Antibacterial efficacy of chlorhexidine, betadine, and probiotic incorporated glass-ionomer cements – An *in vitro* study

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Received - 22 December 2020 Initial Review - 03 January 2021 Accepted - 13 January 2021

ABSTRACT

Background: Addition of antibacterial agents to conventional glass-ionomer cement (GIC) eliminates the recurrence of decay around margins of restoration by inhibiting growth of microbial colonies. **Objective:** The objective of the study was to comparatively evaluate the antibacterial activity of GIC impregnated with betadine, probiotic, and chlorhexidine (CHX) powder against *Streptococcus mutans*. **Materials and Methods:** Chlorhexidine, betadine, and probiotic powder were added to GIC powder to achieve 2.5% CHX-GIC, 2.5% betadine-GIC, and 2.5% probiotic-GIC formulation. Fuji-IX GIC was used as a negative control. The powder and liquid were mixed and inserted in to the wells punched in agar plates. The antibacterial activity of cements was assessed at day 1, day 7, and day 15 using agar diffusion method. **Results:** There was no inhibition zone present on bacterial plates with control GIC. On day 1, mean inhibition zone against *S. mutans* was highest with probiotic-GIC (11.70±0.48 mm), followed by betadine-GIC (10.70±0.48 mm) and CHX-GIC (2.70±1.9 mm). On day 7, mean inhibition zone with probiotic-GIC was 5.30±0.83 mm, betadine-GIC was 3.50±0.71 mm, and CHX-GIC was 1.8±1.03 mm. No inhibition zone was observed with any group on day 15. Wilcoxon signed-rank test showed a significant difference in the inhibition zones on day 1 and day 7 with probiotic-GIC ($p=0.0001$) and betadine-GIC ($p=0.001$). Significant difference was observed between groups using Kruskal–Wallis test and *post hoc* Bonferroni tests ($p<0.05$). **Conclusion:** Probiotic-GIC, betadine GIC is more effective against *S. mutans*. With its enhanced antimicrobial effect, these cements can be considered as an alternative to the conventional GIC'S.

Key words: *Antimicrobial activity, Betadine, Chlorhexidine, Glass-ionomer cement, Probiotic*

Dental caries are the most common disease affecting the oral cavity worldwide. While, *Streptococcus mutans* is considered as a primary cariogenic bacteria for the initiation of dental caries, *Lactobacillus acidophilus* is the principal bacteria responsible for caries progression [1,2]. Among the various restoration techniques, the atraumatic restorative treatment (ART) currently termed interim therapeutic restoration (ITR) has gained attention and is being used by many dental health professionals around the world. It involves the removal of demineralized tooth tissues with hand instruments, followed by filling the cleaned cavity and associated pits and fissures with an adhesive restorative material [3]. Conventional glass-ionomer cements (GIC), introduced in 1972, by Wilson and Kent, is a tooth colored and chemically adhesive material, is the material of choice in ITR approach due to its adhesive property, biocompatibility, and caries protective effect due to fluoride release [2,4]. Various *in vitro* studies have shown that GIC had potential antimicrobial activity and also reduced formation of plaque by *S. mutans* strains [5,6]. However, elimination of all the microorganisms in the residual tissues is unlikely with this technique.

The persisting cariogenic bacteria, with the lack of hermetic seal, can cause recurrent caries, leading to failure of restoration. To overcome this problem, several attempts have been made to enhance the antibacterial effects of GIC by addition of bactericides, such as chlorhexidine (CHX) hydrochloride, cetyl pyridinium chloride, cetrimide, doxycycline, metronidazole, ciprofloxacin, cefaclor, minocycline, and benzalkonium chloride with proven efficacy [7-9]. Among these, CHX has proven to be safe and effective. Studies have shown that incorporation of CHX or its derivatives into GIC improves the antimicrobial effect of the GIC on cariogenic microorganisms [10-13]. However, the addition of CHX has been claimed to interfere with the acid base setting reaction of GIC, resulting in breakdown of the structure [10]. This necessitates the use of additives which do not get released from the cement, yet show antibacterial activity.

Povidone-iodine or Betadine is potent microbicidal agents, it has affinity to the cell membrane which delivers free iodine

(I2) directly to the bacterial cell surface and results in bacterial lysis. It has been shown that application of 10% Povidone-iodine or betadine substantially reduces the risk of dental caries [14]. Similarly, probiotics are living microbes or ingredients containing living microbes that beneficially influence the health of the host. They interact with micro-organisms by competing for adhesion site, thus by reducing the number of this caries pathogen. Several studies suggest that consumption of products containing probiotic lactobacilli or bifidobacteria could reduce the number of *S. mutans* in saliva [15]. In children with extensive dental caries it is essential to introduce an antibacterial agent which will be useful supplement to current techniques of prevention of caries. Therefore, reinforcing the conventional GIC with additional antibacterial agents may be effective in reducing microbial count. Hence, the aim of the study was to compare and evaluate the antibacterial effect of chlorhexidine, probiotic and betadine incorporated GIC against *S. mutans* using an agar diffusion model.

MATERIALS AND METHODS

A conventional restorative GIC (Fuji IX GC –Group I) was used as negative control. Experimental GICs were prepared by incorporating chlorhexidine, probiotic, and betadine powders to GIC powder to achieve 2.5% CHX – GIC, 2.5% probiotic-GIC, and 2.5% betadine-GIC formulations. The agar diffusion method was be used to determine the antibacterial activity of the cements after 1, 7, and 15 days. A total of 40 samples were tested with each group having ten samples each.

In each culture plate, four standardized wells with a diameter of 10 mm and height 4 mm were punched using a sterile metal ring. The powder and liquid of the agents under investigation were mixed according to manufacturer's specification for 30 s with sterile agate spatula on mixing pad and inserted into the wells within 1 min using a centrix syringe. The plates were then incubated at 37°C for 24 h. 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. Four standardized wells were punched into this new agar plate and bacterial inoculation was made over the agar surfaces with 0.5 mL 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 37°C for 24 h, 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 14. The procedure was repeated with fresh agar plates inoculated with microorganisms on the 14th day for obtaining inhibition zones for day 15. The obtained data were then tabulated and statistically analyzed using SPSS software using Wilcoxon signed-rank test, Kruskal–Wallis test and *post hoc* Bonferroni tests wherever appropriate.

RESULTS

On day 1, mean score of inhibition zone against *S. mutans* was highest with probiotic-GIC (11.70±0.48 mm), followed by betadine-GIC (10.70±0.48 mm) and CHX-GIC (2.70±1.9 mm). There was no inhibition zone present on bacterial plates with control GIC. On day 7, mean inhibition zone with probiotic-GIC was 5.30±0.83 mm, betadine-GIC was 3.50±0.71 mm, and least zone of inhibition was noted with CHX-GIC (1.8±1.03 mm). On day 15, no inhibition zone was noted in any group (Fig. 1). Intragroup differences in mean inhibition zone between day 1 and day 7 were assessed using Wilcoxon signed-rank test showed a significant difference in the inhibition zones with probiotic-GIC (p=0.0001) and betadine-GIC (p=0.001). Although there was difference between the mean inhibition zones in CHX-GIC group, the difference was not statistically significant (p=0.244) (Table 1). Intergroup comparison done using Kruskal–Wallis test is summarized in Table 2. Significant difference in the mean inhibition zones was observed between four groups on day 1 and day 7 (p=0.001). Similarly intergroup comparisons carried out using *post hoc* Bonferroni test also showed significant difference in the mean inhibition zones between groups (Table 3).

DISCUSSION

Dental caries is the most common oral disease affecting the population worldwide and is caused by the microorganisms present in the oral microflora. Acid produced by the bacterial reduces the pH of saliva which results in demineralization

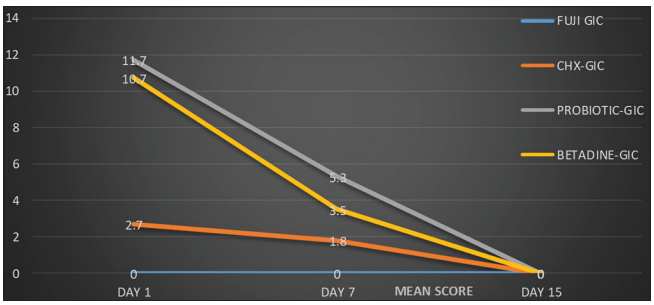

Figure 1: Mean inhibition zones noted on bacterial plates with antibacterial incorporated glass.

Table 1: Intragroup comparison of zone of inhibition

Intervention	Time period	Mean (mm)	SD	P-value
Fuji IX-GIC	Day 1	0	0	-
	Day 7	0	0	
CHX-GIC	Day 1	2.70	1.889	0.244
	Day 7	1.80	1.033	
Probiotic-GIC	Day 1	11.70	0.483	0.001*
	Day 7	5.30	0.823	
Betadine-GIC	Day 1	10.70	0.483	0.001*
	Day 7	3.50	0.707	

of the tooth. It is believed that complete elimination of all the microorganisms in the residual tissues is not achievable during restorative procedures and despite a good restoration, traces of bacteria will be evident in the affected dentine resulting in failed restorations [4,16]. Therefore, restorative materials with antibacterial properties are a preferable choice especially in children with extensive caries which aids in suppressing the growth of bacteria under restorations thereby minimizing the risk of recurrent caries [1,2]. The present study *in vitro* study analyzed the antibacterial effectiveness of antimicrobial (chlorhexidine, betadine, and probiotic) incorporated GIC by observing the zone of inhibition around the experimental samples using an agar diffusion microbiological assay procedure. To the best of our knowledge, no previous research has been done to assess the antimicrobial efficacy of probiotic incorporated GIC.

FUJI IX was used as a negative control in our study since this is the most frequently reported material in *in vivo* and *in vitro* studies in the past [8,17]. It is a high strength posterior restorative material and has been reported to release approximately 10 ppm of fluoride during 48 h; however, this amount of fluoride is too small to exhibit antibacterial action [4]. The previous studies have suggested that conventional GIC with no antimicrobial compounds or elements do not form any inhibition zones [18-21]. CHX has long-term antibacterial properties because of its unique ability to bind to hydroxyapatite, whereby, a gradual release creates a bacteriostatic milieu over a prolonged period of time. CHX diacetate at a concentration of 2.5% is very effective against *L. acidophilus* (60 days) and *S. mutans* (90 days) [2]. Caries inhibiting the effect of the CHX containing glass ionomer, without compromising its physical properties, was reported by Takahashi *et al*., [9]. Deepalakshmi *et al*. [4] have reported that antimicrobial activity was dependent upon the concentration of the disinfectant added. In our study, CHX was preferred to other CHX derivatives, as it is a more stable material, not prone to decomposition, and can be easily blended with GIC powder [16].

Literature suggests the positive effect of betadine/Povidone-iodine in controlling the incidence of new carious lesions in children [22]. Free iodine of the betadine causes disruption of microbial metabolic pathways, as well as destabilization of the structural components of cell membranes, causing irreversible damage to the pathogen. It also inhibits the release of pathogenic factors such as exotoxins, endotoxins, and tissue-destroying enzymes [23]. Irrigation with betadine has shown significant reduction in the rise of *S. mutans* levels from the baseline score in a 12 months follow-up study. The reduction in counts resulted in a lower incidence of caries relapse in these children compared with the deionized water-irrigated controls [24]. Amin *et al*., [14] in their study, evaluated the effect of betadine on *S. mutans* and new caries in young children with a history of extensive caries. They observed a significant decrease in *S. mutans* counts, 6 months after restorative treatment in all children. At 1 year follow-up, only two of the 11 children developed dental caries suggesting positive effect of betadine in controlling dental caries.

Probiotics are viable microorganisms which, when administered in adequate amounts, provide a health benefit to the host. Oral commensals associated with health are likely to be more effective as probiotic species against dental caries than the traditional gut-associated probiotic species. They compete with other bacteria for nutrients and binding sites to the medium, inhibit their growth by producing bacteriocins, and further stimulate the immune response of the host. Probiotics aggravate or delay the colonization of pathogenic bacteria during biofilm formation. Strains of *Lactobacillus*, *Streptococcus,* and *Bifidobacterium* genera have demonstrated the potential to alter colonization of cariogenic bacteria, thereby preventing dental caries [25,26].

Till date, antimicrobial effect of compound GIC is tested against the cariogenic microorganisms such as *S. mutans*, *Lactobacillus casei,* and *Actinomyces viscosus* [9,19,21]. *S. mutans* bacteria are the most cariogenic pathogens as they induce an acid tolerance response that enables this pathogen to survive and grow in low-pH environments. Considering the impact of *S. mutans* as an initiator of the pathological process of dental caries, it was selected as the test organism [16]. The antibacterial activity was evaluated using agar diffusion test which is an accepted method

Table 2: Intergroup comparison of zone of inhibition between four groups

Time period	Intervention	Mean (mm)	SD	p-value
Day 1	Fuji IX- GIC	0[ab]	0	0.001*
	CHX-GIC	2.70[ab]	1.889	
	Probiotic-GIC	11.70[a]	0.483	
	Betadine-GIC	10.70[b]	0.483	
Day 7	Fuji IX- GIC	0[ab]	0	0.001*
	CHX-GIC	1.80[ab]	1.033	
	Probiotic-GIC	5.30[a]	0.823	
	Betadine-GIC	3.50[b]	0.707	

[ab]Same alphabet indicate significant comparison

Table 3: Intergroup comparisons using *post hoc* Bonferroni test

Groups	Groups	Mean difference	Std. Error	p-value	95% Confidence interval	
					Lower bound	Upper bound
Probiotic GIC	Betadine GIC	1.0000	0.4491	0.194	–0.254	2.254
	CHX-GIC	9.0000*	0.4491	0.000*	7.746	10.254
	Fuji IX GIC	11.7000*	0.4491	0.000*	10.446	12.954
Betadine GIC	CHX-GIC	8.0000*	0.4491	0.000*	6.746	9.254
	Fuji IX GIC	10.7000*	0.4491	0.000*	9.446	11.954
CHX-GIC	Fuji IX GIC	2.7000*	0.4491	0.000*	1.446	3.954

*p<0.05

to initially differentiate antibacterial activity between materials. Although the process is relatively inexpensive and can be performed rapidly; it fails to distinguish between bacteriostatic and bactericidal effects. Hence, any information about the viability of the test microorganisms within the inhibition zone cannot be obtained [2,4]. Antimicrobial properties are assessed in the form of zones of inhibition determined in millimeters. Maintaining the specimens in the media for all the time simulates a clinical scenario where the restoration is continually bathed by oral fluids. The use of deionized water for experimental purpose to store GIC has been recommended by various investigators [2]. Similarly, deionized water was used in our study to store the specimens.

The agar diffusion test in our study demonstrated that Fuji IX GIC showed no antibacterial effect against *S. mutans*. These results were consistent with the findings of Mittal *et al*., [16], Dimkov *et al*., [18] Botelho *et al*., [19] Turkun *et al*., [20], and Vermeersch *et al*., [21]. On the contrary, Shashibhushan *et al*. [27] observed some degree of growth inhibition of *S. mutans* by GICs due to the release of fluoride and zinc ions into an aqueous medium. According to Sainulabdeen *et al*., [2] conventional glass ionomers although exhibit antibacterial activity primarily by fluoride release; it does not inhibit acid production by bacteria. In our study, GIC containing 2.5% CHX had superior antimicrobial activity on day 1 and day 7, when compared to the FUJI-IX GIC; however, was significantly lesser compared to betadine-GIC and probiotic-GIC.

The previous studies have suggested that zones of inhibition increases with increased concentration of the antimicrobial uses; however, there is decline of zones with time [19-21]. Similarly, we observed a decline in the zone of inhibition with time. On day 7, there was a substantial decrease in the zone of inhibition as compared to day 1 ($p<0.05$) and on day 15 there was absence of zone of inhibition with all samples. This may be due to the loss of material by elution from the GIC which may also occur in clinical set up due to entry of fluids through any pathway of leakage [2].

The ability of the restorative dental material to withstand the masticatory forces is an important requirement for their long-term clinical performance. To be acceptable clinically, modified materials must provide superior antimicrobial activity without compromising the physical properties [28]. However, we did not assess the physical properties of the modified cement in our study which is the limitation. Further, *in vivo* studies are required to test the clinical efficacy of this concentration before advocating the use of antibiotic-modified GIC in ART procedures. Although, results of our study show a promising antibacterial efficacy of probiotic and betadine incorporated GIC; since this was under experimental conditions and therefore direct correlation cannot be inferred to a clinical scenario. Nevertheless, the study demonstrates a new and effective antimicrobial property of this experimental GIC incorporated with probiotic and betadine. Further *in vivo* research are warranted to evaluate the influence of these modified GICs on chemistry and physico-mechanical properties and efficacy complex biofilms in the oral cavity in a clinical setting.

CONCLUSION

The GIC without incorporation of antimicrobial compounds either forms very small or non-existent inhibition zones. Probiotic-GIC combination had the strongest antibacterial effect closely, followed by betadine-GIC combination, while CHX-GIC had the weakest effect against the bacteria studied. Within the limitations of the present study, it can be concluded that experimental GICs containing antibacterial mixtures such as betadine, probiotics are effective in inhibiting bacteria associated with caries. Hence, the use of these materials could be highly recommended in regular clinical practice.

REFERENCES

1. Khere CH, Hiremath H, Sandesh N, *et al*. Evaluation of antibacterial activity of three different glass ionomer cements on *Streptococcus mutans*: An *in vitro* antimicrobial study. Med Pharm Rep 2019;92:288-93.
2. Sainulabdeen S, Neelakantan P, Ramesh S, *et al*. Antibacterial activity of triclosan incorporated glass ionomer cements-an *in vitro* pilot study. J Clin Pediatr Dent 2010;35:157-62.
3. Frencken JE, van't Hof MA, van Amerongen WE, *et al*. Effectiveness of single-surface art restorations in the permanent dentition: A meta-analysis. J Dent Res 2004;83:120-3.
4. Deepalakshmi M, Poorni S, Miglani R, *et al*. Evaluation of the antibacterial and physical properties of glass ionomer cements containing chlorhexidine and cetrimide: An *in vitro* study. Indian J Dent Res 2010;21:552-6.
5. Boeckh C, Schumacher E, Podbielski A, *et al*. Antibacterial activity of restorative dental biomaterials *in vitro*. Caries Res 2002;36:101-7.
6. Herrera M, Castillo A, Bravo M, *et al*. Antibacterial activity of resin adhesives, glass ionomer and resin-modified glass ionomer cements and a compomer in contact with dentin caries samples. Oper Dent 2000;25:265-9.
7. Prabhakar AR, Prahlad D, Kumar SR. Antibacterial activity, fluoride release, and physical properties of an antibiotic-modified glass ionomer cement. Pediatr Dent 2013;35:411-5.
8. Yesilyurt C, Er K, Tasdemir T, *et al*. Antibacterial activity and physical properties of glass-ionomer containing antibiotics. Oper Dent 2009;34:18-23.
9. Takahashi Y, Imazato S, Kaneshiro AV, *et al*. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. Dent Mater 2006;22:647-52.
10. Palmer G, Jones FH, Billington RW, *et al*. Chlorhexidene release from an experimental glass ionomer cement. Biomaterials 2004;25:5423-31.
11. Hoszek A, Ericson D. *In vitro* fluoride release and the antibacterial effect of glass ionomers containing chlorhexidine gluconate. Oper Dent 2008;33:696-701.
12. Sebnem L, Turkun M. Long-term antibacterial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent 2008;20:29-45.
13. Wicht MJ, Haak R, Kneist S. Suppression of caries-related microorganisms in dentine lesions after short-term chlorhexidine or antibiotic treatment. Caries Res 2004;38:436-41.
14. Amin MS, Harrison RL, Benton TS, *et al*. Effect of povidone-iodine on *Streptococcus mutans* in children with extensive dental caries. Pediatr Dent 2004;26:5-10.
15. Amtha R, Kanagalingam J. Povidone-iodine in dental and oral health: A narrative review. J Int Oral Health 2020;12:407-12.
16. Mittal S, Soni H, Sharma DK, *et al*. Comparative evaluation of the antibacterial and physical properties of conventional glass ionomer cement containing chlorhexidine and antibiotics. J Int Soc Prev Community Dent 2015;5:268-75.
17. Tuzuner T, Kuşgöz A, Kursat ER, *et al*. Antibacterial activity and physical properties of conventional glass-ionomer cements containing chlorhexidine diacetate/cetrimide mixtures. J Esthet Restor Dent 2011;23:46-55.
18. Dimkov A, Gjorgievska E, Nicholson JW, *et al*. Antibacterial effects of conventional glass ionomer cement. Bratisl Lek Listy 2016;117:31-5.
19. Botelho MG. Inhibitory effects on selected oral bacteria of antibacterial agents incorporated in a glass ionomer cements. Caries Res 2002;37:108-14.

20. Turkun SL, Turkun M, Ertugrul F, *et al*. Long-term antibacterial effects and physical properties of a chlorhexidine-cintaining glass ionomer cements. J Esthet Restor Dent 2008;20:29-44.
21. Vermeersch G, Leloup G, Delmee M, *et al*. Antibacterial activity of glass-ionomer cements, compomers and resin composites: Relationship between acidity and material setting phase. J Oral Rehabil 2005;32:368-74.
22. Lopez L, Berkowitz R, Spiekerman C, *et al*. Topical antimicrobial therapy in the prevention of early childhood caries: A follow-up report. Pediatr Dent 2002;24:204-6.
23. Kanagalingam J, Feliciano R, Hah JH, *et al*. Practical use of povidone-iodine antiseptic in the maintenance of oral health and in the prevention and treatment of common oropharyngeal infections. Int J Clin Pract 2015;69:1247-56.
24. Simratvir M, Singh N, Chopra S, *et al*. Efficacy of 10% povidone iodine in children affected with early childhood caries: An *in vivo* study. J Clin Pediatr Dent 2010;34:233-8.
25. Alaker RP, Stephen AS. Use of probiotics and oral health. Curr Oral Health Rep 2017;4:309-18.
26. Krzyściak W, Kościelniak D, Papież M, *et al*. Effect of a Lactobacillus salivarius probiotic on a double-species *Streptococcus mutans* and *Candida albicans* caries biofilm. Nutrients 2017;9:1242.
27. Shashibhushan KK, Basappa N, Reddy VV. Comparison of antibacterial active ity of three fluorides-and zinc-releasing commercial glass ionomer cements on strains of mutans streptococci: An *in vitro* study. J Indian Soc Pedod Prev Dent 2008;26 Suppl 2:S56-61.
28. Marti LM, da Mata M, Ferraz-Santos B, *et al*. Addition of chlorhexidine gluconate to a glass ionomer cement: A study on mechanical, physical and antibacterial properties. Braz Dent J 2014;25:33-7.

Funding: None; Conflicts of Interest: None Stated.

How to cite this article: Patilla AS, Shetty A, Shetty AS, Patilla AS. Antibacterial efficacy of chlorhexidine, betadine, and probiotic incorporated glass-ionomer cements – An *in vitro* study. Eastern J Med Sci. 2021;6(1):18-22.

Doi: 10.32677/EJMS.2021.v06.i01.004