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Research Article

Antibacterial Efficacy of Three Fluoride-Releasing Restorative Materials Against *Lactobacillus acidophilus* and *Streptococcus mutans*: An *In vitro* Analysis

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Abstract

Objective: The study was done to evaluate the antibacterial effects of three fluoride releasing restorative materials, namely seventh generation dentin bonding agent (Futurabond® DC, Voco, Germany), resin modified glass ionomer cement (GC Fuji II™ LC, GC Corporation, Tokyo) and Nanoionomer (Ketac™ N100) against *Lactobacillus acidophilus* [Microbial Type Culture Collection, 10307] and *Streptococcus mutans* [Microbial Type Culture Collection, 497].

Materials and Methods: The antibacterial effect was analysed using agar diffusion test. The experimental groups were three fluoride releasing restorative materials and control against *Lactobacillus acidophilus* (MTCC 10307) (Group A) and *Streptococcus mutans* (MTCC 497) (Group B). The wells in petri dishes were filled with chlorhexidine, resin modified glass ionomer, nanoionomer, bonding agent and were dropped with micropipettes in paper disks, blown dry and light cured. The culture plates were incubated for 24h at 37°c. The antibacterial effect was checked after 24h, 48h and 7days in triplicates..

Results and Conclusion: The results were collected, and statistically analyzed using the ANOVA test to determine the difference between the mean diameters of the inhibition zone observed. All the three restorative materials showed antibacterial activity against Streptococcus mutans and Lactobacillus acidophilus. The antibacterial effect for resin modified glass ionomer and nanoionomer decreased over a period of 7 days whereas for dentin bonding agent there was an increase in the antibacterial effect seen from 24 hours to 48 hours.

Keywords: Antibacterial; Fluoride; Restorative; *Lactobacillus acidophilus*; *Streptococcus mutans*

Introduction

Microbial infection is found to be the main etiologic factor for the inflammation of the dental pulp and the periodontium. Various species of bacteria have been isolated from dental plaque. *Streptococcus mutans* is associated with the initiation and Lactobacillus with the progression of the established carious lesion. These cariogenic bacteria can degrade fermentable carbohydrates to acids which in turn demineralize the tooth structure [1]. *Streptococcus mutans* adheres to a proteinaceous layer called the acquired pellicle that is already present on the enamel.

Since initial adhesion is the first step in biofilm development, research has focused on strategies to prevent initial microbial colonization and subsequently reduce or inhibit biofilm formation. However, as avoiding the initial colonization is almost impossible it is necessary to develop other methods to prevent caries [2].

Secondary caries is a major problem in restorative dentistry which develops at leaky crown margins or insufficient restorations. Cement with antimicrobial potential may aid in preventing secondary caries.[2] The effect of fluoride on demineralization and remineralization of early carious lesions in enamel and dentin is recognized as the vital mechanism of fluoride action. Studies have shown that fluoride released from fluoride-containing restorative materials effectively protects the tooth from demineralization in the areas in close proximity to the restorative materials [3].

Hybrid materials such as resin modified glass ionomer cements (RMGIC) have been developed to help overcome the limitations associated with conventional GIC's such as moisture sensitivity, low initial mechanical properties, and inferior translucency, while retaining their clinical advantages such as fluoride release and adhesiveness. A new category of RMGICs have been introduced for restoration of primary teeth and minimal cavities in permanent teeth. The major innovation is the incorporation of nano-technology, which permits a highly packed filler composition (~69%), predominantly nanofillers.[3] Nanoparticulated ionomer, combines the benefits of resin-modified light-cure glass ionomer cement (RMGIC) and bonded nanofiller particles [4].

The application of a bonding system with antibacterial activity is a promising solution in preventing secondary caries. Several dentin bonding systems are currently available [5].

The purpose of this study is to compare the antibacterial effects of a fluoride releasing restorative material and bonding system (7th generation) against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Materials and Methods

The experimental groups were divided into Group A and Group B.,three fluoride releasing restorative materials and control against *Streptococcus mutans* Microbial Type Culture Collection (MTCC 497) (A) and *Lactobacillus acidophilus* (MTCC 10307) (B). It wasdone in triplicates on a total of 50 Brain Heart infusion agar plates. In the first material,18 plates were used for the 3 materials in triplicates for *S. mutans* and *L. acidophilus*. A single sample was placed in each plate as there was no idea about the size of the zone of inhibition. Six plates of positive control and negative control each were used. So the first cycle consisted of thirty plates. The second and third cycle consisted of six plates each for the three materials in triplicates for *S. mutans* and *L. acidophilus*, as three samples of each material were placed in a single plate. And two plates of positive and negative control each were used. So the second and third cycle consisted of ten plates each.

An agar diffusion test was used for evaluation of antibacterial effect against *S. mutans* (MTCC 497) and *L. acidophilus* (MTCC 10307). the procedures were carried out under asepsis in a laminar airflow chamber. The bacterial suspension were prepared by inoculating a loopful of each bacterial culture in sterile brain heart infusion broth and incubated at 37°c for 24 hours and its turbidity was set to 0.4 Optical density at 530 nm.

Total 18 discs of each material i.e., Resin modified glass ionomer cement, 7th generation dentin bonding agent, and Nanoionomer were prepared. Out of the 18 tablets, 6 tablets each of the test material were used for studying their antimicrobial activity after 24hours, other 6 discs of each material were used for checking activity after 48 hours. The remaining 6 discs of each material were used for checking their antimicrobial activity over the period of 7 days.

The discs of glass ionomer cements, Resin modified glass ionomer cement (GC Fuji II™ LC, GC Corporation, Tokyo) and Nanoionomer (Ketac™ N100) were prepared from 6x4mm sized cylindrical brass molds. The material was placed into the mould. A dental floss was incorporated during fabrication into those discs whose antimicrobial activity was required to be checked over the period of 48 hours and 1 week. The glass ionomer cements were light cured for 20 seconds from above and below the discs. Each disc was immersed in test tube containing 15ml of deionized water and incubated at appropriate time intervals.

20µl of dentin bonding agent (Futurabond® DC, Voco, Germany) was placed with a micropipette on the paper disk and blown dry for 10s using at a distance of 5mm to remove excess material and solvents and bonding agent was light-cured using LED light curing unit (Turbo, Beecool, Confident).

Aqueous 0.2% chlorhexidine digluconate (Hexidine mouthwash-ICPA Health Products Ltd., India) was positive control and empty wells in the agar plate were considered as the negative control. Specimens were UV sterilized for 15 mins. Sterilelaminar airflow chamber was used for the experiment.

To perform Agar diffusion test, pour plate technique was performed. Sterile BHI agar butts were prepared, where each tube contained 20 ml of BHI agar. Each butt was boiled in boiling water bath to obtain molten agar butt, which was later cooled to 40 degrees while it was still in the molten state. At this point 1 ml of culture suspension was added into the molten agar butt, mixed and the whole tube containing the medium and culture is poured into a

sterile petri plate and the medium was allowed to solidify at room temperature. After the medium solidified, three wells of 6mm diameter and 4mm depth were made in agar plate with sterile metal cork borer.

These wells were incorporated with material discs of resin modified glass ionomer cement (GC Fuji II™ LC, GC Corporation, Tokyo), and nanoionomer (Ketac™ N100) cements. Whereas the filter paper disks containing DBA were placed over the uniform inoculated agar surface using sterile metal forceps and then all the plates incubated at 37°c for 24 hours for the first reading. All the paper disks containing DBA were stored in dark, and submersed in PBS at 37°c for 48 hours, 7 days.

Resin modified glass ionomer cement (GC Fuji II™ LC, GC Corporation, Tokyo), Nanoionomer (Ketac™ N100) and the dentin bonding agent (Futurabond® DC, Voco, Germany) were placed on freshly inoculated BHI agar (Hi Media Laboratory Pvt. Ltd., Mumbai, India) after specific time intervals of 48 hours and 7 days after ageing in distilled water and phosphate buffer saline respectively.

After incubation, the average of the diameter of the zones of inhibition around the disks at two locations using a millimeter scale, was calculated. Measurement was repeated thrice and mean was calculated for each well.

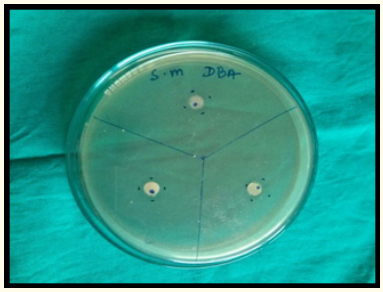


Figure 1: Sample of the zone of inhibition for Dentine bonding agent for *S mutans* at 48hours.

Figure 2: Sample of zone of inhibition for Dentine bonding agent for *Lactobacillus acidophilus* at 48hours.

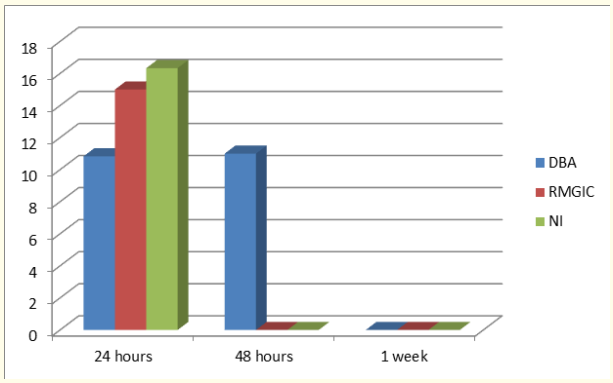
Results

The results were collected, and statistically analyzed using the ANOVA test to determine the difference between the mean diameters of the inhibition zone observed.

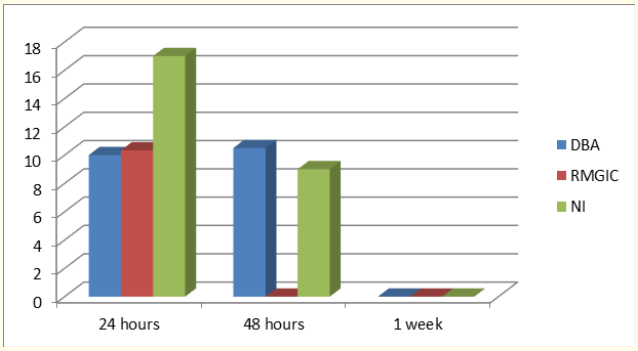
The significance level was fixed around 0.05 and the mean were recorded after 24hours, 48hours and 7 days for the three test materials. LSD test for pairwise comparison was also done for all three materials.

For Group A (*Lactobacillus acidophilus*), after 24 hours, average zone of inhibition obtained from Nanoionomer (16.33mm) was the highest followed by Resin modified glass ionomer cement (15mm) and Dentin bonding agent (10.83mm) (Graph 1) respectively. After 48 hours, only Dentin bonding agent showed zone of inhibition (11.5mm). Whereas Nanoionomer and Resin modified glass ionomer cement did not show any zone of inhibition. And after 7 days none of the three materials showed any zone of inhibition (Graph 1).

For Group B (*Streptococcus mutans*), after 24 hours, average zone of inhibition obtained from Nanoionomer was the highest (17mm) followed by Resin modified glass ionomer (10.33mm)



Graph 1: *Lactobacillus acidophilus*- zones of inhibition for all 3 materials at different time intervals



Graph 2: Streptococcus mutans- zones of inhibition for all 3 materials at different time intervals.

and Dentin bonding agent (10mm) (Graph 2) respectively. After 48 hours, Dentin bonding agent showed even higher zone of inhibition (10.5mm) than Nanoionomer (9mm) and Resin modified glass ionomer cement did not show any zone of inhibition. After 7 days none of the three materials showed any zone of inhibition (Graph 2).

Discussion

Operative work in restorative dentistry currently has an ultra-conservative approach, preserving tooth structure and preventing pulpal injury.[7] However, reduced removal of caries, may leave behind caries in the minimally excavated lesions [7].

Mutans streptococci and lactobacilli are acid producing bacteria and hence cause an ideal environment creating the risk for cavities [8]. Studies have reported significant correlation of Lactobacilli in root surface plaque and root caries lesion [9]. *L. acidophilus* is the primary organism responsible for the continuation of deep dentinal caries [7].

Care was taken to keep the plates for 2 hours at room temperature to allow the permeation of agents through the agar and incubated under appropriate gaseous conditions at 37°c [10].

The strength of antibacterial activity is deciphered by the readings in millimeter diameter of the inhibition zone around the material [5]. The size of the inhibition zone depends antibacterial properties, the quantity used, and diffusion potential of the material across the culture medium. Larger inhibition zones were directly proportional to the quantity and diffusion rate. While the quantity of material applied can be easily controlled thanks to the use of an automatic pipette, the final result may be influenced by the diffusion potential.

The development of dentin bonding agents with improved micromechanical bonding to enamel and dentine has revolutionized new treatment modalities. Dental adhesives may come into direct contact with the residual carious dentine and so the concept of Dentin bonding agents possessing antimicrobial properties seems a logical one. The addition of DBA s with antimicrobial properties into the restoration process would therefore be an added advantage. This has led to the development of DBA s with antibacterial components such as fluoride and 12 methylacryloyloxydodecylpuridiniumbromide (MDPB). For etch and rinse DBA it might be expected that the etchant, usually >30% phosphoric acid, would exert a significant antibacterial effect on the microflora of the infected dentine [11].

The tooth-restoration interface created when using the dentin bonding agents does not eliminate microleakage and bacterial colonization, allowing an influx of cariogenic bacteria and their toxins into the dentin, permeating dentine leading to secondary caries. Dentin bonding with antibacterial properties is an auxilliary in preventing secondary caries as it may hinder or delay the ingress of bacteria via microleakage at the tooth restoration interface [7]. Reduction in bacterial counts is associated with decreased caries incidence. The seventh-generation all-in-one adhesives combined etch, prime and bonding procedures into a single-step application.

A self-etching dentin bonding system (Futurabond DC) used needed simple application procedure and low technique sensitivity and reduced the risk of the primed surface contaminated with saliva. In this self-etching dentin bonding systems demonstrated antibacterial action which may be due to their acidity or chemical composition. The viscosity, diffusion capacity, and presence of antibacterial agents in the bonding systems may also be factors involved [7].

The antibacterial performance of Futurabond DC was probably due to it having the low ph. Due to low pH, SEPs and SEAs generally exert some antibacterial effect in dentin substrate before the restoration, because dentin is not etched and rinsed with water, and the smear layer is significantly incorporated into the hybrid layer [12].

Both Resin modified glass ionomer cement (GC Fuji II™ LC, GC Corporation, Tokyo) and Nanoionomer (Ketac™ N100) evaluated in this study showed zones of inhibition which was highest after 24 hours. The zones of inhibition due to amounts of fluoride release, which was highest after 24 hours after which the release reached to a low steady level and called as "Burst Effect". The burst effect originates from the initial acid-base reaction between the glass and polyalkenoic acid and is associated with the release of fluoride which is loosely bound in the glass ionomer cement. The later gradual release results from a balance between erosive leaching of glass particles in the bulk of the cement and diffusion of the leached fluoride through the matrix.

The burst effect of fluoride release is crucial for remineralization and for reduction of viability of bacteria left behind in the inner carious dentine [13].

According to De Araujo FB., *et al*. [14]. and Di-az Arnold et al.[15] resin modified glass ionomer cements release less fluoride at the initial few hours compared to the conventional glass ionomer cement.

The inclusion of fluorite and/or cryolite as fluxes in the RMGIC during the manufacturing process, may enhance the release of F - ions into the matrix during the setting reaction in the initial 24h period. The liquid component of RMGIC conventionally contains hydroxylethyl methacrylate, may aid the antibacterial effect by providing a low initial pH. The low pH (2.2-3.6) of initial mix cement rises to neutrality during continuation of the setting reaction. Initial acidity may play a major role in its antibacterial effect [6].

Nanotechnology has become the most highly energized disciple in science and technology [5]. Nanoparticulated ionomer are resin-modified glass ionomer cements, which combine the benefits of RMGIC and bonded Nanofiller particles in the range of 0.1 to 100 nanometers in size. This broad range of filler particle can influence strength, optical properties, abrasion resistance, and increased fluoride release. Also less number of voids, cracks, and microporosities are found on the surface in Nanoionomer cement [4].

Paschoal., *et al*. [16]. observed that nanoparticulated glass ionomer cement show a steady release of low levels of fluoride compared to other resin modified glass ionomer cements. The translucency of material is reduced by flourides and low levels of fluoride content are incorporated in the cement, to improve the esthetic property of the material. This may validate for the low release of fluoride by the nanoionomer cement.

Colonisation of bacteria at the tooth/restoration interface or leakage through a marginal gap might not occur for the three materials, for a specific period of time, as observed by the zones of inhibition in the first 24-48 hours. Significantly, the effect that the tested materials on residual bacteria left in the tooth preparation prior to the restorative procedure, as in a clinical situation, may also be noted [7]. It may be speculated that the antibacterial effects may depend on the components of the material added to promote adhesion or improve its physical properties. The minimum amount of fluoride required for preventing or arresting a carious lesion has not been well established. However it might not be correct to conclude whether the lesser amount of fluoride released by nano ionomer, resin modified glass ionomer, and fluoride containing 7th generation dentin bonding agent may be adequate or not as an antibacterial agent. Further studies, both clinical and laboratory, are required to establish the antibacterial effect of nano ionomer and seventh generation bonding agent.

Conclusion

Within the limitations of this study, it can be concluded that all the restorative materials showed antibacterial efficacy against both *Streptococcus mutans* and *Lactobacillus acidophilus* in the first 24hours.

After 48hours, the 7th generation dentin bonding agent and Nanoionomer showed zones of inhibition against *Streptococcus mutans*. Dentin bonding agent demonstrated increase in zones of inhibition against *Lactobacillus acidophilus*.

At 7 days, none of the three tested materials showed any zone of inhibition i.e., did not exhibit antibacterial activity.

The antibacterial effect for Resin modified glass ionomer cement and nanoionomer decreased over a period of 7 days whereas for dentin bonding agent there was an increase in the antibacterial effect seen from 24 hours to 48 hours.

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