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Comparative Evaluation of Antibacterial Efficacy of Hybrid Tooth Coloured Restorative Materials against Streptococcus Mutans: An In Vitro Analysis

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ABSTRACT:
Introduction: The purpose of this study was to evaluate the antibacterial effect of three restorative materials, conventional glass ionomer cement, resin-modified glass ionomer cement, and bioactive fluoride-releasing composite against Streptococcus mutans. **Method:** Thirty test specimens for each of the three dental materials were prepared withaqueous 0.2% chlorhexidine digluconateused as a positive control. The antibacterial activity was evaluated by the agar disc diffusion test against Streptococcus mutans (MTCC 890) on BHI agar supplemented with 5% defibrinated sheep blood. Four wells 6.5mm in diameter were made and assigned groups according to the materials inserted in them, Group I (conventional GIC), Group II (RM GIC), Group III (bioactive fluoride-releasing composite), and Group IV (0.2% chlorhexidine as positive control). The bacterial suspension was evenly poured and the culture plates were incubated at 37°C and evaluated at 24 hours, 48 hours, and 7 days for each group. The zones of bacterial inhibition were recorded in millimetres using a digital Verniercalliper for each culture plate and the mean was calculated. **Statistical Analysis:** Comparison of zones of inhibition between the groups at various time intervals was done using one-way ANOVA followed by pairwise comparison using Tukey post hoc test. **Results:** Glass ionomer cement showed the highest antibacterial activity against S. mutans followed by the bioactive fluoride-releasing composite and resin-modified glass ionomer cement with the highest value at 24 hrs followed by 48 hrs & least for 7 days. **Conclusion:** At all time intervals the antibacterial activity of the positive control was significantly higher than the experimental groups withmaximum antibacterial efficacy at 24 hours that progressively decreased with time.

KEYWORDS: Streptococcus mutans, conventional glass ionomer cement, RMGIC, chlorhexidine.

I. INTRODUCTION

Dental caries is an infectious microbial disease of the teeth that results in localized dissolution and destruction of calcified tissues. The etiology of dental caries is multifactorial of which Streptococcus mutans which is a commensal of the oral microbial flora has been often implicated to play an important part.[1] Restorative dentistry aims at re-establishing the tooth to its proper form, function, and aesthetics which is achieved by employing various techniques of cavity preparation and subsequent use of the best available restorative materials. Micro-organisms at the tooth restoration interface and restoration margins lead to the initiation of secondary caries which is one of the major causes for restoration failures. Hence restorative materials with added antibacterial effect were proposed to minimize such failures.

This biomimetic approach aims at innovating restorative materials that react according to the changes in the oral environment releasing ions aiding in remineralization as an approach towards reversing the initiation of the caries process. The introduction of antibacterial agents in certain restoratives like fluorides incorporated in glass ionomer cements leads to

remineralization of early enamel lesions with increased resistance to further acid challenge. Fluorides also act as antibacterial[2] agents by inhibiting enzyme enolase, irreversibly. Composite resins and resin-modified glass ionomer cements were developed having better aesthetics, superior mechanical properties, and low solubility but with a high coefficient of thermal expansion and polymerization shrinkage leading to microleakage. To inhibit secondary caries caused due to microleakage restorative materials with antibacterial potential were developed.

Newer fluoride-releasing composites were developed to achieve the antibacterial effect by modifying the conventional materials. Although various studies have been done to explain the antibacterial activity of GIC, there is little information regarding this activity in hybrid restorative materials. On this background, the present study was carried out to compare the antibacterial effect of hybrid tooth-colored restorative materials against Streptococcus mutans and also to evaluate their antibacterial activity as a function of time.

II. MATERIALS AND METHOD

Ethical clearance to conduct the study was obtained from the college research ethics committee. All the procedures for this study were carried out in a Grade II biosafety cabinet to prevent contamination of the samples and that of the pure cultured bacteria.[3,4]

Collection and preparation of S. mutans strain:

Indicator strains of S. mutans (MTCC 890) in the form of lyophilized culture were obtained, rehydrated in 15 ml of Luria Bertani broth for 48 hours at 37°C and then placed in 5 ml of Brain Heart Infusion broth for 24 hours at 37°C to form a suspension (inoculum), corresponding to 106 colony-forming units/mL using the McFarland scale.

Sample preparation:

Thirty test specimens each of the three groups of restorative materials conventional GIC (GC Fuji IX™ GP, GC Corporation, Tokyo, Japan), RMGIC (GC Fuji II™ LC, GC Corporation, Tokyo, Japan), and fluoride-releasing composite (ActivaTM-Bioactive Restorative) were prepared using a custom made teflon ring mold with a diameter of 6.5 mm and thickness of 2 mm. Thirty specimens of 10 µlof aqueous 0.2% chlorhexidine digluconate(Hexidine mouthwash-ICPA Health Products Ltd., India) were loaded on sterile filter paper discs and used as a positive control. All test specimens were sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes. The agar diffusion test was used to evaluate the antibacterial effect of the materials, in which a base layer containing 15 ml Brain Heart Infusion agar supplemented with 5% defibrinated sheep blood was evenly spread to a thickness of 4 mm on thirty sterile petri dishes followed by making four wells 6.5mm in diameter after solidification using the blunt end of a micropipette tip.

These wells were assigned groups according to the materials inserted in them,

Group I: Conventional glass ionomer cement
Group II: Resin modified glass ionomer cement
Group III: Bioactive fluoride-releasing composite
Group IV: 0.2% chlorhexidine (as positive control)

The bacterial suspension was poured with a micropipette and it was spread evenly using the plate spreader followed by incubation for 24 hours at 37°C.

Evaluation of antibacterial activity:

The antibacterial activity was evaluated at 24 hours, 48 hours, and 7 days for each group. The zones of bacterial inhibition were recorded in millimeters using a digital verniercaliper measuring the greatest distance between two points at the outer limit of inhibition halo formed around the wells. This measurement was repeated for each culture plate and the mean was calculated.

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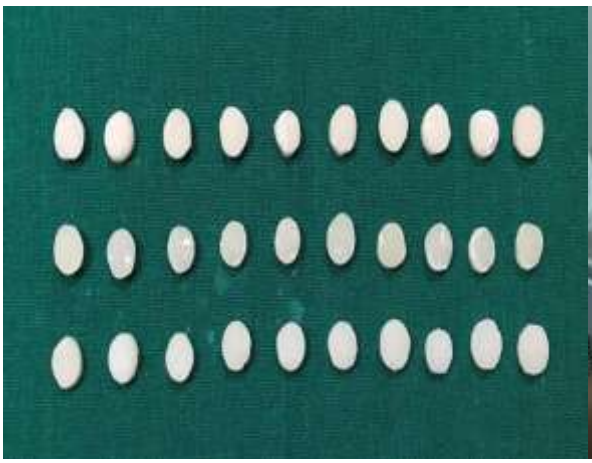

Fig. 1 SAMPLES OF RESTORATIVE MATERIAL

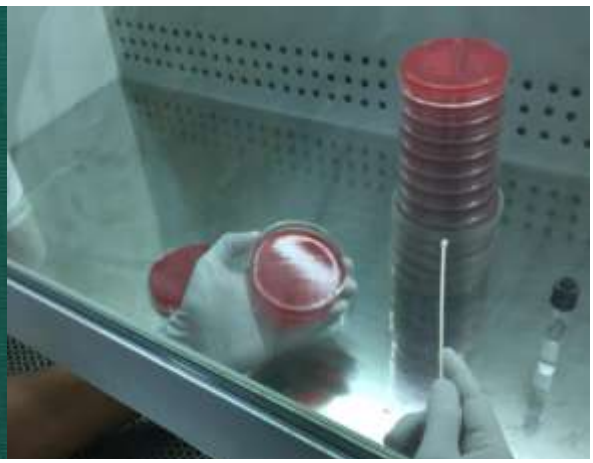

Fig. 2 SPREADING THE BACTERIAL INOCULUM ON BHI AGAR SUPPLEMENTED WITH 5% SHEEP BLOOD

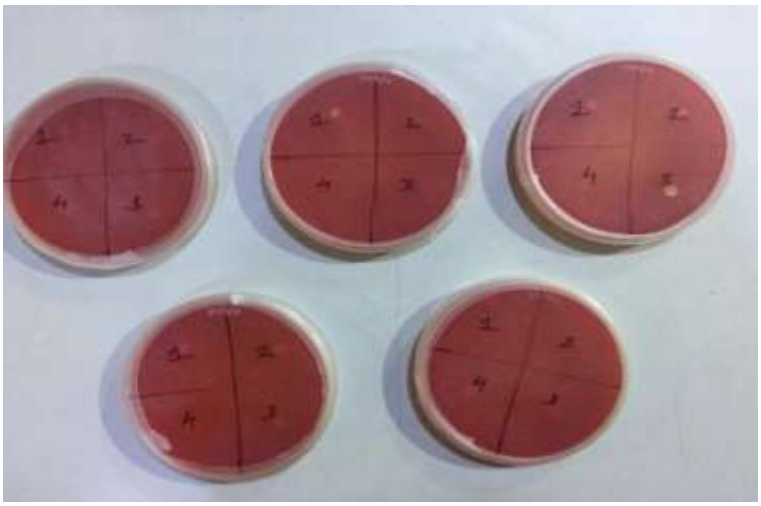

Fig. 3 NUMBERING THE SAMPLES ACCORDING TO THE GROUPS

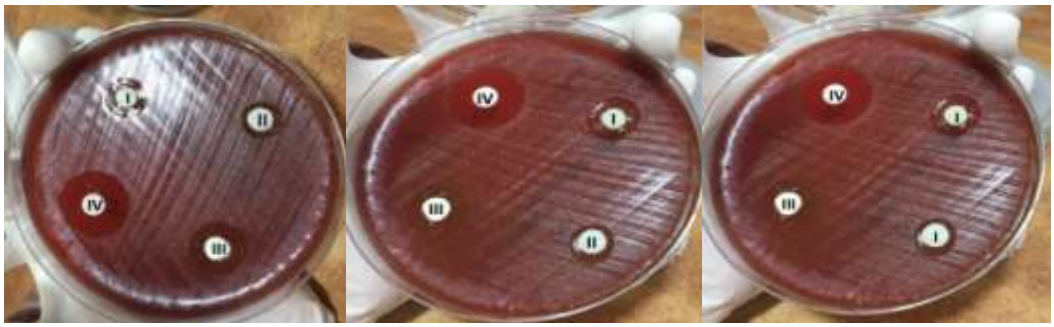

Fig. 4a ZONES OF INHIBITION AT 24 HOURS **Fig. 4b** ZONES OF INHIBITION AT 48 HOURS **Fig. 4c** ZONES OF INHIBITION AT 7DAYS

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Fig. 5 MEASUREMENT OF ZONES OF INHIBITION BY DIGITAL VERNIER CALIPER

III. STATISTICAL ANALYSIS

The data obtained was found to have a normal distribution, hence parametric tests were used. Comparison of zones of inhibition between the groups at various time intervals was done using one-way ANOVA followed by pairwise comparison using post hoc Tukey's test. Intergroup comparison of zones of inhibition across time for each group was done using repeated measures ANOVA followed by pairwise comparison using paired t-test. For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

IV. RESULTS

Intragroup comparison:

- There was a statistically significant difference seen between zones of inhibition when compared with various time intervals ($p<0.01$) with the highest value at 24 hrs followed by 48 hrs & least for 7 days for all the three test materials,
- There was a statistically significant difference seen between zones of inhibition when compared within 24 hrs vs 48 hrs & 24 hrs vs 7 days ($p<0.01$, $p<0.05$) for group IV. However, there was a nonsignificant difference between 7 days vs 48 hrs ($p>0.05$)

Intergroup comparison:

- There was a statistically significant difference seen between zones of inhibition between all 4 groups at 24 hrs ($p<0.01$) with means highest in CHX followed by GIC, fluoride-releasing composite (Activa), and least for RMGIC.
- There was a statistically significant difference seen between zones of inhibition between all 4 groups ($p<0.01$) except for GIC vs fluoride-releasing composite (Activa) ($p>0.05$) at 48 hours.
- There was a statistically significant difference seen between zones of inhibition between group I and group II ($p<0.02$) and a highly significant difference between Group I, II, and III with group IV ($p<0.01$) except for GIC vs fluoride-releasing composite (Activa) ($p>0.05$) at 7 days.

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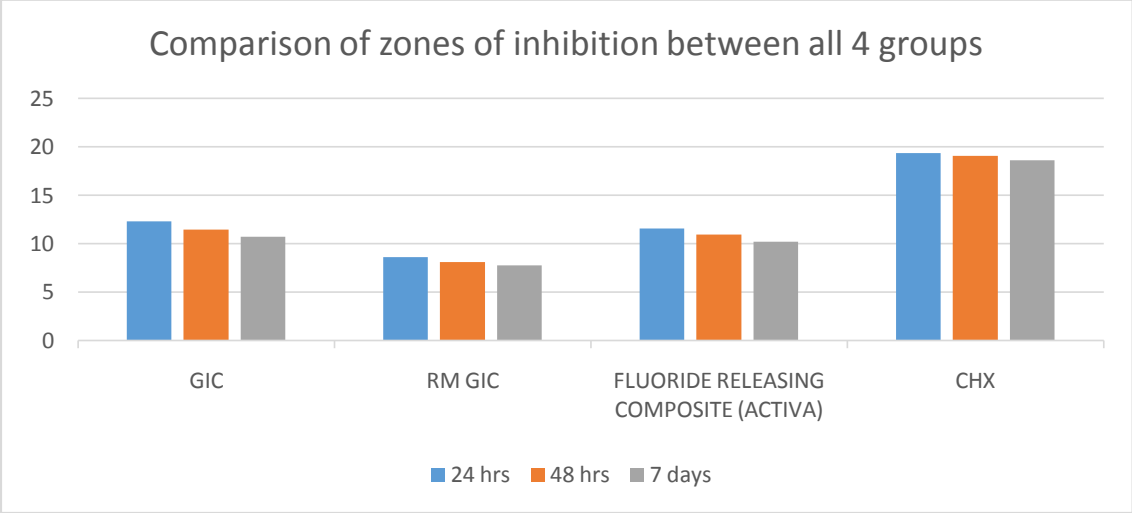

Graph 1: Intragroup and intergroup comparison of zones of inhibition between all 4 groups at 24 hours, 48 hours and 7 days

V. DISCUSSION

Dental caries has been regarded as the most prevalent, chronic, pandemic, polymicrobial and multifactorial disease of the oral cavity. An ideal restorative material should not allow or rather inhibit microbial adhesion and growth. Newly developed materials claim to have a very high degree of antimicrobial activity against the most common etiological agent implicated in dental caries ie; Streptococcus mutans by the virtue of which the material will not allow colonization of microorganisms in the form of plaque and actively combat oral bacteria to prevent secondary caries. This also reduces the overall caries incidence owing to its fluoride release.

The antibacterial activity of GIC, has been previously well established by various studies. Though it has a high initial fluoride release it reduces significantly with time with some antibacterial activity retained. Resin modified glass ionomer cements were developed to overcome the problems of conventional GICs such as moisture sensitivity and low wear resistance, and at the same time maintain their clinical advantages such as fluoride release and adhesiveness. The light-cured composite resin materials also underwent an evolution by the addition of compounds that released fluoride on the drop in pH in an attempt to incorporate antibacterial activity within these materials to prevent secondary caries due to increased marginal gaps.

The present study evaluated the antibacterial effect of conventional glass ionomer cement, resin-modified glass ionomer cement, and bioactive fluoride-releasing composite against Streptococcus mutans. 0.2% chlorhexidine, was chosen as the positive control because of its widespread clinical use[5,6] and a common point of reference[7,8] for comparisons with others studies. A positive control helps to determine the appearance of the zone of inhibition on agar.

GIC and RM GIC were used in the capsulated pre-proportioned form whereas fluoride-releasing composite was used in the syringe form according to the manufacturer's instructions. The capsulated form was used to overcome the inability to standardize the mixing time and powder: liquid ratio and also to reduce the incorporation of air bubbles as compared to the hand-mixed manipulation.[9] Agar disc diffusion was the method of choice to evaluate zones of inhibition because it allowed both solid (test) and liquid (control) materials to be assayed[10,11] together. The depth of the agar medium was standardized at approximately 4 mm as recommended by Barry and Fay (1973)[12] to eliminate the diffusion differences in the vertical dimension of the materials under study. Streptococcus mutans (MTCC 890) acquired from the Institute of Microbial Technology was the microbial strain used for this study. The review of literature implicates Streptococcus mutans to be the normal commensal of the oral cavity which plays a major role in dental caries[1] and was thus selected for this study. The bacterial inoculum density was standardized to 0.5 concentration using the McFarland scale to produce reliable and reproducible results.[13] Owing to the uniformly circular zone findings as also seen in previous studies[14] zones of inhibition were evaluated by measuring the diameter of the zone around the wells.

In this study, the results obtained showed a highly statistically significant difference between the antibacterial activity of Group IV (0.2% chlorhexidine) and Group I, Group II and Group III, with the highest antibacterial activity observed in Group IV at all the time intervals. This is due to the predictable and high susceptibility of chlorhexidine to S. mutans.[5] The antibacterial activity of GIC, RMGIC, and fluoride-releasing composite progressively decreasing as a function of time has been in accordance with the previous studies of Takahashi et al (1993)[15], Vermeersch etal(2005)[16]Davidovich et al (2007)[17]

Also, it showed an initial peak in the fluoride release and antibacterial activity of GIC and RMGIC followed by a significant reduction after one week. This could be attributed to the possible mechanism of fluoride release from these materials.[18] GIC and RMGIC show an initial fluoride burst effect from the surface after which the elution is markedly reduced, accompanied by a second bulk diffusion process by which small amounts of fluoride continue to be released into the surrounding media. There was a statistically significant difference between the antibacterial activity of GIC and RMGIC at 24 hours, 48 hours, and 7 days against S. mutans with GIC showing significantly higher zones of inhibition than RMGIC which is explained as

- The fluoride release rate may be adversely affected by the replacement of part of the water in the cement by resin.[19]
- Acid-base reaction is more extensive in conventional GIC with a defined matrix layer which leads to higher fluoride release and subsequently higher antibacterial activity.[20]

A statistically significant difference was seen between the antibacterial activity of GIC and fluoride-releasing composite at 24 hours with the highest antibacterial activity shown by GIC followed by the fluoride-releasing composite. GICs showed maximum fluoride release and low initial pH in the first 24 hours.[18] At 48 hours and 7 days there was no statistically significant difference in the antibacterial activity of GIC and fluoride-releasing composite. The resin composite used in this study was a novel pH-dependent ion releasing smart composite Activa Bioactive restorative (pulpdent) which is capable of releasing fluoride, calcium, phosphate ions, and of buffering acids by the release of hydroxyl ions when the pH drops thus helping in neutralizing the acid produced by the bacteria and acting as an antibacterial agent.[21] This explains the high antibacterial effect of this smart resin composite which is almost comparable to that of conventional GICs.

Fluoride releasing composite showing significantly higher zones of inhibition than RMGIC against S. mutans at 24 hours, 48 hours and 7 days which can be attributed to lesser total fluoride content seen in the glass ionomer matrix available for fluoride release in resin-modified glass ionomers whereas the higher antibacterial activity of Activa could be attributed to the release of fluoride and hydroxyl ions on-demand to lower pH values due to microbial acid production.[21]

The low initial pH that increases as setting reaction proceeds could explain the taper in the antibacterial activity of specimens with time in Group I, Group II with the highest antibacterial activity seen at 24 hours. For Group III the antibacterial activity decreasing as a function of time was observed in accordance with the study by Boeckh et al (2002)[22] which could be postulated to be a result of the superficial rinsing effect of the fluoride from the smart composite resin material. This being an in vitro study there were no means of fluoride recharge of the dental materials used.

VI. CONCLUSION

Glass ionomer cements showed the highest antibacterial activity against S. mutans followed by the bioactive fluoride-releasing composite and resin-modified glass ionomer cement at 24 hours, 48 hours, and 7 days. The antibacterial efficacy of all the tested restorative materials progressively decreased with time although all of them retained some antibacterial activity even at the end of 7 days. Results obtained from the present in vitro study cannot be directly extended to clinical situations as the oral environment is subjected to dynamic conditions with a diverse microbial challenge, fluoride rechargeability, and oral prophylaxis measures. Hence further research is needed to substantiate the results of the present study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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