10.5005/jp-journals-10024-1332

ORIGINAL RESEARCH

Comparison of Antibacterial Activity of Glass-ionomer Cement and Amalgam in Class Two Restorations by *Streptococcus mutans* Count Analysis at Fixed Intervals: An *in vivo* Study

Veeresh S Tegginmani, Beenarani Goel, Virendra Uppin, Priya Horatti, LS Vijay Kumar, Abhinav Nainani

ABSTRACT

Aim: The purpose of the present study was to determine the influence of glass ionomer cement and amalgam restoration on the level of *Streptococcus mutans* in the interproximal plaque at periodic intervals and also to compare these values.

Materials and methods: Seventeen adult patients having two proximal carious lesions on any quadrant of the jaw (either opposing or contralateral) were selected for this study. Carious lesions were diagnosed clinically and from bitewing radiographs. Of the two carious lesions, one was restored with glass ionomer cermet cement and another with amalgam. Plaque samples were collected from interproximal areas before and at 1 month and 3 months post-treatment in a test tube containing 5 ml of modified Stuart's liquid transport fluid. Identification of organisms in the colony was done after Gram staining.

Results: Comparison of values before restoration and after restoration at 1 month interval showed a statistically significant decrease ($p < 0.001$). Similarly, comparison of values before and after restorations at 3 months also showed statistically significant decrease ($p < 0.02$). But comparison of restorations of 1 and 3 months intervals showed no statistical significant difference ($p > 0.05$).

Conclusion: Glass ionomer restorations have definite advantage over the amalgam, as the tunnel preparation is more conservative and fluoride release from the glass ionomer inhibits the growth of *S. mutans* in the plaque.

Clinical significance: Glass ionomer cement should be preferred over amalgam in conservatively prepared restorations as it reduces the microbial activities due to fluoride release.

Keywords: Glass ionomer cement, Amalgam, *Streptococcus mutans*, Fluoride.

How to cite this article: Tegginmani VS, Goel B, Uppin V, Horatti P, Kumar LSV, Nainani A. Comparison of Antibacterial Activity of Glass-ionomer Cement and Amalgam in Class Two Restorations by *Streptococcus mutans* Count Analysis at Fixed Intervals: An *in vivo* Study. J Contemp Dent Pract 2013;14(3): 381-386.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

Mutans streptococci are considered to be the most important group of bacteria initiating caries lesions even though it has been debated lately. Chemical adhesion and release of fluorides are two major properties that make glass ionomer superior from other restorative materials.[1,2] This release of fluoride has been shown to have a cariostatic effect. It is certain that fluoride aids in the remineralization of damaged enamel and it showed that fluoride releasing effect changes the composition of bacterial plaque and biochemistry by altering the carbohydrate metabolism.[3-5] The influence of fluoride is found in zone of resistance to demineralization which is at least 3 mm around a glass ionomer which had shown decreased prevalence of *Streptococcus mutans* in saliva after restoration with glass ionomer.[6-10] Continued improvements in dental materials in recent years have promoted renewed interest in the tunnel type restoration for class II carious lesions. The presence of copper in amalgam reduces plaque acidogenicity and it also accumulates in dental plaque and inhibits the growth of *S. mutans* in high copper amalgam.[11-14] A low prevalence of *S. mutans* in plaque from approximal non-gamma-2 amalgam than in conventional amalgam has been noticed.[15,16] The purpose of the present study was to determine the influence of glass ionomer cement (GIC) and amalgam restorations on the levels of *S. mutans* in the interproximal plaque at periodic interval and also to compare these values.

MATERIALS AND METHODS

Seventeen adult patient having two proximal carious lesions on any quadrant of the jaw (either opposing or contralateral) were selected for this study. These carious lesions on posterior teeth were diagnosed clinically and from bitewing radiographs. Of the two carious lesions, one was restored with GIC and another with amalgam.

For amalgam restorations, conservative class II cavities were made (Fig. 1). Here the occlusal margins were located on the occlusal inclined planes of the involved marginal ridge. The facial and lingual margins occlusally were limited in their extent and were always on the inclined planes of the involved ridge. Proximal preparation included removal of all carious and undermined tooth structure and was placed either supragingival or subgingival depending upon the carious extent. Wherever necessary, pulpal protection was offered by zinc polycarboxylate and varnish. All the cavities were later filled with amalgam (Amalcap Plus, Vivadent).

For class II tunnel restoration (Fig. 2), preparation was made through occlusal fossa which did not involve the marginal ridge. The initial approach was made through fossa with a small round diamond point, without involving marginal ridge. The entry point was kept atleast 2 mm from the marginal ridge, leaving a strong occlusal rim of enamel. With a diamond point (1/4 or 1/2) directed diagonally toward the lesion, entry was made. It was not held parallel to the long axis of the tooth, to avoid pulpal exposure and excessive removal of healthy tissue. The entry point was extended buccolingually within the fossa area to make the carious lesion more visible and wide enough to have a free access to the carious lesion.

With no. 1 or 2 tungsten carbide bur, the caries was removed using low speed and tactile sensation. Fiber-optic diagnostic kit (NSK Japan) was used to examine the cavity and to remove any caries left during cavity preparation (Fig. 3).

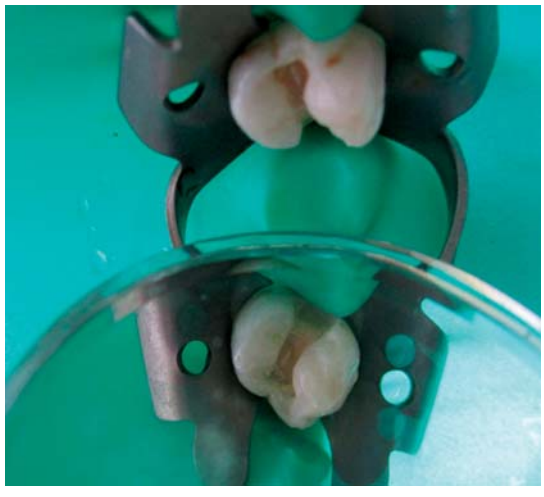

Fig. 1: Conservative class II preparation

Fig. 2: Class II tunnel preparation

Fig. 3: Fiber-optic diagnostic kit (NSK Japan)

Restoration phase consisted of cleaning the cavity preparation first with a 25% polyacrylic acid solution for 10 seconds. The cavity was washed with water and dried.

Next, a matrix band was tightly wedged against the proximal surface. The glass ionomer cermet cement (Chelon-Silver ESPE, Germany) was mixed according to the manufacturer's instructions and carried to the cavity. To achieve a proper condensation for this small cavity, a sharp probe was made blunt by cutting the tip and used as condenser. A space was created at the occlusal surface by removing the cement prior to the placement of posterior composite resin. The cement was allowed to set for at least 5 minutes prior to the etching. The enamel and cement floor was etched with a 37% phosphoric acid gel for 30 seconds, washed with air/water jet and dried for 10 seconds. Composite resin (Heliomolar, Vivadent) was applied and adapted accurately to the occlusal margins and cured for 40 seconds. The composite resin was finished with diamond stones, occlusion checked and adjusted if necessary.

Plaque samples were collected from interproximal areas before the treatment with 0.5 ml of saliva and was transferred to a test tube containing 5 ml of modified Stuart's liquid transport fluid (STF Dynamicro, Thane, India). The same

procedure was followed after restoring these class II cavities at 1 and 3 months interval. Sterile periodontal curettes were used to collect these interproximal plaques, taking care not to touch any other areas.

The samples in the laboratory were dispersed and sonicated. One loop full of the above obtained solution was placed on Mitis Salivarius agar with bacitracin (MSB agar, Dynamicro, Thane, India). These plates were incubated anaerobically in an anaerobic jar with charge (which releases carbon dioxide after adding 20% sulphuric acid). The jar with the plates was kept in incubator jar for 3 days at 37°C.

The plates were opened after 3 days and colonies were identified on the MSB agar plates. The characteristics of these colonies were raised, convex, undulate, opaque colonies of the light blue.

RESULTS

In data analysis, a descriptive statistics, i.e. means, SDs, medians and ranges were calculated for CFU/ml of the interproximal plaque in two groups at different time intervals. The comparison of antimicrobial activities of two restorations was performed by Mann-Whitney U-test and Wilcoxon's matched pairs test was performed between the different time intervals separately in each restoration. A statistical significance was set at 5% level of significance.

The mean of CFU/ml of interproximal plaque before amalgam restoration was ($1.3 \times 10^5 \pm 1.6 \times 10^5$) followed by $4.9 \times 10^4 \pm 4.7 \times 10^4$ at 1 month and $5.8 \times 10^4 \pm 4.1 \times 10^4$ at 3 months. However, the median of CFU/ml before restoration was 9×10^4 as compared to 4×10^4 at 1 month and 5×10^4 at 3 months interval (Table 1).

From the results of the above table, it can be seen that, a significant difference was observed between before and after restoration at 1 month interval ($p < 0.001$), before and after restoration at 3 months interval ($p < 0.001$). It means that, a significant reduction in CFU/ml was observed from before to 1 month and before to 3 months interval restorations. But no significant difference was observed between the restoration at 1 and 3 months interval ($p > 0.05$) (Table 2).

Comparison of values before and after restoration at 3 months also showed statistically significant decrease after 3 months interval ($p < 0.002$). But comparison of restoration at 1- and 3-month intervals showed no statistical significant difference ($p > 0.05$). The mean of CFU/ml of interproximal plaque before restorations with GIC was ($1.1 \times 10^5 \pm 8.9 \times 10^4$) followed by $4.2 \times 10^4 \pm 2.8 \times 10^4$ at 1 month interval and $5.3 \times 10^4 \pm 4.1 \times 10^4$ at 3 months interval. Further, the median value of CFU/ml before GIC restoration was 1.0×10^5 as compared to 4.0×10^4 in 1 month and 4.0×10^4 in 3 months interval (Table 3).

It can be seen from the above table that a significant difference was observed between before and after GIC restoration at 1 month ($p < 0.001$), before and after GIC restoration at 3 months ($p < 0.001$) and also from 1 and 3 months interval ($p < 0.05$). It means that a significant reduction in CFU/ml was observed from before to 1 and 3 months interval GIC restorations (Table 4).

DISCUSSION

It was interesting to see that all the amalgam restorations showed decrease in *S. mutans* count after treatment at 1 and 3 months intervals. This reduction in microbial count of interproximal plaque may be due to the removal of carious

Table 1: The CFU/M/F interproximal plaque and various parameter readings for amalgam restoration before and after restorations

Parameter	*Initial interval (before restoration)*	*1 month interval*	*3 months interval*
Mean	1.3×10^5	4.9×10^4	5.8×10^4
Median	9×10^4	4×10^4	5×10^4
Range	$2 \times 10^3 – 6 \times 10^5$	$2 \times 10^3 – 1.7 \times 10^5$	$7 \times 10^3 – 1.5 \times 10^5$
Standard deviation (SD)	1.6×10^5	4.7×10^4	4.1×10^4

Table 2: Comparison of interproximal plaque before and after amalgam restoration by Wilcoxon matched pairs test by ranks

Interval	*Wilcoxon test (T)*	*Number (n)*	*p-value*
0 and 1 month	08	17	<0.001
0 and 3 months	20	17	<0.02
1 and 3 months	68	16	>0.05

Table 3: Various parameter readings of the interproximal plaque before and after restorations with glass ionomer cement

Parameter	*Initial interval (before restoration)*	*1 month interval*	*3 months interval*
Mean	1.1×10^5	4.2×10^4	5.3×10^4
Median	1×10^5	4×10^4	4×10^4
Range	$3 \times 10^4 – 4 \times 10^5$	$1 \times 10^4 – 1.1 \times 10^5$	$1 \times 10^4 – 1.5 \times 10^5$
Standard deviation (SD)	8.9×10^4	2.8×10^4	4.1×10^4

Table 4: Comparison of interproximal plaque before and after GIC restoration by Wilcoxon matched pairs test by ranks

Interval	*Wilcoxon test (T)*	*Number (n)*	*p-value*
0 and 1 month	1	17	<0.001
0 and 3 months	14	17	<0.001
1 and 3 months	48	14	<0.05

lesion, restoration of the part and presence of copper in amalgam restorations.

Oppermann and Johnson have reported that copper accumulation in dental plaque can reduce plaque acidogenicity and inhibit the growth of *S. mutans*, also modern non-gamma-2 amalgam releases more copper than the conventional low copper amalgam especially at low pH.[14] The present study confirms their findings. Copper has minimal bactericidal action at 400 ppm. Whereas, zinc in high copper amalgam also changes the protein structure and lead to inhibition of specific metabolic enzymes, thereby causing growth inhibition (Shashibhushan et al).[17] This cariostatic effect can contribute to reduction of secondary caries. A similar view was expressed by Wallman et al.[18] The Wallman-Björklund et al showed greater proportions of *S. mutans* in conventional amalgam restorations as compared to non-gamma-2 amalgam restorations.[16]

It had been proved that the ability of enamel adjacent to glass ionomer restoration for fluoride uptake helps in resisting demineralization. *In vitro* studies conducted by Hattab et al and Dionysopoulos et al proved less demineralization around the restoration and absence of recurrent wall lesion in teeth filled with GICs when compared to composite and amalgam.[19]

Glass ionomer restorations at 1 and 3 months also showed decrease in CFU as compared to the values before restorations. This reduction in microbial count can be attributed to the release of fluoride which effects a variety of vital enzymetic cell function both directly and indirectly. Fluoride inhibits the various bacterial enzymes like enolase, phosphates, proton extruding ATPase and pyrophosphatase. It also influences the bacterial composition and alters the plaque ecosystem (Jeevarathan et al).[20] Naturally occurring fluoride does not significantly influence the bacterial composition of plaque but higher level of fluoride could eliminate susceptible microorganisms and modify plaque ecosystem. *In vitro* animal studies have shown that fluoride affect the carbohydrate metabolism of mutans streptococci by acidogenic plaque microflora. The *S. mutans* count in saliva decrease after the placement of glass ionomer restorations. Hattab et al[19] have also shown an increase on salivary fluoride concentration after treatment for short period of 8 days.[19] Freshly mixed GIC has 3- to 10-fold more fluoride release. It had been reported that 10-fold fluoride increase in glass ionomer when compared to other restoration, which may be due to the presence of soluble fluoride in which ion exchange occurs not only on the surface, but possibly some small distance into the material.

The greatest proportion of cumulative total fluoride is release at first 24 hours after mixing (DESchepper et al). Palenik et al had shown that the fluorides when measured for a period of 7 days inhibited the growth and adherence of oral bacteria.[21,22] Our study showed that short-term fluoride release levels were positively correlated with growth inhibition. A sustained fluoride release and intimate contact of restoration to the tooth margins are needed to facilitate the exchange of fluoride with hydroxyapatite of enamel. McCourt et al in his study on glass ionomer and fluoride release reported statically significant difference. Materials showed largest release called 'burst effect' on the first and second day and then decrease significantly.[23] The above said studies show that fluoride release is for a short period. Findings of this study were found similar to the study by Berg et al which gave decreased level of proximal plaque level of *S. mutans* at 1 week which were almost at the same level after 1 month interval and stabilize at 3-month post-treatment.[10] According to Svanberg et al around 4 weeks, both the total viable count and proportions of mutans streptococci were significantly lower in plaque samples from newly made GIC restorations than in plaque samples from newly made amalgam restorations.[24] It has been shown previously that the microbial composition of plaque can be affected by different materials, thus the prevalence of mutans streptococci in early *in vivo* plaque on test pieces was higher on composite than on enamel or amalgam (Skzorland and Sonju).[25] The level of plaque fluoride is dependent on exposure to fluoride (Tatevossian).[26] Since glass ionomer release large amounts of fluoride within the first few days (Swartz et al Forsten), it is conceivable that the fluoride level in plaque adjacent to glass ionomers may increase at least some time after placement of the restoration.[27] The antimicrobial property of glass ionomer is due to release of silver ions and fluoride ions from the cermet restorations (Opperman RV et al).[14] Silver is released in an ionized state which inhibits plaque acidogenicity even at low concentrations.

The antibacterial activity of GICs may not be limited to Streptococci but also against other cariogenic bacteria and

other orodental pathogens; therefore the use of GICs can be indicated in the restorative treatment of dental caries (T Menon et al).[28] The antibacterial and cariostatic properties of GICs are associated with the amount of fluoride released. Therefore, fluoride release from a restorative material for extended periods of time is considered favorable (Neelakantan et al).[29] Glass ionomer materials have been shown to be able to release fluoride at a sustained rate for long periods of time (at least 5 years) (Theodore P Croll et al).[30] The use of 1.25% CHX significantly improved the antibacterial effects of the evaluated RMGIC, without causing any detrimental effects to the odontoblast-like cells and on the mechanical properties. This RMGIC and CHX combination completely eliminated mutans streptococci after 3 months.[31]

CONCLUSION

Glass ionomer restorations have definite advantage over the amalgam, as the tunnel preparation is more conservative and fluoride release from the glass ionomer inhibits the growth of *S. mutans* in the plaque. Copper release and accumulation in the plaque from high copper amalgam is responsible for antibacterial effect of this restoration. Long-term investigations to compare and to evaluate the influence of these two materials for their antibacterial effect need to be carried out.

REFERENCES

1. McLean JW, Gasser O. Glass cermet cements. Quint Int 1985;16:333-343.
2. Wilson AD, Groffman DM, Kuhn AT. The release of fluoride and other chemical species from a glass ionomer cement. Biomaterials 1985;6:431-433.
3. Horowitz HS. A review of systemic and topical fluorides for the prevention of dental caries. Community Dent Oral Epidemiol 1973;1(3):104-114.
4. Horowitz HS, Heifetz SB. Evaluation of a combination of self-administered fluoride procedures for the control of dental caries in a nonfluoride area: findings after 4 years. J Am Dent Assoc 1979 Feb;98(2):219-223.
5. Ingram GS, Nash PF. A mechanism for the anticaries action of fluoride. Caries Res 1980;14:298-303.
6. Kidd EAM. Cavity sealing ability of composite and glass ionomer cement restorations. An assessment in vitro. Br Dent J 1978;144:139-142.
7. Hotz PR. Experimental secondary caries around amalgam, composite and glass ionomer cement fillings in human teeth. SSO Schweiz Monatsschr Zahnheilkd 1979; 89(9):965-986.
8. Hicks MJ, Flaitz CM, Silverstone LM. Secondary caries formation in vitro around glass ionomer restorations. Quintessence Int 1986;17:527-532.
9. Koch G, Hatibovic-Kofman S. Glass ionomer cements as a fluoride release system in vivo. Swed Dent J 1990;14:267-273.
10. Berg JH, Farrell JE. Class II glass ionomer/silver cermet restorations and their effect on interproximal plaque growth of mutans streptococci. Ped Dent 1990;12:20-23.
11. Afseth J, Opperman RV, Rolla G. The in vivo effect of glucose solutions containing Cu++ and Zn++ on the acidogenicity of dental plaque. Acta Odontal Scand 1980;38:229-233.
12. Afseth J, Oppermann RV, Rolla G. Accumulation of Cu and Zn in human dental plaque in vivo. Caries Res 1983;17:310-314.
13. Afseth J. Some aspects of the dynamics Cu and Zn retained in plaque as related to their effect on plaque pH. Scand J Dent Res 1983;91:169-174.
14. Opperman RV, Johansen JR. The effect of fluoride salt of copper, silver and tin on the acidogenicity of dental plaque in vivo. Scand J Dent Res 1980;88:476-480.
15. Maltz M, Emilson CG. Susceptibility of oral bacteria to various fluoride salts. J Dent Res 1982 Jun;61(6):786-790.
16. Wallman-Björklund C, Svanberg M, Emilson CG. Streptococcus mutans in plaque from conventional and from non-gamma-2 amalgam restoration. Scand J Dent Res 1987;95:266-269.
17. Shashibhushan KK, Basappa N. Comparison of antibacterial activity of three fluoride– and zinc releasing commercial glass ionomer cements on strains of mutans streptococci: An in vitro study. J Indian Soc Pedod Prevent Dent 2008;26 Suppl 2:S56-S61.
18. Wallman C, Afseth J, et al. Copper in approximal plaque from conventional and non-gamma-2 amalgam restoration. Acta Odontal Scand 1992;50:79-82.
19. Hattab FN, el Mowafy OM, et al. An in vivo study on the release of fluoride from glass ionomer cement. Quintessence Int 1991;22:221-224.
20. Jeevaranthan J, Deepti A. Effect of fluoride varnish on Streptococcus mutans counts in plaque of caries free children using Dentocult SM strip mutans test: a randomized controlled triple blind study. J Indian Soc Pedod Prevent Dent 2007 Oct-Dec;25(4):157-163.
21. DeSchepper EJ, Berr EA, Cailleteau JG, Tate WH. A comparative study of fluoride release from glass ionomer cement. Quintessence Int 1991;22:215-219.
22. Palneik CJ, Behnen MJ. Inhibition of microbial adherence and growth by various glass ionomer in vitro. Dent Mater 1992 Jan;8(1):16-20.
23. McCourt JW, Cooley RL, Huddleston AM. Fluoride release from fluoride-containing liners/bases. Quintessence Int 1990;21(1): 41-45.
24. Svanberg M, Krasse B, et al. Mutans streptococci in interproximal plaque from amalgam and glass ionomer restorations. Car Res 1990;24:133-136.
25. Skjorland KK, Sonju T. Effect of sucrose rinse on bacterial colonization on amalgam and composite. Acta Odontal Scand 1982;40:193-196.
26. Tatevossian A. Fluoride in dental plaque and its effects. J Dent Res 1990 Feb;69 Spec No:645-652; discussion 682-683.
27. Forsten L. Short- and long-term fluoride release from glass ionomers and other fluoride-containing filling materials in vitro. Scand J Dent Res 1990;98:179-185.
28. Menon T, Kumar C, Dinesh K. Antibacterial activity of glass ionomer restorative cements and polyacid modified composite resin against cariogenic bacteria. Indian J Med Microbiol 2006;24:150-151.
29. Neelakantan P, John S, Anand S, Sureshbabu N, Subbarao C. Fluoride release from a new glass ionomer cement. Operat Dent 2011;36:80-85.
30. Croll TP, Nicholson JW. Glass ionomer cements in pediatric dentistry: review of the literature. Pediatric Dentistry. 2002; 24(5):423-429.
31. de Castilho AR, Duque C, Negrini Tde C, Sacono NT, de Paula AB, de Souza Costa CA, et al. In vitro and in vivo investigation of the biological and mechanical behaviour of resin-modified glass ionomer cement containing chlorhexidine. J Dent 2013 Feb;41(2):155-163.

ABOUT THE AUTHORS

Veeresh S Tegginmani (Corresponding Author)

Professor and Head, Department of Conservative and Endodontics ACPM Dental College, Dhule-424001, Maharashtra, India, Phone: +918888816650, e-mail: dr.viresh@gmail.com

Beenarani Goel

Private Practitioner, Endodontist, Belgaum, Karnataka, India

Virendra Uppin

Professor, Department of Conservative and Endodontics, KLE's VK Institute of Dental Sciences, Belgaum, Karnataka, India

Priya Horatti

Professor, Department of Conservative and Endodontics, SDM College of Dental Sciences, Dharwad, Karnataka, India

LS Vijay Kumar

Reader, Department of Conservative and Endodontics, MA Rangoonwala College of Dental Sciences and Research Centre, Pune Maharashtra, India

Abhinav Nainani

Postgraduate Student, Department of Conservative and Endodontics ACPM Dental College, Dhule, Maharashtra, India