

Evaluation of the Antibacterial Efficacy of Two Different Glass Ionomer Cements on S.Mutans - An in-Vitro Study

Vasudha T.H¹, Jayalakshmi K.B², Arul Selvan³, Prasanna Latha Nadig⁴, Poorani Nagalakshmi⁵

¹Postgraduate student of krishnadevaraya college of dental sciences, Bangalore, India
vasu93gdc[at]gmail.com

²Head of the Department, Department of Conservative Dentistry and Endodontics, Krishnadevaraya College of dental Sciences, Bangalore, India
vishwajaya[at]gmail.com

³Head of the Department, Department of Microbiology, Krishnadevaraya College of Dental Sciences, Bangalore, India

⁴Professor, Krishnadevaraya College of Dental Sciences, Bangalore, India

⁵Senior Lecturer, Krishnadevaraya College of Dental Sciences, Bangalore, India

Abstract: ***Aim:** The present study was conducted to assess and compare the antimicrobial activity of two commercially available GICs against S.mutans bacteria. **Materials and Methods:** The test materials used in the study were divided into two groups namely Group 1:GC EQUIA FORTE Group 2: GC FUJI IX GP EXTRA. Direct contact test was done by placing a standardized suspension of s.mutans on the cement disc placed in a sterile 2ml flat bottomed Eppendorf tube and incubated for 1 hour at 37°C with 5-10% CO₂. 10 µl of culture was transferred to a sterile BHI Agar plate and uniformly spread using a sterile L spreader. The plates were then incubated at 37°C for 18 hours with 5-10% CO₂. The colonies were counted using digital colony counter and CFU/ml was calculated. Data collected were analysed using Independent Student t Test to compare the mean OD values and CFUs / micro litre between 2 groups. **Results and Conclusion:** The test results demonstrated that GC Fuji IX GP Extra showed significantly lesser mean OD Values and CFUs/micro litre as compared to GC Equia Forte. Within the limitation of this study, it can be concluded that both the GIC's evaluated demonstrated antibacterial activity against S. mutans. The superior antimicrobial activity was demonstrated by GC Fuji IX GP Extra. Hence, it could be advantageous in patients with high caries risk.*

Keywords: Gc equia forte, Gc fuji IX GP extra, antibacterial, direct contact test

1. Introduction

Dental caries is considered as one of the most prevalent chronic oral diseases in humans worldwide and can be distracting and painful. It can reduce a person's working efficiency, which in turn affects the economy.^[2,4]

Microorganisms play an important role in its initiation and progression. *Streptococcus mutans* has a profound effect on the incidence of dental decay in the human population. Under less severe sucrose exposure, the metabolic activity of *S. mutans* can potentiate the postprandial pH drop at the plaque– enamel interface, thereby interfering with the normal salivary remineralizing system and leading eventually to dental decay.^[1]

Those restorative materials that prevent both bacterial growth and surface colonization are preferred. As acid-producing bacteria may result in tooth demineralization, it ultimately leads to formation of secondary caries, which occurs at the junction of restoration and the tooth surface. Averages of 50% of dental restorations fail within 10 years, mainly due to secondary caries. Thus, restorative materials must have good antibacterial property.^[4]

Glass ionomer cements (GICs) possesses certain unique properties that make them useful as restorative and adhesive materials, including adhesion to the tooth structure and base

metals, anticariogenic properties due to fluoride release, thermal compatibility with the tooth enamel, and biocompatibility. The antibacterial activity of GICs may be attributed to the low pH of the cements before setting and/or their fluoride release.^[2]

Hence this study was aimed to evaluate and compare the antibacterial efficacy of GC EQUIA FORTE and GC FUJI IX GP EXTRA against S.mutans.

2. Aim of the study

To evaluate the antibacterial efficacy of two different glass ionomer cements on S.mutans.

3. Objectives of the study

To assess and compare the antibacterial efficacy of two different glass ionomer cements against S.mutans.

4. Materials and methodology

This in vitro study was conducted in the Department of Conservative dentistry and Endodontics and Department of

Microbiology at Krishnadevaraya College of Dental Sciences, Bangalore.

4.1 Isolation of *S. mutans* bacteria from caries sample

Collection of samples

Caries sample from ten different patients were collected in the department of conservative dentistry and endodontics, using sterile spoon excavator and were immediately placed in ten different tubes containing 1 ml of sterile phosphate-buffered saline with pH 7.0. Samples were stored in cool place and processed within 1-2 hour after the collection.

One hundred microliter of undiluted samples were spread on the surface of mitis-salivarius agar (MS – agar) plates using sterile swabs. Cultures were incubated anaerobically for 48 hrs at $35 \pm 2^\circ\text{C}$ and aerobically overnight at $35 \pm 2^\circ\text{C}$. Count of more than 250 colonies (104 cells/ml) was considered as positive samples.

Identification of isolates

Colonies from positive samples were subcultured on the surface of blood-agar plates for further purification and incubated anaerobically for two days at $35 \pm 2^\circ\text{C}$.

Isolates were first identified depending on their gram-staining, microscopic examination and catalase test. The streptococci are gram- positive, individual cocci which are spherical or ovoid and are arranged in chains under light microscope and may be considered as catalase negative bacteria as indicated by identification scheme of Friedrich. Depending on the colonial shape and form on the surface of agar media, *S. mutans* could be identified as hard coherent, raspberry like high refractile, raised colonies.

Inoculation suspension of *S. mutans*

An inoculation suspension was made by suspending it in BHI broth and incubated at 37°C in 5-10% CO_2 for 4 hours. The culture suspension will be adjusted to McFarland 0.5 opacity standard to obtain a culture containing approximately 1.5×10^8 CFU/ml.

Division of experimental group and sample preparation

The samples were divided into two experimental groups:

Group 1: GC EQUIA FORTE

Group 2: GC FUJI IX GP EXTRA

Both the cements are available in capsule form and mixed using an amalgamator. Twenty discs were prepared using cylindrical polyethylene tubes with a diameter of 5mm and thickness of 2mm.

Direct Contact Test

10 μl of the adjusted culture was inoculated on the resin disc placed in a sterile 2ml flat bottomed Eppendorf tube using sterile microtips and incubated for 1 hour at 37°C with 5-10% CO_2 . 10 μl of culture was transferred to a sterile BHI Agar plate and uniformly spread using a sterile L spreader. The plates were then incubated at 37°C for 18 hours with 5-10% CO_2 . The colonies were counted using digital colony counter and CFU/ml was calculated.

Statistical Analysis

Independent Student t Test was used to compare the mean OD values and CFUs / micro litre between 2 groups. The level of significance [P-Value] was set at $P < 0.05$.

5. Results

Table 1

Comparison of mean CFUs (per ml) hrs between 02 groups using Independent Student t Test								
Group	N	Mean	SD	Mean Diff	95% Conf. Interval		t	P-Value
					Lower	Upper		
GC Equia Forte	10	252.20	7.41	45.50	38.45	52.55	13.557	<0.001*
GC Fuji IX GP Extra	10	206.70	7.60					

*-statistically significant

The test results demonstrate that GC Fuji IX GP Extra showed significantly lesser mean OD Values (0.5596 $\pm$ 0.0151) as compared to GC Equia Forte (0.6495 $\pm$ 0.0134) at 24hrs with a mean difference of 0.0899(95% CI, 0.0765-0.1033). This mean difference was statistically significant at $P < 0.001$

Mean Optical Density Values at 24 hrs b/w 02 groups

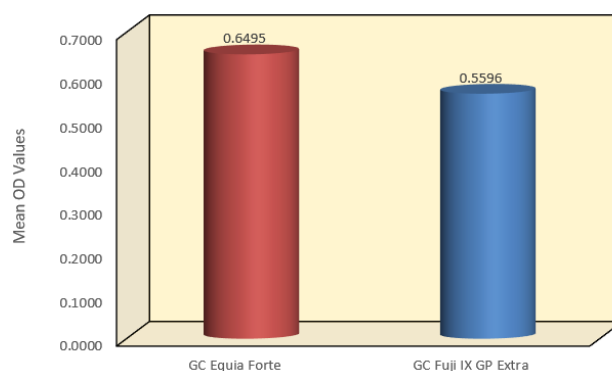
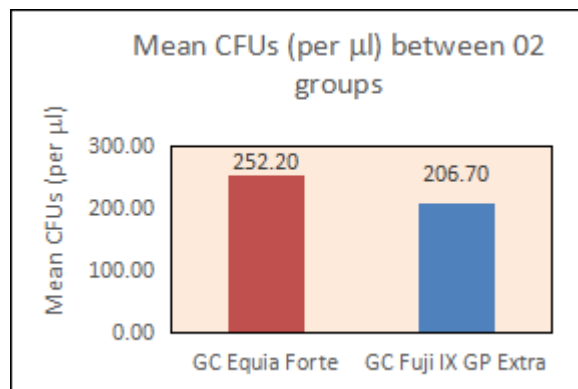


Table 2

Comparison of mean Optical Density Values at 24 hrs b/w 02 groups using Independent Student t Test								
Group	N	Mean	SD	Mean Diff	95% Conf. Interval		t	P-Value
					Lower	Upper		
GC Equia Forte	10	0.6495	0.0134	0.0899	0.0765	0.1033	14.092	<0.001*
GC Fuji IX GP Extra	10	0.5596	0.0151					

The test results demonstrated that GC Fuji IX GP Extra showed significantly lesser mean CFUs/micro litre (206.70 $\pm$ 7.60) as compared to GC Equia Forte (252.20 $\pm$ 7.41) with a mean difference of 45.50(95% CI, 38.45-52.55). This mean difference was statistically significant at $P < 0.001$



9. Discussion

Secondary caries process is difficult to diagnose and cannot be permanently treated by operative management. Clinical experience has indicated that very few or no secondary carious lesions are observed around the glass ionomer restorations.^[1]

Glass ionomer cements have proven antibacterial activity against *S. mutans*, *S. oralis*, *S. salivarius*, and *Streptococcus* species. Different in vitro methods have been used in the past to study the antimicrobial activity of dental materials with the aim of inhibiting secondary caries at the restoration margin.^[3]

This study determined the antibacterial effectiveness of different glass ionomers used in restorative dentistry. In this study we used the DCT to determine the bacterial growth after incubation of a small amount of bacterial suspension on the materials surface. Bacteria were allowed to come in direct contact, under controlled conditions, with the tested material, to study the kinetics of bacterial growth.

The materials used in this study were GC EQUIA FORTE, GC Fuji IX GP Extra. There are scarce reports to assess the antimicrobial efficacy of these two restorative materials.

Recently new improved high viscosity glass ionomer GC-EQUIA Forte has been introduced that are designed to be used in the posterior area. In 2015, Equia Forte was launched as a new material based on glass hybrid technology, consisting of a highly viscous conventional GIC combined with a nanofilled coating material (Equia Forte coat). Equia's powder consists of 95% strontium fluoroaluminosilicate glass, including the newly added highly reactive small particles, and 5% polyacrylic acid. The liquid component consists of 40% aqueous polyacrylic acid. Strontium is responsible for increased radiopacity and it does not have any undesired effects on the appearance of the cement. This substitution of calcium with strontium has enhanced fluoride release. For the fluoride to be released, the salt needs to dissociate and diffuse through the bulk cement. Since calcium is more electropositive than strontium, CaF_2 is less soluble than SrF_2 .

It was noted that the Equia Forte samples released somewhat more F^- ions than other GICs. The reason could be a replacement of Ca^{2+} with Sr^{2+} ions which slightly enhances

fluoride release rate because SrF_2 is more readily dissociated in less acidic environment than CaF_2 resulting in a higher fluoride release^[6]

GC Fuji IX GP EXTRA is the latest addition to the well established family of glass ionomers that offer unsurpassed wear resistancy, compressive strength, and durability and has fastest setting time. It has sufficient strength to resist masticatory stress. It has smaller glass particle size and these small mean particles size increases the surface area for polymeric acid and glass interaction which leads to faster maturation and higher hardness. Because of the high content of fluoride it shows excellent tendency to release fluoride ion and have potential to prevent the caries development. The amount of fluoride release is about $1200\mu\text{g cm}^2$ which is much higher compared to other experimental group. Also it can absorb fluoride ion from surrounding and act as reservoir of fluoride. The adsorption of fluoride increases with decrease in pH.^[4]

In the present study, among two experimental restorative materials GC Fuji IX GP Extra exhibited the highest antibacterial activity.

10. Conclusion

On the basis of the results of the present study, we can conclude that the GIC demonstrated antibacterial activity with differences according to the material. Furthermore, GC IX GP EXTRA cement showed the better antibacterial activity when compared to GC EQUIA Forte, which could be an advantage in terms of its use in regular clinical practice.

References

- [1] Hugar SM, Assudani HG, Patil V, Kukreja P, Uppin C, Thakkar P. Comparative Evaluation of the Antibacterial Efficacy of Type II Glass Ionomer Cement, Type IX Glass Ionomer Cement, and AMALGOMER™ Ceramic Reinforcement by Modified "Direct Contact Test": An in vitro Study. International journal of clinical pediatric dentistry. 2016 Apr;9(2):114.
- [2] Naik RG, Dodamani AS, Khairnar MR, Jadhav HC, Deshmukh MA. Comparative assessment of antibacterial activity of different glass ionomer cements on cariogenic bacteria. Restorative dentistry & endodontics. 2016 Nov;41(4):278.
- [3] Beyth N, Pilo R, Weiss EI. Antibacterial activity of dental cements containing quaternary ammonium polyethylenimine nanoparticles. Journal of Nanomaterials. 2012 Jan 1;2012.
- [4] Khere CH, Hiremath H, Sandesh N, Misar P, Gorie N. Evaluation of antibacterial activity of three different glass ionomer cements on streptococcus mutans: an in-vitro antimicrobial study. Medicine and pharmacy reports. 2019 Jul;92(3):288.
- [5] Cosgun A, Bolgul B, Duran N. In vitro investigation of antimicrobial effects, nanohardness, and cytotoxicity of different glass ionomer restorative materials in

- dentistry. Nigerian journal of clinical practice. 2019 Mar 1;22(3):422.
- [6] Brzović Rajić V, Miletić I, Gurgan S, Peroš K, Verzak Ž, Ivanišević Malčić A. Fluoride release from glass ionomer with nano filled coat and varnish. Acta stomatologica Croatica. 2018 Dec 19;52(4):307-13.
- [7] Ferreira GL, Freires I, Alves LA, Jovito VC, Carvalho FG, Castro RD. Antibacterial activity of glass ionomer cements on cariogenic bacteria—an in vitro study. Int J Dent Clin. 2011 Jul;3:1-3.
- [8] Gaybarao R, Dodamani M, Khairnar R, Chaitram H, Jadhav MA. Comparative assessment of antibacterial activity of different glass ionomer cements on cariogenic bacteria.
- [9] Wang SP, Ge Y, Zhou XD, Xu HH, Weir MD, Zhang KK, Wang HH, Hannig M, Rupf S, Li Q, Cheng L. Effect of anti-biofilm glass-ionomer cement on Streptococcus mutans biofilms. International journal of oral science. 2016 Jun;8(2):76-83.
- [10] Jenkinson HF, Lamont RJ. Streptococcal adhesion and colonization. Critical Reviews in Oral Biology & Medicine. 1997 Apr;8(2):175-200.
- [11] Murai M, Ikeda M, Yanagihara T, Hara G, Kato K, Nakagaki H, Robinson C. Fluoride uptake in human dentine from glass-ionomer cement in vivo. Archives of oral biology. 1993 Dec 1;38(12):1093-8.
- [12] Kanjevac T, Milovanovic M, Volarevic V, L Lukic M, Arsenijevic N, Markovic D, Zdravkovic N, Tesic Z, Lukic A. Cytotoxic effects of glass ionomer cements on human dental pulp stem cells correlate with fluoride release. Medicinal Chemistry 2012 Jan 1;8(1):40-5.
- [13] Yadiki JV, Jampanapalli SR, Konda S, Inguva HC, Chimata VK. Comparative evaluation of the antimicrobial properties of glass ionomer cements with and without chlorhexidine gluconate. International journal of clinical pediatric dentistry. 2016 Apr;9(2):99.
- [14] Fúcio SB, Carvalho FG, Sobrinho LC, Sinhoreti MA, Puppim-Rontani RM. The influence of 30-day-old Streptococcus mutans biofilm on the surface of esthetic restorative materials—An in vitro study. Journal of dentistry. 2008 Oct 1;36(10):833-9.
- [15] Dionysopoulos D. The effect of fluoride-releasing restorative materials on inhibition of secondary caries formation. Fluoride. 2014 Jul 1;47(3):258-65

