is a cost-effective and efficient method for evaluating the antimicrobial efficacy of experimental GICs, as it can be performed quickly, even with a large number of specimens, and the size of the inhibition zone reflects the susceptibility of the isolates and the diffusion rate of the chemical additive.^{10, 14-16} GICs can inhibit the growth of *S. mutans* through the release of fluoride and zinc ions, but intrinsic and extrinsic factors such as material preparation, P/L ratio, manipulation time, temperature, specimen geometry, and surface protection can affect ion release.¹³

The antibacterial activity of experimental GICs was evaluated after 48 h, and all groups demonstrated significant antibacterial activity, with cetrimide incorporated GIC (Group 3) exhibiting the highest inhibitory effect, possibly due to its bactericidal activity and residual antimicrobial effect. These findings were similar to those of Botelho et al.¹¹ who investigated the antimicrobial efficacy of combining antibacterial agents with GIC and found that cetrimide-GIC showed the greatest inhibitory effect against *Streptococcus*, *Lactobacillus*, and *Actinomyces* over time.

Deepalakshmi M. et al⁵ evaluated antibacterial property of GIC incorporated with CHX and cetrimide at different concentration against *Lactobacillus casei* that showed GIC containing 2% w/w cetrimide exhibited significantly greater inhibition followed by GIC containing 2% w/w CHX.

Ribeiro & Erickson¹⁷ investigated the antibacterial properties of GICs containing different concentrations of CHX tested *in-vitro* against strains of *Lactobacillus casei* and *Mutans streptococci* and concluded that the higher the concentrations of CHX in the mixture, the longer the time of antibacterial effect on *Streptococcus mutans*.

Shashibhushan et al¹⁸ demonstrated that some degree of growth inhibition of mutans streptococci is exhibited by GICs due to the release of fluoride

and zinc ions into an aqueous medium, which may inhibit the growth of *S mutans*.

One of the main limitations of this study is the inability of the method to distinguish between the bactericidal and bacteriostatic effect, which cannot be determined using the agar diffusion method.

CONCLUSION

In conclusion, this study successfully identified and confirmed the strain of *S. mutans*. The data obtained from the one-way ANOVA and Tukey post hoc test indicate that experimental GICs incorporated with 2% CHX and 2% cetrimide significantly inhibit the growth of *S. mutans*. GIC + 2% cetrimide demonstrated the highest mean antibacterial activity after 48 h. These results suggest that the use of antibacterial additives in GICs could be an effective approach to prevent caries-associated bacteria. However, further investigations are necessary to understand the effects of antibacterial GICs on complex biofilms and their interactions with the physical properties of GICs. Nonetheless, this study provides useful insights into the potential use of GICs in dentistry.

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