Effect of Addition of Bioactive Glass to Glass Ionomer Cement on Accumulation of Streptococcus mutans and Lactobacilli around Orthodontic Bands

Maryam Shirazi¹, Fatemeh Fotoohi-Ghazvini², Keivan Khazaei³, Mozhgan Izadi³

¹Department of Orthodontics, Qazvin University of Medical Sciences, Qazvin, Iran, ²Department of Microbiology, Qazvin University of Medical Sciences, Qazvin, Iran, ³Department of Periodontics, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Bioactive glass (BAG) is used as an antibacterial agent. This study aimed to assess the effect of addition of BAG to glass ionomer cement (GIC) on accumulation of Streptococcus mutans (S. mutans) and Lactobacilli around orthodontic bands. Material and Method: This clinical trial was conducted on 10 patients requiring orthodontic treatment with lingual arch and molar bands. In five patients, Fuji II SC GIC was used for cementation of the right and Fuji II SC containing 30wt% BAG was used for cementation of the left molar band. In the remaining five patients, Fuji II LC was used for cementation of the left and Fuji II SC containing 30wt% BAG was used for cementation of the right molar band. Formation of S. mutans and Lactobacillus colonies in the culture medium was assessed and number of colonies was counted using a colony counter. The data were analyzed by using paired t-test. Results: The mean number of total bacteria, aerobic Lactobacilli, anaerobic Lactobacilli and S. mutans was significantly different between the two cements with and without BAG such that the mean count of total bacteria, aerobic Lactobacilli, anaerobic Lactobacilli and S. mutans in Fuji II SC plus BAG was less than that in plain Fuji II SC cement. Conclusion: Addition of BAG to GIC decreases the accumulation of S. mutans and anaerobic and aerobic Lactobacilli around orthodontic bands.

Key Message: The used cement can be effective in reducing the damaging effect of orthodontics' bands.

Key Words: Bioactive glass, Orthodontic cement, Orthodontic brackets, Streptococcus mutans, Lactobacillus

Introduction

Dental caries and periodontal disease are among the most important chronic diseases worldwide [1]. Dental caries is a microbial infectious disease of the teeth, which is characterized by dissolution and destruction of tooth structure by the acidogenic bacteria that create an acidic environment and cause demineralization of tooth structure [2]. Dental caries and periodontal disease are the result of imbalance in microbial flora of the mouth, which can lead to emergence of potentially pathogenic bacteria [3]. Environmental and personal factors such as nutrition, use of fluoride, oral hygiene maintenance, saliva flow and immunologic factors can affect the oral micro flora and development of caries [4]. Orthodontic treatment is among the factors that can change the balance of oral micro flora. Also, risk of dental caries, gingivitis and periodontitis increases following the use of orthodontic appliances [5]. These changes result in an increase in level of microorganisms especially Streptococcus mutans (S. mutans) in the saliva and dental biofilm [6].

Over 600 bacterial species are present in the oral cavity which result in formation of oral microbial plaque [7]. It has been reported that risk of dental caries increases as the result of increase in cariogenic microflora and bacterial plaque [8]. Cariogenic bacteria such as S. mutans and Lactobacilli and bacteria related to periodontitis such as Eubacterium, Fusobacterium and Treponema have been isolated from the orthodontic dental plaque using polymerase chain reaction (PCR) based on their DNA characteristics as well as other molecular techniques [9-11].

Glass ionomer cements (GICs) are composed of acid soluble calcium-fluoroaluminosilicate glass and polyacrylic acid with high viscosity, and are used as the liner, base or cement. Resin modified glass ionomers are stronger than conventional glass ionomers, better adhere to tooth structure and are more resistant to dissolution and disintegration [12]. The main advantage of GICs is their fluoride release potential for a long period of time and their optimal biocompatibility [13]. Evidence shows that the fluoride release potential of GICs is sufficient for protection of teeth against caries and exerts anti-bacterial effects as well [14].

Bioactive glass (BAG) is an implant constituent of implants introduced in 1971. It has optimal biocompatibility and excellent biological properties in bone and soft tissue. Recently, BAG was suggested for use in areas at high risk of microbial infections. It has several types with different molecular compositions. In this study, 45S5 BAG with the following composition was used: 46.1 mol% SiO₂, 26.9 mol% CaO, 24.4 mol% Na₂O and 2.5 mol% P₂O₅. It has wide-spectrum antibacterial activity against supragingival and sub gingival bacteria. For this reason, antibacterial activity of BAG has been the topic of many investigations in dental materials [15-19]. The role of type of cement used in orthodontic treatment in colonization and decolonization of bacteria has yet to be fully understood. Therefore, this study aimed to assess the effect of addition of BAG to GIC on the count of S. mutans and Lactobacilli around orthodontic bands.

Li et al., in 2003 evaluated the antimicrobial effects of GC Fuji II and Fuji II LC GICs mixed with 0wt%, 10wt%, and 30wt% BAG powder on S. mutans and Candida albicans (C. albicans) count in vitro. They evaluated growth inhibition on agar medium. The effect of materials was also evaluated in aqueous environment. The effect of extract of materials on acidic products was evaluated using cell suspensions. Antimicrobial activity of materials was evaluated by incubation of cell suspensions with the powder. In the agar

Corresponding author: Mozhgan Izadi, Assistant Professor, Dental Material Research Center, Department of Periodontics, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran, Tel:00989131012493; E-mail: mozhgan.izadi.1165@gmail.com
medium, only GIC containing 30wt% BAG inhibited the growth and proliferation of S. mutans. In the culture medium, the products had no inhibitory effect on S. mutans. Only GIC without BAG inhibited acid production by S. mutans. The tested materials had insignificant effect on C. albicans. Only BAG was effective against C. albicans when incubated with C. albicans in suspension [20]. Huang et al., in 2012 evaluated the antimicrobial activity and physicochemical properties of GIC and resin modified GIC combined with chlorhexidine and BAG. The results showed that the group with 1% chlorhexidine caused a significant reduction in optical density of bacterial suspension; consequently, destruction of S. mutans biofilm was enhanced. However, the group containing 10% BAG had no significant effect on optical density and biofilm formation. Mechanical properties of materials and their polymerization were not affected by the addition of chlorhexidine. However, compressive strength of materials was low following the use of BAG. They concluded that GIC plus chlorhexidine can not only preserve the mechanical properties of the materials, but it can also decrease biofilm formation by S. mutans. Also, controlled release/sustained release technology is required to control biofilm formation by S. mutans following the use of GIC plus BAG [21].

White spot lesions (WSLs) are seen in about 50% of orthodontic patients. Long-term antibacterial properties of orthodontic cements can decrease the occurrence of WSLs. Dastjerdie et al., in 2012 compared the antimicrobial activity of three commercially available GICs namely Resilience, Band-Tite and Ariadent with three commercially available zing phosphate cements namely Harvard, Hoffman’s and Aridant for a specific period of time against C. albicans and S. mutans. They performed direct contact test to assess the antibacterial and antifungal properties of cements after two and seven days of incubation. The results showed that all cements had antibacterial activity but the antimicrobial activity of GIC was higher than that of zine phosphate cement. The C. albicans count after two days in GIC group was significantly less than that in the control group but no significant difference was found at seven days between GIC and control group. They showed that antimicrobial and antifungal effects of cements were not long-term [22].

Komori et al., in 2012 determined the composition of supragingival bacterial plaque and its acidogenic potential in molar teeth of patients with orthodontic band, bracket or without them. The plaque sample obtained from the surface of mandibular and maxillary first molars with orthodontic band or bracket or without them was anaerobically cultured on blood agar in six patients (age 11-30 years). The isolated bacteria were identified using 16SrRNA gene sequence. The acidogenic potential of isolated bacteria was determined using anaerobic agar plate containing bromocresol. The bacterial count was 6.6 ± 6.5, 6.9 ± 7.1 and 7.4 ± 7.6 log CFUs/mg in molar teeth with bands, brackets or without them (control), respectively. Actinomycyes (43.5% and 40%) and Streptococcus (23.5% and 34.7%) were the most common bacterial species isolated from the orthodontic bracket groups and the control group. In contrast, Streptococcus count (44.4%) was significantly higher than that of Actinomycyes (17.6%) in molar teeth with bands (P<0.01). The percentage of acidogenic bacteria in the plaque of molar teeth with band, bracket and control teeth was 74.5%, 71.3% and 81.6%, respectively; although this difference was not statistically significant. Their results showed that differences exist in microbial composition and acidogenic potential of supragingival plaque of molar teeth with band, bracket or none of them and showed that supragingival plaque of teeth with bracket was mainly composed of bacteria related to periodontitis rather than bacteria related to dental caries [22].

Sargolzaee et al., in 2014 evaluated changes in subgingival microbial plaque in fixed orthodontic patients using real time PCR and concluded that treatment with fixed orthodontic appliances may temporarily increase the growth and proliferation of periodontal pathogenic bacteria and cause inflammation of the gingiva but has no destructive effect on periodontal tissue [23].

Materials and Methods

This split mouth clinical trial was conducted on 10 patients who were randomly selected. This study approved by ethical committee of Qazvin University of Medical Sciences with ethical number of IR.QUMS.REC.1395.300. There is no conflict with ethical considerations.

The inclusion criteria were patient consent for participation in this study, good oral hygiene, absence of active caries or periodontal disease, no mouth breathing, absence of cleft lip or palate, absence of conditions affecting oral microbial flora, absence of conditions affecting tooth brushing and no use of chlorhexidine mouthwash or antibiotics within three weeks prior to this study or during the course of study. Wash out of cement and loosening of appliances on molar teeth were also among our exclusion criteria.

Patients were first clinically examined for dental caries and periodontal disease using a dental explorer and a periodontal probe. Patients who met our inclusion criteria were thoroughly informed about the study and signed informed consent forms. In the first treatment session, patients were thoroughly examined for presence of supragingival calculus and absence of periodontitis using a dental mirror and a Williams periodontal probe. In case of presence of supragingival calculus, it was removed by an ultrasonic scaler and all patients underwent prophylaxis. After oral hygiene instruction, they were requested to brush their teeth twice a day for three days and then come back for the second session. Orthodontic bands were autoclave-sterilized prior to use. According to the manufacturer’s instructions, one spoon of powder was mixed with two drops of liquid in 3.2 to 1 weight ratio. The powder was divided into two portions and each portion was mixed with the liquid for 10 seconds until a glossy consistency was obtained.

In half the patients, the molar band in the right side was cemented using Fuji II SC (GC Crop. Tokyo, Japan) and the molar band in the left side was cemented using Fuji II SC and 30wt% BAG. In the remaining patients, the molar band in the left side was cemented using Fuji II SC and the molar band in the right side was cemented using Fuji II SC and 30wt% BAG [20].
The patients were examined four months after placement of lingual arch on the first molar teeth. The four-month time was selected to allow adequate proliferation of bacteria without wash out of cement or loosening of bands. The area was isolated using cotton rolls. The visible dental plaque was removed from the buccal surface of teeth and a sample was collected from the subgingival plaque using a sterile paper point for 15 seconds [23]. The samples were placed in 4 mL of Stewart’s transport medium. Ten-fold dilutions of the samples to $10^{-6}$ were prepared. To count the total number of bacteria, 0.1 mL of each dilution was incubated in blood agar. To determine the suitable culture medium, a pilot study was performed. After ensuring that the culture medium was suitable, the samples were cultured in the media to assess the presence of \textit{S. mutans} and \textit{Lactobacilli}. For identification and counting of \textit{S. mutans} colonies, each dilution was transferred to Mitis Salivarius agar including 0.001% tellurite solution, 15% sucrose and 0.2 U/mL bacitracin and incubated for 48 hours at 37°C under anaerobic conditions. The suspected colonies (based on their macroscopic characteristics, large and white) were biochemically tested (microscopic assessment by Gram staining, motility test, catalase test and glucose test). After their identification, number of the colony forming units (CFUs) per milliliter was determined using the formula below:

$$\text{CFUs} = \text{number of counted colonies} \times \text{inverse of the dilution}$$

The dilution factor is inverse of the dilution of culture medium for bacterial counting. Colonies were counted using a colony counter [24]. It should be noted that the examiner only placed the lingual arch and was not aware of the type of cement used.

The mean, standard deviation and percentage were calculated for descriptive findings. Paired t-test was used to compare Fuji II SC with and without 30wt% BAG. Data were analyzed using SPSS version 21.

### Results

The mean total bacterial count was $3.08 \times 10^4 \pm 1.34 \times 10^4$ in Fuji II SC plus BAG and $16.95 \times 10^4 \pm 10.02 \times 10^4$ in Fuji II SC group.

The mean count of anaerobic \textit{Lactobacilli} was $1.59 \times 10^4 \pm 1.5 \times 10^4$ in Fuji II SC plus BAG and $5.07 \times 10^4 \pm 3.82 \times 10^4$ in Fuji II SC group.

The mean count of aerobic \textit{Lactobacilli} was $0.475 \times 10^4 \pm 0.624 \times 10^4$ in Fuji II SC plus BAG and $1.08 \times 10^4 \pm 1.11 \times 10^4$ in Fuji II SC group.

The mean count of \textit{S. mutans} was $0.401 \times 10^4 \pm 0.56 \times 10^4$ in Fuji II SC plus BAG and $2.25 \times 10^4 \pm 1.58 \times 10^4$ in Fuji II SC group (Table 1).

### Table 1. Bacterial colony count in the two groups of Fuji II SC cements with and without BAG.

<table>
<thead>
<tr>
<th>Cement type</th>
<th>Total bacteria</th>
<th>Anaerobic lactobacillus</th>
<th>Aerobic lactobacillus</th>
<th>\textit{S. mutans}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuji II SC + BAG</td>
<td>Mean: 3.08</td>
<td>1.59</td>
<td>0.475</td>
<td>0.401</td>
</tr>
<tr>
<td></td>
<td>Standard deviation: 1.36</td>
<td>1.5</td>
<td>0.624</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Minimum: 1.56</td>
<td>0.0001</td>
<td>0.002</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Maximum: 5.55</td>
<td>3.52</td>
<td>1.4</td>
<td>1.25</td>
</tr>
<tr>
<td>Fuji II SC</td>
<td>Mean: 16.95</td>
<td>5.078</td>
<td>1.08</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>Standard deviation: 10.02</td>
<td>3.82</td>
<td>1.11</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>Minimum: 8.96</td>
<td>1.36</td>
<td>0.092</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Maximum: 35.63</td>
<td>11.26</td>
<td>2.9</td>
<td>4.26</td>
</tr>
</tbody>
</table>

The results of comparison of the mean count of total bacteria, aerobic \textit{Lactobacilli}, anaerobic \textit{Lactobacilli} and \textit{S. mutans} between the two groups of cements by paired t-test are shown in Tables 2-5. The mean count of total bacteria, aerobic \textit{Lactobacilli}, anaerobic \textit{Lactobacilli} and \textit{S. mutans} was significantly different between the two cement groups (P<0.05). The mean count of total bacteria, aerobic \textit{Lactobacilli}, anaerobic \textit{Lactobacilli} and \textit{S. mutans} was significantly less in Fuji II LC plus BAG group compared to the Fuji II SC cement group.

### Table 2. Comparison of the mean total bacterial count in the two cement groups.

<table>
<thead>
<tr>
<th>Cement type</th>
<th>Mean</th>
<th>T</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuji II SC + BAG</td>
<td>$3.08 \times 10^4$</td>
<td>3.3</td>
<td>0.022</td>
</tr>
<tr>
<td>Fuji II SC</td>
<td>$16.95 \times 10^4$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Comparison of the mean number of anaerobic \textit{Lactobacillus} in the two resin cement groups.

<table>
<thead>
<tr>
<th>Cement type</th>
<th>Mean</th>
<th>T</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuji II SC + BAG</td>
<td>$1.59 \times 10^4$</td>
<td>3.1</td>
<td>0.026</td>
</tr>
<tr>
<td>Fuji II SC</td>
<td>$5.078 \times 10^4$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Comparison of the mean number of aerobic \textit{Lactobacillus} in the two resin cement groups.

<table>
<thead>
<tr>
<th>Cement type</th>
<th>Mean</th>
<th>T</th>
<th>P-value</th>
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<tbody>
<tr>
<td></td>
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<td></td>
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</tbody>
</table>
Orthodontic appliances cause accumulation of biofilm on tooth surfaces and parts of the appliance (such as retainers, clasps, springs and bands). This results in enamel demineralization and gingivitis inflammation. BAG added to orthodontic cement undergoes reactions in aqueous environment and subsequently some changes occur in its chemical composition, causing its antibacterial activity and prevention of gingivitis and periodontitis [15,24]. This study assessed the effect of addition of BAG to GIC on accumulation of \textit{S. mutans} and aerobic and anaerobic Lactobacilli around orthodontic bands. Since this study had an in vivo design, its results are highly important. In this study, the mean total bacterial count was \(3.08 \times 10^4\) and \(1.08 \times 10^4\) for Fuji II SC plus BAG and 1.59 \(\times 10^4\) and 1.5 \(\times 10^4\) in Fuji II SC group. The mean count of \textit{S. mutans} was 0.401 \(\times 10^4\) \pm 0.56 \(\times 10^4\) in Fuji II SC group. The mean number of total bacteria, aerobic Lactobacilli, anaerobic Lactobacilli and \textit{S. mutans} was significantly different between the two cement groups. The mean count of total bacteria, aerobic Lactobacilli, anaerobic Lactobacilli and \textit{S. mutans} count was significantly less in Fuji II LC plus BAG group compared to the Fuji II SC cement group. Our results were in agreement with those of Huang et al. [21]. They evaluated the antimicrobial activity and physicochemical properties of GIC and resin modified GIC in combination with chlorhexidine and BAG and showed that use of GIC along with chlorhexidine and BAG prevented biofilm formation by \textit{S. mutans} and maintained physical properties of GIC. Zayed et al. [25] reported the same results as ours. They evaluated the antibacterial potential of GIC against colony formation by \textit{S. mutans} and reported that although Biodentine showed the strongest antibacterial effect, bioactive GIC and calcium hydroxide had a strong inhibitory effect on \textit{S. mutans} and subsequently prevented dental caries.

Stoor et al. [24] were the first to report the inhibitory effect of BAG on gingivitis and periodontitis. They showed that exposure to 40% BAG for 10 minutes caused three-fold reduction in count of Actinobacillus actinomycetemcomitans and after 60 minutes it completely inhibited the growth of this microorganism. The same results were obtained for Porphyromonas gingivalis. Exposure of Actinomyces naeslundii to BAG for 10 minutes resulted in deactivation of bacteria as seen for \textit{S. mutans}. Allan et al. (CCVI) reported that use of 300-500 mm of BAG for one hour resulted in deactivation and killing of \textit{S. mutans}, \textit{S. sanguinis}, \textit{P. gingivalis}, \textit{Fusobacterium nucleatum}, \textit{Prevotella intermedia} and \textit{A. actinomycetemcomitans}. The percentage of elimination of bacteria ranged from 51% for \textit{S. mutans} to 100% for \textit{Prevotella intermedia}.

Allan et al. [15] also evaluated the effect of BAG on a combination of different bacterial biofilms isolated from the human saliva and found that the biofilm was significantly deactivated. They also found that toothpastes containing BAG particles (Novamin) decreased the percentage of viable planktonic bacteria, \textit{S. mutans}, \textit{A. Naeslundii}, \textit{F. nucleatum} and \textit{S. sanguinis}. Exposure to a tooth paste containing 3% and 10% BAG for two minutes caused 4.5-fold reduction in activity of \textit{F. nucleatum} while it completely inhibited \textit{S. sanguinis}. Eberhard et al. [26] showed that topical application of SS45 BAG in humans with gingivitis improved the clinical signs and symptoms of inflammation. Tai et al. [27] indicated that Novamin toothpaste containing BAG significantly improved oral health and decreased gingival bleeding and supragingival plaque compared to a toothpaste without BAG during the six-week course of treatment. In aqueous environment, BAG particles exchange their sodium ions with \(\text{H}^+\) or \(\text{H}_2\text{O}^+\) in the environment, which results in faster release of \textit{Fusobacterium nucleatum} and \textit{A. actinomycetemcomitans}. The percentage of elimination of \textit{S. sanguinis} and \textit{P. intermedia} was 100% for 100% BAG, 90% for 50% BAG and 80% for 25% BAG. This study evaluated the effect of combination of different bacterial biofilms isolated from the human saliva and found that the biofilm was significantly deactivated. They also found that toothpastes containing BAG particles (Novamin) decreased the percentage of viable planktonic bacteria, \textit{S. mutans}, \textit{A. Naeslundii}, \textit{F. nucleatum} and \textit{S. sanguinis}. Exposure to a tooth paste containing 3% and 10% BAG for two minutes caused 4.5-fold reduction in activity of \textit{F. nucleatum} while it completely inhibited \textit{S. sanguinis}. Eberhard et al. [26] showed that topical application of SS45 BAG in humans with gingivitis improved the clinical signs and symptoms of inflammation. Tai et al. [27] indicated that Novamin toothpaste containing BAG significantly improved oral health and decreased gingival bleeding and supragingival plaque compared to a toothpaste without BAG during the six-week course of treatment. In aqueous environment, BAG particles exchange their sodium ions with \(\text{H}^+\) or \(\text{H}_2\text{O}^+\) in the environment, which results in faster release of \textit{Fusobacterium nucleatum} and \textit{A. actinomycetemcomitans}. The percentage of elimination of \textit{S. sanguinis} and \textit{P. intermedia} was 100% for 100% BAG, 90% for 50% BAG and 80% for 25% BAG. This study evaluated the effect of combination of different bacterial biofilms isolated from the human saliva and found that the biofilm was significantly deactivated. They also found that toothpastes containing BAG particles (Novamin) decreased the percentage of viable planktonic bacteria, \textit{S. mutans}, \textit{A. Naeslundii}, \textit{F. nucleatum} and \textit{S. sanguinis}. Exposure to a tooth paste containing 3% and 10% BAG for two minutes caused 4.5-fold reduction in activity of \textit{F. nucleatum} while it completely inhibited \textit{S. sanguinis}. Eberhard et al. [26] showed that topical application of SS45 BAG in humans with gingivitis improved the clinical signs and symptoms of inflammation. Tai et al. [27] indicated that Novamin toothpaste containing BAG significantly improved oral health and decreased gingival bleeding and supragingival plaque compared to a toothpaste without BAG during the six-week course of treatment. In aqueous environment, BAG particles exchange their sodium ions with \(\text{H}^+\) or \(\text{H}_2\text{O}^+\) in the environment, which results in faster release of \textit{Fusobacterium nucleatum} and \textit{A. actinomycetemcomitans}.


