Effect of Acid and Fluoride Release from Four Glass Ionomer Cements on Streptococcus mutans: An In Vitro Study

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INTRODUCTION

Atraumatic restorative treatment (ART) has been developed for the treatment of caries in parts of the world with limited resources through the use of dental hand instruments and glass ionomer cement (GIC). However, hand instruments do not remove carious dentin as effectively as rotary burs. Thus, cavities treated by ART may have residual bacteria. Hence, it is highly desirable for GIC to possess antibacterial activity. The antibacterial activity of GICs has been attributed to the longevity of the fluoride release. Recent research has also shown that the antibacterial property of GICs also depends on the acid release from the GIC. However, both fluoride and acid release decrease progressively as the cement ages. Hence, this study was conducted with the following objectives: (i) To determine the fluoride and acid release from GICs over time and (ii) to evaluate and compare the relationship of the fluoride and acid release from the four GICs with their activity against Streptococcus mutans over time.

MATERIALS AND METHODS

An In vitro study was carried out. The study was conducted over a period of 3 months. Four GICs, GIC (Fuji IX GP), Miracle Mix, Glass Ionomer Restorative Cement (d-tech: dtechasia, Pune) and Ketac Molar were used as the study materials. Pure culture of S. mutans was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. Ethical clearance was obtained for Institutional Review Board.

Evaluation of Fluoride and Acid Release

The cements were mixed according to manufacturer’s instructions and placed in molds to create samples measuring 5 mm diameter and 3 mm height. The samples were placed in 50 ml of deionized distilled water and left undisturbed for 24 h. After 24 h, the fluoride content and pH in each water sample were determined. The fluoride content was measured using ion electrode method with total ionic strength adjusted buffer (Orion 9409) and the pH was measured using the digital pH meter. The procedure was repeated daily for the next 15 days.

Evaluation of Activity against S. mutans

The microorganism S. mutans was used. Strains were grown in the brain heart infusion broth and incubated anaerobically for 18 h at 37°C. Strains were then grown and sub-cultured on blood agar. Prepared samples were placed in 50 ml of deionized distilled water and were left undisturbed for 1, 3, 5, 7, 9, and 11 days. The
water was replaced every 24 h. Samples of each of the four GICs, either fresh or aged, were placed on the inoculated agar plates. Inhibition zones were measured after 48 h of incubation at 37°C.

**Statistical Analysis**

Mean and standard deviation of the fluoride release, the pH and the zone of inhibition of the different samples were calculated. Pearson’s correlation coefficient was used for correlating the fluoride and acid release from the four GICs with the change in their activity against *S. mutans* over time. *P* < 0.05 was considered statistically significant. The analysis of data was done by SPSS version 16.0 (Statistical Package Software).

**RESULTS**

The antimicrobial activity of the four GICs was greatest immediately after the cements were mixed. The fluoride release from all the four cements was maximum in the first 24 h after which it reduced drastically to similar levels [Figure 1]. The greatest fluoride release was from d-tech, followed by Miracle Mix, Ketac Molar, and Fuji IX. Similar results were shown by all the samples with respect to the acid release. The greatest acid release was from d-tech followed by Miracle Mix and Fuji IX, and the least was from Ketac Molar [Figure 2]. The antibacterial property of all the test materials decreased as the fluoride release from the materials decreased [Figure 3]. The antibacterial property of the test materials reduced as the acid release from the test materials reduced [Figure 4].

**DISCUSSION**

The agar diffusion test has been widely used to evaluate the antibacterial property of dental materials. In this method, the zones of inhibition provided by the materials depend on the toxicity of the material against the bacteria tested and the diffusibility of the material across the culture medium used. This method allows measurement of the inhibitory activity against bacteria colonizing a surface, which imparts clinical relevance if *in vitro* data are being extrapolated to oral bacteria colonizing around or on restorative materials.[3]

The results of this study show that the antibacterial property of GICs decreased as the acid release from the material decreased. This finding is consistent with a similar study conducted by Fischman and Tinanoff in USA in 1994.[2] Fraga *et al.* conducted a study in Brazil in 1996 to test the antibacterial effects of photocured GICs during setting and found a similar relation between the antibacterial activity and acid release from GICs.[3]

Studies conducted by Marczuk-Kolada *et al.* in Poland in 2006 and Berg *et al.* in USA in 1988 to evaluate the fluoride release from GICs and its effect on the growth of *S. mutans* around the material, show results similar to this study.[6,7]

Studies conducted by Tobias *et al.* in the UK in 1985 and Seppä *et al.* in Norway in 1993 showed that the maximum antibacterial activity was exhibited by freshly mixed samples of restorative materials and there was drastic reduction in the antibacterial activity after day 1. These results confirm the findings of the present study.[6,7]

Yap *et al.* in Singapore in 1999 showed that the amount of fluoride release from GIC decreased drastically after 1-week, which is consistent with the results of the present study.[8] In another study conducted in Japan by Nakajo *et al.*, indicate that GIC fillings in the oral cavity, reduce bacterial acid production, and the subsequent bacterial growth may decrease, creating the cario-static environment.[9]

In a study conducted by Chau *et al.*, results suggest that the anti-cariogenic biofilm activity of GICs is closely correlated with their fluoride release rate during biofilm formation.[10,11]

The antimicrobial activity of the GICs could be related to the synergistic action of acid and fluoride release of the cement.
samples, since the bacterial inhibitory effect of fluoride increases as the pH decreases.

**CONCLUSIONS**

The acid release, fluoride release, and the antibacterial activity were at their peak immediately after mixing the cements. Both the factors, acid and fluoride release that contribute to the antibacterial activity of GICs reduce significantly as the cement ages, thereby reducing its antibacterial activity.

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