Original Article

Adhesion of Streptococcus Mutans to Glass Ionomer, BisCem Cement and Enamel: An in Vitro Study

Ezzatollah Jalalian[1], Ghazal Mofrad[2]✉, Sogol Ahmadpour[3]

[1]Associate Professor, Department of Fix Prosthodontics, Member of Implant Research Center, Dental Branch, Azad University of Tehran, Iran
[2]Dentist, MPH, University of Nevada, LV, School of Community Health Sciences, Nevada, USA
[3]Dentist, Resident of Oral & Maxillofacial Radiology, Department of Oral & Maxillofacial Radiology, Islamic Azad University, Tehran Dental Branch, Tehran, Iran

Abstract

Objectives: Considering the adhesion of some microorganisms such as Streptococcus mutans (S. mutans) to restorative materials and the unrecognized consequences of this phenomenon, and due to the controversies in this regard, it is important to discover the materials to which the lowest adhesion of S. mutans occurs. The objective of this study was to assess the level of adhesion of S. mutans to glass ionomer (GI), BisCem Cement and enamel.

Materials and Methods: In this in vitro experimental study, 12 specimens including five GI blocks (GC America Inc., Alsip, IL, USA), five BisCem blocks (Bisco Inc., Schaumburg, IL, USA) and two enamel blocks were exposed to a bacterial suspension (1×10^6 mg/mL). After incubation for one hour at 37˚C, the swab samples were taken and cultured in blood agar. The S. mutans colonies were counted by unaided vision after 48 hours of incubation. The results were analyzed using ANOVA followed by the Tukey's test.

Results: The number of colonies attributed to enamel, GI, and BisCem blocks was 24±2, 24.2±2.7 and 14.8±1.7 colonies/mm^2, respectively. There was no difference between enamel and GI in terms of adhesion of S. mutans (P=0.08 and P>0.001, respectively); however, the difference between these two and BisCem was statistically significant (P= 0.00075 and P<0.001, respectively).

Conclusion: Within the limitations of this study, BisCem cement is superior to GI for the cementation of indirect restorations.

Keywords: Bacterial Adhesion; Streptococcus Mutans; Glass Ionomer Cements; BisCem Cement

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✉ Corresponding author:
G. Mofrad, MPH, University of Nevada, LV, School of Community Health Sciences, Nevada, USA

mofrad@unlv.nevada.edu

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INTRODUCTION

Bacterial adhesion plays a considerable role in the incidence of tooth deterioration, calculus formation, and gingivitis [1-4]. Streptococcus mutans, which is the dominant microorganism among the populations of bacteria forming the dental plaque contributes the most to the process of development of dental caries and periodontal problems in patients with active caries and is mainly responsible for tooth decay [5-8]. Nowadays, resin cements play a crucial role when applying indirect restorations of all types, especially all kinds of tooth-colored crowns and bridges that are widely employed [9-11]. A strong and stable bond between the luting cement and dentin will contribute to the clinical performance of bonded indirect restorations [12].

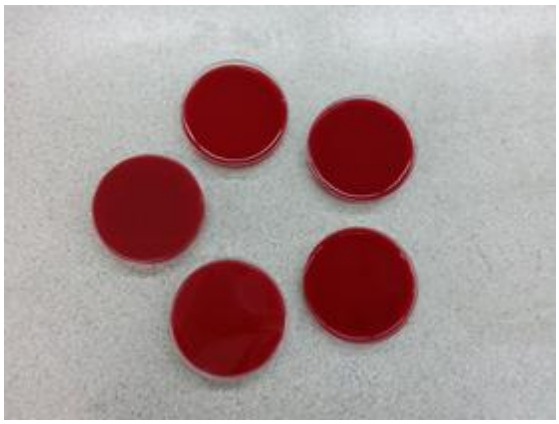

Fig. 1. Blood agar plates prior to culturing

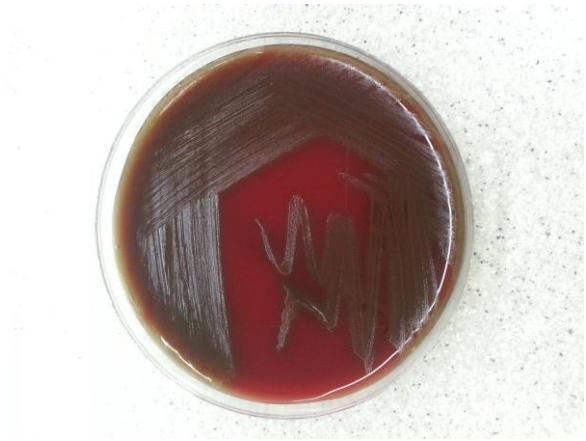

Fig. 2. Bacterial (S. mutans) growth in blood agar culture

Resin cements have been selected for their advantageous mechanical and adhesive properties compared with conventional luting cements [13]. The main advantage of these cements is their simple handling, since they can adhere to the tooth structure without the need for using an adhesive or etchant; thus, by applying them, we will overcome the technique sensitivity associated with the use of multi-step systems [14-16]. The most routine purpose of using these materials is for the cementation of indirect tooth-colored restorations. They provide a bond that reduces microleakage, postoperative sensitivity, marginal staining and recurrent caries, and also reinforces the structures of both the tooth and the restoration [17]. Aside from the effective bond to the tooth structure, these cements have been proven to show good marginal adaptation [18]. Recently, the indications of indirect restorations and other new treatments have increased such as Maryland bridges introduced in the 1980s, and ceramic restorations introduced in the 1990s. Better marginal sealing, decreased postoperative sensitivity, low solubility and superior mechanical properties of resin cements are some of the advantages of indirect restorations [19-21]. However, inadequate polymerization of resin cements will lead to postoperative sensitivity, microleakage, and recurrent caries [22,23], and such restorations will be more prone to degradation, discoloration and decrease in mechanical properties. Due to the distinct nature of dentin and enamel, recent changes in the mentioned characteristics might lead to higher rates of leakage and adhesion of microorganisms. Yet, if the recent types of cements with improved characteristics indicate low rates of leakage as well, applying them for indirect restorations would be preferred. Selecting the appropriate resin cement with low affinity for S. mutans is critical, especially in patients who show high amounts of such colonization by nature. Several studies have been done on different materials applied in restorative dentistry [1,2,4,24] and orthodontics [25]; yet, only a few have assessed the adhesion of microorganisms to cements. In some researches, GI was proven to demonstrate the least bacterial adhesion [26], while some other studies claimed that light-cured cements were better in this regard compared to GI [27-29]. Some investigations stated that light-cured cements as well as self-cured cements showed the least bacterial adhesion [30]. A research in 2004 revealed that although the release of fluoride ions from GI is far more than from other substances, this fact does not have any effects on reducing the adhesion of S. mutans [31]. Another study in

Fig. 3. Bacterial (S. mutans) growth in blood agar culture

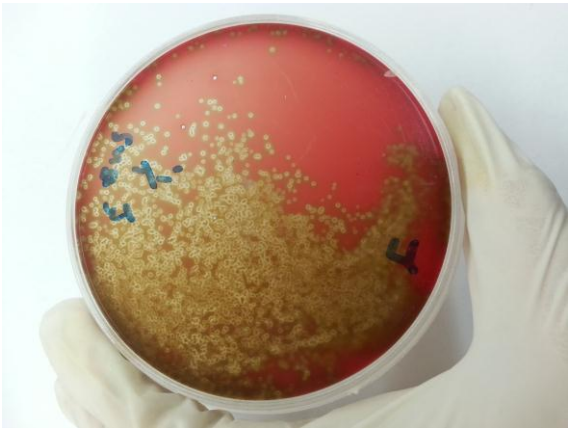

Fig. 4. S. mutans colonies to be counted

1991 also proved that the amount of fluoride released from GI was not high enough to stop the aggregation of S. mutans on the adjacent surfaces [32]. Another research in 2004 proved that the amount of plaque accumulation is in direct relationship with the surface roughness of the restoration; however, there was no relationship between this factor and the number of colonies [33]. In 2010, the effect of various polishing methods on the surface roughness and bacterial adhesion was studied and among all the methods, the diamond rotary catching instrument was proven to create the roughest surface [34].

MATERIALS AND METHODS

This experimental in vitro study was approved by the ethical committee of the institution in which it was performed. Five cylindrical blocks of GI (GC America Inc., Alsip, IL, USA) and five cylindrical blocks of BisCem cement (Bisco Inc., Schaumburg, IL, USA) were prepared with the diameter of 2mm and height of 5mm. All the dimensions were calibrated by a gauge. BisCem is a self-adhesive resin cement that can bond to a variety of substrates, such as metals, composites, porcelain and amalgam. It does not require etching, priming or bonding of the prepared surface [35]. Also, two enamel blocks were made with similar dimensions as the control specimens. They were carved out of two premolars using diamond disks. The premolars were free of any caries, fillings or stains and were extracted due to orthodontic reasons. All specimens were fabricated by the same technician. The number of samples was chosen according to previous researches [36]. All the 12 specimens were rinsed with distilled water and sterilized in an autoclave. Then they were exposed to the bacterial suspension with the standard code of RTCC1683 and a concentration of 1×10^6 mg/mL (0.5 McFarland). The procedure was as follows: 500mL of the bacterial suspension was poured into the test tube and then the specimen was placed inside the tube. Afterwards, 500mL of human whole saliva was added. The saliva was taken from a healthy person who showed no periodontal disease or active caries, was undoubtedly not using any kind of medication during the time of study, and had no history of antibiotic intake or fluoride therapy in the past two weeks, since the composition of normal bacteria of oropharynx and nose can be interrupted by the frequent use of broad-spectrum antibiotics [4,37]. High levels of fluoride could also modify the plaque ecosystem [4]. Saliva samples were taken in the

same morning, and stored at 4˚C. The specimens were incubated along with a control for each group (the control for each group was a block of enamel in 500mL of saline plus 500mL of human whole saliva) at 37˚C for one hour. Then they were all rinsed by immersion in normal saline for 20 seconds, and secondly, each sample was shaken for one minute in a fresh normal saline solution. Then swab samples were taken from each of the specimens of GI, BisCem, and enamel. In order to culture the microorganisms, each swab was streaked across the surface of the blood agar. Also 0.1mL of the final saline solution was cultured in blood agar. At the end, each block was pushed into blood agar in order to ensure it was contaminated. All culture plates were then incubated at 37˚C for 48 hours. The colonies were counted by unaided vision (Figs. 1-4), and the results were analyzed using ANOVA followed by the Tukey's test. P-values of less than 0.001 were considered significant [7,15,23-41].

RESULTS

Five GI blocks (GC America Inc.) and 5 BisCem blocks (Bisco Inc.) were exposed to bacterial suspension of 1×10^6 mg/mL in two groups; one enamel block was considered as the control in each group. The mean adhesion levels of bacteria to enamel, GI, and BisCem blocks were 24± 2, 24.2±2.7, and 14.8±1.7 colonies/mm2, respectively (Table 1).

The post hoc Tukey's test revealed that there was no significant difference in terms of the level of adhesion between GI and enamel blocks (P=0.08); however, the difference between these two and BisCem was statistically significant (P= 0.00075). Hence, the P-value achieved from comparing the three groups of this study was 0.00075. The level of homogeneity of adhesion was equal in both groups of cements, and the enamel specimens showed the highest level of homogeneity.

DISCUSSION

Microleakage, which is the penetration of saliva and microorganisms through the tooth-restoration interface is the major concern when applying cemented restorations due to the fact that it can cause secondary caries, pulp irritation and post-operative sensitivity [15]. Resin cements play a major role in the success or failure of indirect restorations [15]. Continuous cement margins without any gaps are important for the durability of the restoration; however, no luting agent can provide a perfect marginal seal [38]. Therefore, the adhesion of S. mutans to resin cements plays a crucial role in the occurrence of enamel decay, formation of calculus, gingivitis and pulp irritation [1,2,9,10]. Resin cements are applied widely today for various types of crowns and bridges [9,10]. The current study was done considering the increasing application of resin cements, and due to the lack of knowledge about the level of adhesion of S. mutans to these materials. The objective of this study was to assess the level of adhesion of S. mutans to GI, BisCem cement, and enamel. The ANOVA revealed that the higher levels of adhesion observed in specimens of enamel and GI were statistically significant compared to those of BisCem (P<0.001). Also, ANOVA

Table1. The Adhesion level of S. mutans based on the type of material (Enamel, GI, BisCem)

Type of Material	Minimum Adhesion Level	Maximum Adhesion Level	Mean Adhesion Level (colonies/mm^2)	CV
Enamel	23	25	24±2	8
GI	21.1	26.3	24.2±2.7	11
BiCem Cement	13.3	17.2	14.8±1.7	11

CV: Coefficient of variation

proved that the levels of bacterial adhesion were variable in the three groups. The minimum level was found in BisCem and the maximum in GI. Therefore, when all the factors affecting bacterial adhesion are similar, such as marginal integrity, emergence profile, gingival contour and the finishing line, lower levels of bacterial affinity will lead to healthier gingival and periodontal tissues in long-term. In patients with poor oral hygiene, since bacterial adhesion to GI is higher than to BisCem and enamel, inflammatory reactions of gingival and periodontal tissues are more ubiquitous. In the study by Montanaro et al, in 2004, in which the adhesion levels of S. mutans to different restorative materials [three flowable composites: Filtek Flow, Tetric Flow, and Arabesk Flow, three microhybrid composites: Clearfil APX, Solitaire 2, and Z250, two glass-ionomers: Fuji IX, Fuji IX fast, a compomer (F2000), an ormocer (Admira), and surface treated polystyrene as the control reference] were studied, it was proven that the level of adhesion to Admira ormocer and the Fuji IX fast glass ionomer was more considerable than the rest of the specimens, although Admira ormocer and the Fuji IX fast GI released more fluoride ions compared to other substances [31]. These results were similar to the results of the current study with regard to considerable adhesion to GI. This agreement between our results and those of previous studies indicates correct methodology and control of confounding parameters namely exposure to bacteria, the washing, and the two incubation steps. Eick et al, also studied the bacterial adhesion of S. mutans to Ariston, Tetric, Dyract, Compoglass, Vitremer, Aqua Ionofil, Ketac Fil, amalgam, Galloy, and ceramics as controls, and the surface roughness of each material as well. They concluded that the adhesion level to glass ionomers was higher, and the ions released from GI did not effectively inhibit the adhesion of S. mutans [33]. These results regarding higher affinity of bacteria to GI is in accordance with our findings, which can be attributed to the comparable basic methodology of the two studies with regard to the specimens, saliva, and the bacterial suspension. Ahn et al, in 2010 reported that S. mutans adhesion to GI was significantly more than to three non-fluoride-releasing composites, one fluoride-releasing composite, and one polyacid-modified composite (compomer), but there was no significant difference among the composites [39]. Each material was incubated with whole saliva and cariogenic streptococci in their study and their findings also attest our results. Hassan studied five luting cements namely Panavia Ex, Marycol, Superbond, poly-carboxylate and GI [40]. The maximum adhesion was found in poly-carboxylate and not in GI. This controversy might be due to the measuring instruments, or the different types of resin cements tested. Klai et al. compared the bacterial adhesion to two glass ionomer luting cements namely GC Fuji I and 3M ESPE Ketac Cem Easymix, and three experimental GI cements called A, B, and C. They also used enamel as the reference and concluded that the GI specimens A, B, and C showed an adhesion level close to that of the enamel [41]. The findings of their study were in agreement with ours. They also used human saliva and incubated specimens at 37℃ as we did. There can be an explanation about the close levels of S. mutans adhesion to GI and enamel according to the study by Ahn et al, [39] in 2007; the differences in surface characteristics of various orthodontic materials might provide valuable information on bacterial adhesion to them. Among the materials tested, bovine enamel, and GI showed high bacterial affinity, which was attributed to the surface roughness of the former, and the high surface free energy of the latter [42]. No study has been done regarding the adhesion of S. mutans to BisCem cement; however, the amount of microleakage associated with this cement has been assessed. Nemati-Anaraki et al. conducted a study in 2015 to compare the effect of Panavia F2,

Biscem and Maxcem on the microleakage of ceramic inlays, and concluded that gingival microleakage of BisCem was less than that of Maxcem [15]. This might also provide evidence that less bacterial adhesion occurs at the margins of BisCem compared to Maxcem.

The weakness of the current study was its in vitro design, which could not ideally simulate what happens in vivo, since some parameters appear to be different in the oral cavity. However, the results gained from an in vitro study can be applied to clinical situations with caution. Its strong point was that the colonies were studied quantitatively, and the enamel blocks were used as the control group. Also, storage of the specimens in human saliva helped simulate in vivo setting.

CONCLUSION

1.Enamel and GI showed similar results in terms of S. mutans adhesion (P=0.08).

2. Within the limitations of this study, it is preferred to use BisCem cement rather than GI for cementing indirect restorations when all the factors affecting bacterial adhesion are similar, since the level of adhesion of S. mutans to BisCem is less than to GI. By applying a luting cement with less bacterial affinity, the durability of restorations would increase.

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