DENTAL MEDICINE

Evaluation of antibacterial activity of three different glass ionomer cements on streptococcus mutans: an in-vitro antimicrobial study

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Abstract

Aim. Purpose of this in-vitro study was to assess and compare the antimicrobial activity of three different glass ionomer cements (GIC) against streptococcus mutans (S. mutans) bacteria using agar plate diffusion test.

Methods. Thirty blood agar plates were prepared and three wells of 4mm diameter were made on each agar plate. Three different GIC (Micron bioactive, GC Fuji IX GP Extra, Bioglass r) were mixed and filled into the wells. These plates were inoculated with S. Mutans and incubated at 37°C for 24 hours. Bacterial growth inhibition zone around each well were recorded in millimeters using Hiantibiotic Zonescale-C.

Result. All the restorative material used in the study exhibited antimicrobial property against S. Mutans. GC Fuji IX GP Extra showed superior antimicrobial efficacy with 17.3±2.6 mm mean diameter of bacterial inhibition zone, followed by Micron bioactive 14.4±1.07 mm and Bioglass r 10.8 ± .91 mm inhibition zone respectively.

Conclusion. Within the limitation of this study, it can be concluded that all the GIC 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: glass ionomer cement, Streptococcus mutans, bacterial inhibition zone

Introduction

Dental caries is considered as one of the most prevalent chronic oral diseases in humans worldwide [1,2]. It can be distracting and painful which deleteriously affects the patient's quality of life. According to the World Health Organization, about three quarters of the world population suffer from dental caries. A major percentage of school children (60-90%) and nearly 100% of adults are affected by dental caries [3,4]. It is a huge health problem, particularly among the underprivileged groups in developed and developing countries without access to treatment care [5].

The main factors responsible for the occurrence of dental caries are bacteria, type of food consumption and the immune response of the host [6]. Its etiology also includes disturbance of the micro-ecological balance of dental plaque. Microorganisms play an important role in its initiation and progress. Several strains of oral streptococci may lead to the formation of dental plaque biofilms and causes cavity formation [7,8]. De Paz et al. have indicated that streptococcus mutans (S. mutans) is the primary cariogenic bacteria for the initiation of dental caries [9]. The dental plaque biofilm grows on all the surfaces of oral cavity, including the teeth, mucosa and restorative materials.

Extensive research in the field of modern dentistry has led to the development of various restorative materials and different treatment modalities for dental caries [10,11]. Preferable restorative materials are those that prevent both bacterial growth and surface colonization. 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.

Glass ionomer cements (GIC)

DOI: 10.15386/mpr-1113

Manuscript received: 25.07.2018
Received in revised form: 03.01.2019
Accepted: 29.01.2019

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possess certain unique properties that make them useful as restorative materials, including adhesion to the tooth structure which potentially reduces the microleakage, anticariogenic properties due to fluoride release, thermal compatibility with the tooth enamel, and biocompatibility [12,13]. The antibacterial activity of GIC may be due to the low pH of the cements before setting and their ability to release fluoride [14].

The present in–vitro study evaluated the antimicrobial activity of three different GIC on S. Mutans.

The null hypothesis states that there is no difference in the antimicrobial effect of three different restoratives GIC on S. mutans.

Materials and methods

This in-vitro experimental study was conducted to assess and compare the antimicrobial efficacy of three different GIC on S. mutans. The three different GIC used were Micron bioactive (Prevestdenpro, India), GC Fuji IX GP Extra (GC CO. Ltd. America) and Bioglass r (Biodenāmica, Portugal) (Table I). The antibacterial effect of each GIC was evaluated against S. Mutans by using an agar plate diffusion test.

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, Sri Aurobindo College of dentistry, Indore, using sterile spoon excavator and were immediately placed in a ten different tubes containing 1 ml of sterile pH 7.0 phosphate-buffered saline. Samples were stored in cool place and processed within 1-2 hour after the collection.

Isolation of S. mutans bacteria

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 ± 2ºC and aerobically overnight at 35 ± 2ºC. Count of more than 250 colonies (10^4 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 ± 2ºC.

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 [15].

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.

Colonies S. mutans were differentiated from that of other streptococci like Streptococcus. sanguis by spreading the test solution of (10%) mannitol and (4%) of 2,3,5-triphenyltetrazolium chloride (TTC) on the agar plates. A change in color to a dark pink was considered as an indicator for the presence of mutans streptococci [16].

Inoculation suspension of S. mutans

An inoculation suspension was made by harvesting the organism from agar plate and suspending it into Brain Heart Infusion (BHI) broth for another 24 hrs at 35 ± 2ºC, corresponding to 10^6 CFU/ml using the McFarland scale.

Blood agar plates (n=10) were prepared and divided into 3 equal parts and marked as A (Micron bioactive), B (GC Fuji IX GP Extra) and C (Bioglass r) using permanent marker. Blood agar plates were inoculated with S. mutans inoculums. The inoculum was uniformly spread all over the agar plate by using the lawn culture method. Each GIC was mixed according to the manufacturer's instructions and filled in respective wells of blood agar plate (Figure 1).

A Teflon-coated instrument was used for condensing the GIC (4 mm diameter) into each well respectively (Figure 2(A)). The culture plates were placed in the incubator for 24 hours at 37°C. After incubation, the plates were taken out of the incubator and the bacterial inhibition zone were recorded in millimeters by using Hiantibiotic zonescale - C (PW297) (HiMedia Lab. Pvt. Ltd. Mumbai). Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the material (Figure 2(B)).

Table I. Restorative material used in this study.

Material	Manufacture	Filler	Liquid
Bioglass r	Biodenāmica, Portugal	Calcium Barium Aluminum Fluorosilicate and inorganic filler	Polyacrylic acid
GC Fuji IX GP Extra	GC CO. Ltd. America	Smartglass	Polyacrylic acid
Micron bioactive	Prevestdenpro, India	FluoroAlumino silicate glass powder and hydroxyapatite powder	Polyacrylic acid

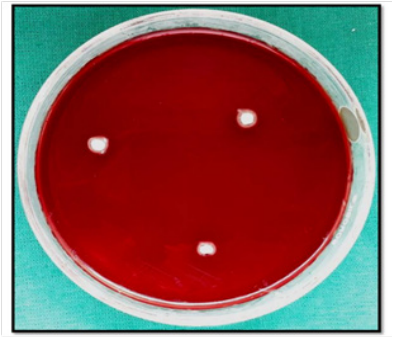

Figure 1. Blood agar plate with different glass ionomer cements disc.

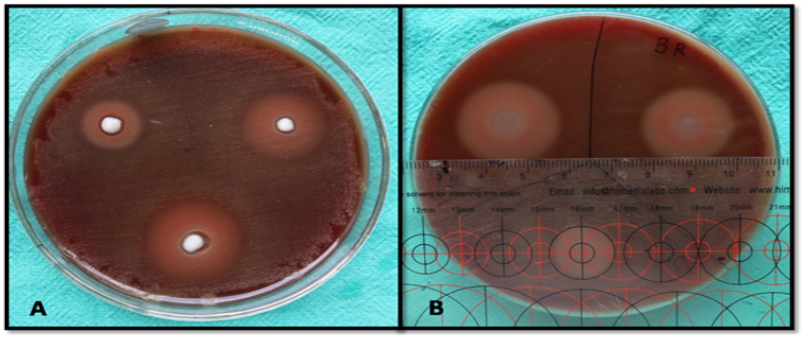

Figure 2. (A) Inhibition zone of three glass ionomer cements against streptococcus mutans (B) Measuring the diameter of inhibition zone using Hiantibiotic scale- C (in mm).

Statistical analysis

The homogeneity of variance in each group was confirmed before analyzing the data. The mean and the standard deviation of each experimental group were determined. The mean inhibition zones of the materials against the bacterial strain were compared for the three groups using analysis of variance (ANOVA). The post hoc Tukey test was performed to compare the differences in the zone of inhibition of three experimental group using software SPSS version 16.

Results

In the present study, the antimicrobial analysis as well as statistical analysis demonstrated that all the GIC had antibacterial properties against S. mutans. The mean and the standard deviation of diameter of inhibition zone for S. Mutans of three experimental restorative materials were calculated (Table II).

A comparison using ANOVA exhibited a highly statistically significant difference ($p< 0.001$) in the mean diameters of the zone of inhibition for S. Mutans among the three experimental restorative materials. Among experimental group GC Fuji IX GP Extra showed the largest diameter of the growth inhibition zone against the bacteria followed by Micron bioactive and Bioglass r (Table III).

In the pair-wise comparison using the post hoc Tukey test, the difference in the mean diameter of the zone of inhibition for S. Mutans between three experimental restorative materials was found to be statistically significant (Table IV).

Table II. Mean diameter and standard deviation of inhibition zone for S. Mutans of three restorative materials.

Experimental Materials	N	Minimum (mm)	Maximum (mm)	Mean (mm)	Std. Deviation
Micron bioactive	10	13.00	16.00	14.4000	1.07497
Bioglass r	10	10.00	12.00	10.8000	.91894
GC Fuiji IX GP Extra	10	15.00	21.00	17.3000	2.16282

Table III. Comparing inhibition zone for streptococcus mutans of three experimental groups using ANOVA test.

	Mean (mm)	Std. Deviation	F	Sig.
Between Groups	14.4000	1.07497	47.636	.000
Within Groups	10.8000	.91894		
Total	17.3000	2.16282		

Table IV. Post hoc Tukey's comparison of inhibition zone for streptococcus mutans of three experimental groups.

Experimental Materials	Mean Difference	Sig.
Micron bioactive Vs Bioglass r	3.60000*	0.001
Micron bioactive Vs GC Fuji IX GP Extra	-2.90000*	0.001
Bioglass r Vs GC Fuji IX GP Extra	-6.50000*	0.001

Discussion

The present study determined the antibacterial effectiveness of different GIC used in restorative dentistry by observing the zone of inhibition around the experimental samples in the culture plates by using an agar diffusion microbiological assay procedure.

Dental caries is the most common oral disease which leads to patient's poor quality of life. Microorganisms play a very important role in the initiation and progress of dental caries. Streptococcus mutans is the primary cariogenic bacteria responsible for the initiation of dental caries [17]. It produces acid which in turn lowers the local pH below solubility limit of teeth and hence harms the tooth. Control of the acid produced by the bacteria would lead to a control of caries [18].

In the present study, the MS agar plate was used to isolate S. mutans from caries sample of the patients. Identification of the S. mutans strains was primarily done depending on the gram staining, microscopic examination and catalase test. The presence of S. mutans was confirmed by spreading the test solution of 10 % mannitol and 4% TTC over the agar plate which leads to the color change to dark pink. This is due to the unique ability of S. mutans to hydrolysis of mannitol to acid by the enzyme mannitol-1-phosphate dehydrogenase and a reduction of TTC. The differentiation was also done using the colony shape and form on the agar plate as S. mutans bacteria are easily distinguished from other bacteria due to their unique colony morphology [16].

Bacterial biofilm can grow on all the surfaces of oral cavity, including the teeth, mucosa and restorative materials. Secondary caries occurs due to colonization of cariogenic bacteria at tooth restorative interface [19,20]. The occurrence of secondary caries is one of the leading causes for the failure of dental restorative materials. Thus, preferably restorative materials should be selected that can prevent both bacterial growth and surface colonization of bacteria.

Fluoride can inhibit acid production and offers the anticariogenic effect. It also stimulates remineralization and inhibits demineralization [21,22]. The effect of fluoride in caries control may be due to disruption of the ecological balance in the mouth, through prevention of adsorption of salivary glycoproteins to hydroxyapatite or perhaps by inhibiting growth of S. mutans directly [23]. Fluoride caused inhibition of growth rate of S. mutans with glucose as the primary energy and carbon source. Metabolism of glucose or lactose requires enolase enzyme [24]. It is the most fluoride-sensitive enzyme in the glycolytic pathway. Fermentative growth which is dependent upon glycolysis is inhibited by fluoride and thus blocks phosphoenolpyruvate (PEP) synthesis [25]. Since PEP is used for both energy and transport in S. mutans, fluoride is detrimental to growth for S. mutans [26]. Thus restorative material should have fluoride releasing capacity to inhibit the caries formation and progression.

GIC are great example of fluoride releasing material which offer fluoride around restoration and inhibit development of secondary caries. The elution of fluoride from GIC is a complex process. It can be affected by several intrinsic and experimental variables, such as resin matrix and filler composition, solubility and porosity of the material, powder-liquid ratio used in preparing the material, method of mixing, amount of exposed surface area of the material [27-29].

In the present study antibacterial efficacy of restorative material was done using agar plate diffusion test. The Hiantibiotic zone scale – C (PW297) (HiMedia Lab. Pvt. Ltd. Mumbai) was used to measure the bacterial inhibition zone. It is an easy, reliable handy tool with size 200 X 95 mm. It can measure zones in the range of 10-40 mm. It has circles of different diameter used by placing the scale over the inhibition zone and matching the appropriate size of the zone.

The materials used in this in vitro study were Micron bioactive (Prevestdenpro, India), GC Fuji IX GP Extra (GC CO. Ltd. America) and Bioglass r (Biodenāmica, Portugal). To the best of our knowledge, no previous research has been done to assess the antimicrobial efficacy of these restorative materials.

Micron bioactive (Prevestdenpro, India) is GIC incorporated with hydroxyapatite crystals and has good mechanical property. It has better handling property and biocompatibility to tooth structure [30].

Due to presence of higher amount of fluoride in this material; it has the potential to increase the amount of fluoride release from the set GIC and has good antimicrobial efficiency. In this present study, micron bioactive exhibited antimicrobial activity against streptococcus bacteria in agar – plate diffusion test, the mean diameter of zone of inhibition against bacteria was found to be 14.4±1.07 mm, which is lesser than the GC Fuji IX GP Extra but higher than the Bioglass r.

GC Fuji IX GP Extra (GC CO. Ltd, America) has smaller glass particle size and sets faster. It exhibits superior physio-mechanical property and good wear resistance thus gives it sufficient strength to resist masticatory stress [31]. 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 μg cm^2 which is much higher compared to other experimental groups [31]. Also it can absorb fluoride ion from surrounding and act as reservoir of fluoride. The adsorption of fluoride increases with decrease in pH. In the present study, among three experimental restorative materials GC Fuji IX GP Extra exhibited the largest diameter of the zone of growth inhibition against the bacteria. The mean diameter of zone of inhibition was found to be 17.3 ± 2.6 mm.

Bioglass r (Biodenāmica, Portugal) exhibit better anticariogenic properties due to the release of fluoride, thermal compatibility with tooth enamel, biocompatibility

and low toxicity. In this present study, Bioglass r exhibited antimicrobial activity against streptococcus bacteria in agar – plate diffusion test. The mean diameter of zone of inhibition against bacteria was found to be 10.8 ± .91 mm, which is least among the three experimental restorative materials [32].

All of the evaluated GICs showed an antibacterial activity according to the agar-plate diffusion test, inhibiting the growth of the selected cariogenic bacteria, probably associated with the solubility of organic and inorganic components. The factors that influence solubility include filler concentration and mean particle size, coupling agents, the nature of the filler particles type of solvent and the monomer conversion degree [33].

As all the evaluated GICs showed antimicrobial efficacy, these materials can be highly recommended to their use in regular clinical practice as they can prevent secondary caries formation around the restoration. The antimicrobial efficiency of GC Fuji IX GP Extra proved to be better when compared to other experimental group. Thus, it could be an advantage in terms of its use in high caries risk patients.

Conclusion

On the basis of the results of the present in vitro study, it can be concluded that all the GICs evaluated demonstrated antibacterial activity with variable effect of action against S. mutans. Thus these materials could prevent the secondary caries formation around the restoration. Hence the use of these materials could be highly recommended in regular clinical practice. The superior antimicrobial activity was demonstrated by GC Fuji IX GP Extra. Hence, it could be advantageous in patients with high caries risk.

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