Buffering capacity and antibacterial properties among bioactive glass-containing orthodontic adhesives

Tanawan WANTWISUTCHAIB, Naruporn MONMATURAPOJ2, Ratchatip SRISATJALUK3, Kittitad SUBANNAJUI4, Surachai DECHKUNAKORN1, Niwat ANUWONGNUKROH1 and Pong PONGPRUEKSA5

1 Department of Orthodontics, Faculty of Dentistry, Mahidol University, 6 Yothi, Ratchathewi, Bangkok 10400, Thailand
2 Assistive Technology and Medical Devices Research Center, National Science and Technology Development Agency, 111 Phahonyothin, Klong Luang, Pathum Thani 12120, Thailand
3 Department of Oral Microbiology, Faculty of Dentistry, Mahidol University, 6 Yothi, Ratchathewi, Bangkok 10400, Thailand
4 Material Science and Engineering Program, Multi-Disciplinary Unit, Faculty of Science, Mahidol University, Phuttamonthon 4, Salaya, Nakhon Pathom 73170, Thailand
5 Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, 6 Yothi, Ratchathewi, Bangkok 10400, Thailand

Corresponding author, Pong PONGPRUEKSA; E-mail: pong.pon@mahidol.edu

This study was to evaluate the acid-buffering capacity and antibacterial properties of orthodontic adhesives containing bioactive glasses (BAGs) (45S5, 45S5F, S53P4), Hydroxyapatite, beta-tricalcium phosphate, and Canasite. Fillers comprising 15 wt% bioactive glasses, HAp, β-TCP, and Canasite incorporated with 55 wt% silanated glass were added to a mixture of UDMA/TEGDMA. Acid-buffering capacity was tested by exposing disc-shaped samples of each adhesive to medium of bacteria-produced acids, and pH changes were recorded at 24 and 48 h. Antibacterial properties were assessed by indirect testing by exposing polymerized adhesive samples to a medium and direct testing by immersing the specimens in solutions containing S. mutans and S. sanguinis. A significant buffering capacity was shown by the 45S5, 45S5F and S53P4 BAG adhesives. The antibacterial properties were not significant in all experimental adhesives. Therefore, the experimental orthodontic adhesives containing BAGs demonstrated a significant buffering capacity but did not show significant antibacterial properties against S. mutans and S. sanguinis.

Keywords: Bioactive glasses, Buffering Capacity, 45S5, 45S5F, S53P4

INTRODUCTION

Enamel decalcification or white spot lesions due to orthodontic treatment has been attributed to the irregular surfaces of brackets, bands, wires and other attachments that provide areas for bacterial and plaque accumulation1. The presence of these small and complex appliances makes tooth brushing and cleaning more challenging, where if done improperly, will promote the accumulation of cariogenic bacteria which produce acids, resulting in demineralization of tooth structure2. The incidence of decalcification is reported to be around 50% in orthodontic patients3. Human saliva can induce demineralization and remineralization due to its natural self-cleaning action. Besides saliva, the oral musculature and tongue contribute towards the remineralization process and neutralization of acids in the oral cavity4. To address the issue of enamel demineralization from orthodontic treatment, fluoride-releasing orthodontic adhesives such as glass-ionomer cements (GICs) and resin-modified glass-ionomer cements (RMGICs) were developed5. However, though these materials confer the advantage of fluoride release, their bond strengths are substantially lower compared to the conventional resin adhesives6,7.

The use of bioactive glasses (BAGs) in dentistry has been increasingly developed. BAGs have been used for over 40 years for its excellent biocompatibility as bone substitutes in orthopaedics and now in dentistry8,9. Composite adhesives incorporated with BAGs have shown biomimetic properties, where BAGs which come into contact with bodily fluids result in the formation of tooth-like hydroxyapatite. BAGs release ions that interact with each other in the surrounding solution to produce a supersaturated solution and calcium phosphate precipitate. This phenomenon has the potential for the remineralization of demineralized enamel10,11. Moreover, BAGs in aqueous solution increase pH levels to prevent demineralization12,13. Antibacterial properties of BAGs have also been reported14,15, but seem to be strongly correlated to the pH of the supernatant16 and high silicon ion levels17.

Therefore, the purpose of this study is to evaluate the buffering capacity and antibacterial properties of BAGs incorporated into experimental orthodontic resin adhesives compared to other calcium phosphate compositions and a commercial product. These properties are of interest as they may prevent initial enamel demineralization as well as reduce the bacterial load around orthodontic appliances.

MATERIALS AND METHODS

Materials preparation

Experimental orthodontic adhesives containing 15 wt% each of Hydroxyapatite (HAp; Ca10(OH)2(PO4)6)2, beta-tricalcium phosphate (β-TCP; β-Ca3(PO4)2), Canasite (CaNa4K3Si12O36(OHF)4; 62.7%SiO2, 17.8%CaO, 5Na4K2Si12O30) glasses, HAp, β-TCP, and Canasite. Fillers comprising 15 wt% bioactive glasses, HAp, β-TCP, and Canasite incorporated with 55 wt% silanated glass were added to a mixture of UDMA/TEGDMA. Acid-buffering capacity was tested by exposing disc-shaped samples of each adhesive to medium of bacteria-produced acids, and pH changes were recorded at 24 and 48 h. Antibacterial properties were assessed by indirect testing by exposing polymerized adhesive samples to a medium and direct testing by immersing the specimens in solutions containing S. mutans and S. sanguinis. A significant buffering capacity was shown by the 45S5, 45S5F and S53P4 BAG adhesives. The antibacterial properties were not significant in all experimental adhesives. Therefore, the experimental orthodontic adhesives containing BAGs demonstrated a significant buffering capacity but did not show significant antibacterial properties against S. mutans and S. sanguinis.
8.4%CaF₂, 5.2%K₂O, 3.9%Na₂O, 2.1%P₂O₅), 45S₅ (45%SiO₂, 6%P₂O₅, 24.5%Na₂O, 24.5%CaO), 45S₅F (45%SiO₂, 6%P₂O₅, 24.5%Na₂O, 7.5%CaO, 12.25%CaF₂), and S₅₃P₄ (53%SiO₂, 4%P₂O₅, 23Na₂O, 20%CaO), were fabricated by the Biomedical Engineering Research Unit, National Metal and Materials Technology Center, Thailand. For the glass samples, the required proportions of the raw materials were mixed together, melted in a covered Pt-10%Rh crucible at 1,450°C for 2 h and then quenched in cold water to produce frit. Fine glass powder was produced by ball milling with a zirconia ball as the grinding media to achieve an average particle size (D₀.₅) of 5.81 µm upon being analyzed by laser diffraction technique (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). These additive fillers were combined with 55 wt% of 0.7 µm silanized glass filler (Esstech, Essington, PA, USA). A resin matrix was prepared with a mixture of 80 wt% urethane dimethacrylate (UDMA), 20 wt% triethylene glycol dimethacrylate (TEGDMA), 0.5 wt% camphorquinone (CQ), 0.5 wt% trimethylbenzoyl phosphine oxide (TPO) and 0.5 wt% dimethylaminobenzoic acid ethyl ether (EDMAB). All of these raw materials were purchased from Sigma-Aldrich (Steinheim, Germany). The fillers were added in small amounts to the resin matrix in a centrifuge (Universal 32 R, Hettich, Singapore) at 3,500 rpm for 60 s until reaching the desired filler concentration, followed by food blender mixing for an additional 60 s. A commercially-available orthodontic adhesive (Transbond XT, 3M Unitek, Monrovia, CA, USA) was used as a control.

Cylindrical specimens were prepared from each experimental adhesive using a polytetrafluoroethylene mould (2 mm thickness, 6 mm diameter) and covered with a glass slide to prevent oxygen inhibition and to control surface smoothness. The specimens were cured for 20 s on each side with a polywave LED light-curing unit (Bluephase N, Ivoclar-Vivadent, Schaan, Liechtenstein) set to ‘High’ mode (output of approximately 1,100 mW/cm²). The specimen surface was wet-sanded with 1000, 1500 and 2500- grit SiC paper (CarbiMet, Buehler, Lake Bluff, IL, USA), respectively, as a surface control.

Buffering capacity towards bacteria-produced acids
An overnight culture of S. mutans in a custom-made buffer-free brain heart infusion (BHI) broth was centrifuged, suspended, and adjusted to a concentration of 1.5×10⁷ CFU/mL. After subculturing for 24 h at 37°C and 5% CO₂, the supernatant containing bacteria-produced acids was collected for pH measuring using a pH meter (Micro pH meter, Mettler Toledo, Greifensee, Switzerland). Specimens of each adhesive sample which had been sterilized using ultraviolet (UV) light for three hours on each side were exposed to 250 µL of the supernatant from the bacterial culture in 48-well plates. The pH values were measured in triplicate at 24 and 48 h. The negative controls were the supernatant from 24-h S. mutans cultures. Statistical analysis was carried out by Kruskal-Wallis tests. A schematic of this procedure is presented in Fig. 1.

Antibacterial properties: Indirect technique
The pH of the custom-made buffer-free BHI was adjusted to a pH of 5 by adding lactic acid (Sigma-Aldrich) to imitate the acidic conditions produced by the biofilm bacteria. The UV-sterilized specimens were immersed in 250 µL of the acidic BHI media for 24 h at 37°C. The media was removed, subsequently filter-sterilized. An aliquot of 100 µL of the media and either S. mutans or S. sanguinis culture (1.5×10⁷ CFU/mL) were mixed separately in 96-well plates. The plate was incubated at 37°C, 5% CO₂ for 24 h. The optical density (OD) of this bacterial suspension was measured using a spectrophotometer (BioTek ELx800, BioTek, Winooski, VT, USA) at 630 nm. Both bacterial cultures in custom-made BHI at pH 5 and 7 without the material sample served as the controls. These experiments were repeated triplicates for each bacterial species. Statistical analysis was conducted by Kruskal-Wallis tests. An illustration of this indirect technique procedure is presented in Fig. 2.

Antibacterial properties: Direct technique
All UV-sterilized material specimens were immersed in 350 µL of S. mutans and S. sanguinis in BHI at concentrations of 1.5×10⁷ CFU/mL in a 48-well plate.

Fig. 1 Schematic illustration demonstrating the acid-buffering properties from the bacteria-produced acid test method.
The bacterial cultures without the tested specimens were used as the controls for bacterial growth. The plate was incubated at 37°C, 5% CO₂ for 24 h. An aliquot of 100 µL of each sample solution was transferred to a new 96-well plate. The OD value at 630 nm was measured in triplicate using a spectrophotometer. The adhesive specimens were stained with crystal violet dye (0.5%) for 15 min, then washed three times with PBS. The crystal violet was dissolved from the stained biofilms with 95% ethanol. An aliquot of 100 µL was transferred to a new 96-well plate. The OD value at 575 nm was measured in triplicate using a spectrophotometer. Statistical analysis was done using Kruskal-Wallis tests. Figure 3 shows a schematic of this direct technique procedure.

Correlation between pH and bacterial growth
The relationship between the pH values of the acidic media obtained after exposure to the test adhesive materials at 24 h and the OD values representing the amount of the bacteria obtained from the indirect technique was statistically analyzed with Spearman's rank correlation coefficient tests.

RESULTS
Buffering capacity towards bacteria-produced acids
The buffering capacity towards bacteria-produced acids was assessed by incubating the specimens in the supernatant from the overnight culture of S. mutans, containing bacteria-produced acids. The initial pH of the supernatant was 4.95±0.05. The pH levels of the supernatant for all BAG adhesives (45S5, 45SSF, and S53P4) were significantly increased at both 24 and 48 h (p<0.05), while the pH for HAp, β-TCP, Canasite-containing adhesives, and Transbond XT were similar to the control (Fig. 4).
Antibacterial properties: Indirect technique
Antibacterial properties of the adhesive materials were tested on *S. mutans* and *S. sanguinis* by the indirect technique. When the bacteria were cultured with the acid media that had been exposed to the adhesive materials, the growth was observed by measuring the turbidity of the cultures and expressed as OD values at 630 nm. The baseline OD before incubation was approximately 0.1. The results from *S. mutans* showed that all the adhesive materials did not have significant antibacterial properties. The media for all samples showed OD values higher than baseline OD indicating that *S. mutans* growth had occurred. Only OD of the media from 45S5 materials was significantly higher than the control medium at pH 5, and was comparable to the control medium at pH 7 (Fig. 5). In the case of *S. sanguinis*, the OD of all experimental BAG adhesives (45S5, 45S5F, and S3P4) were significantly increased in relation to the pH 5 control. When compared to the pH 7 control however, all adhesives did not show statistically different OD values (Fig. 6).

Antibacterial properties: Direct technique
Regarding the direct technique used to assess antibacterial properties of the adhesive materials, *S. mutans* and *S. sanguinis* cultures were directly incubated with the adhesive materials. The amount of the bacteria was observed by the OD values at 630 nm. In addition, the biofilm formation on the adhesive materials was evaluated by crystal violet staining. For the *S. mutans*, the amount of the bacteria in the media HAp and β-TCP adhesives was significantly higher than the others. The biofilms on HAp and 45S5 adhesive were significantly greater than the others (Fig. 7). For the *S. sanguinis*, all experimental adhesives did not show any significant difference in the bacterial density in solution. However, the amount of biofilm was found significantly greater on 45S5 than the others (Fig. 8). The antibacterial property testing among the experimental adhesives similarly showed negative results for both *S. mutans* and *S. sanguinis* as tested in the washed solution as well as biofilm formation.

Correlation between pH and bacterial growth
The buffering capacity of the adhesive materials towards the growth of bacteria was statistically analyzed with Spearman's rank correlation coefficient tests. A
Fig. 7 Direct antibacterial properties of adhesives to *S. mutans* in the solution (left) and in the biofilm (right). The same letters indicate no statistically significant difference (*p* > 0.05).

Fig. 8 Direct antibacterial properties of adhesives to *S. sanguinis* in the solution (left) and in the biofilm (right). The same letters indicate no statistically significant difference (*p* > 0.05).

A moderate positive correlation was found between *S. mutans* growth and pH values at *r* = 0.42 (Fig. 9), while the correlation between *S. sanguinis* and the pH value was higher at *r* = 0.72 (Fig. 10).

**DISCUSSION**

As we developed experimental orthodontic adhesives incorporated with BAGs, this study aimed to evaluate the buffering capacity and antibacterial properties of these experimental orthodontic adhesives compared to other adhesives containing HAp, β-TCP, Canasite, and the commercially available Transbond XT. Regarding buffering capacity, the BAG-containing adhesives (45S5, 45S5F, and S53P4) showed great buffering capacity, which was absent in other adhesives. However, all
experimental and commercial adhesives did not display antibacterial properties towards both tested bacterial species \textit{S. mutans} and \textit{S. sanguinis}.

A previous study suggested that BAG particles incorporated into a Bis-GMA/TEGDMA are able to induce reactivity in an aqueous environment\(^{19}\). In this study, an UDMA/TEGDMA matrix was used to avoid bisphenol A-based materials, due to rising concerns about the effects of leaching from such materials on patients’ health\(^ {20,21}\). The authors inferred that a 20 wt% loading of BAGs in the adhesives might have favored the establishment of a constant high-pH environment\(^ {19}\), thus conferring antimicrobial properties\(^ {16,22}\). By increasing the particle content in the matrix, the higher its viscosity and the higher the rate of active exchange between the BAG particles and surrounding environment can be expected\(^ {19}\). However, our study chose to load the experimental adhesives with 15 wt% of BAGs as an earlier study has shown that this filler volume was suitable to maintain the mechanical properties of the adhesive\(^ {23}\). Another point of concern is the leaching of BAG ions with ageing, leading to the degradation in mechanical properties, making it not much better or worse than many commercial composites\(^ {21}\). This is an issue as the adhesives need to withstand the applied orthodontic forces for tooth movement throughout the course of treatment.

Three different types of BAGs were chosen for our study: 45S5 is one of the first tested BAG compositions with substantial success, 45SSF has additional fluoride ions which aid in increasing apatite formation\(^ {24,25}\), and S53P4 is composed of the highest amount of SiO\(_2\). Besides, 45S5 has displayed antibacterial activity which is strongly correlated with the pH of the supernatant and high silicon ion levels\(^ {16,17}\). Other materials, i.e., HAp, \(\beta\)-TCP, and Canisite, with excellent biocompatibility and non-toxicity were also included in our study, where their calcium phosphate compositions are of interest due to their similarity to bones and teeth\(^ {26}\). HAp and \(\beta\)-TCP have frequently been used due to their osteoconductivity, crystallographic structures, and chemical composition similar to that of skeletal tissue\(^ {27,28}\). Canisite is a glass-ceramic with high-silica content, where the silicate chains demonstrate high fracture toughness which both reinforces the material as well as imparts aesthetic advantages\(^ {29}\).

The buffering ability in adhesives is a desirable property as it would potentially offer resistance towards dental caries\(^ {30}\). The present study has shown that BAG-containing adhesives (45S5, 45SSF, and S53P4) provided a considerable buffering capacity against \textit{S. mutans}-produced acids after contact for 24 and 48 h. These results are in agreement with a previous study which demonstrated that BAG particles embedded in a resin matrix were successful in increasing the pH values of buffered saline solution\(^ {19}\). It is suggested that this results from the release of alkaline ions, of which are mainly sodium ions (Na\(^+\)), into the surrounding environment\(^ {22}\), thus reducing the acidity and creating favorable conditions to reduce the risk of enamel demineralization. This increase in pH has also been suggested to depend on the concentration of BAGs, where a previous study demonstrated that the addition of 10 wt\% BAG particles induced a pH increase to 8.7, while 20 wt\% BAG particles resulted in a pH of 10.8 which remained constant throughout 21 days\(^ {19,31}\). Our study which used 15 wt\% BAGs increased the pH values to approximately 7.25 within 24 h (Fig. 4). This might be influenced by different resin matrix types, photoinitiators, BAG particle size, composition, and the surrounding environment. The monomer also affects the effectiveness of BAGs as the process and degree of
polymerization of the monomer can minimize the BAG activity and release\(^\text{32}\). We chose to use CQ/amine and TPO as photo-initiators due to their optimal strength and high degree of polymerization\(^\text{38}\), however the latter may also alter the efficacy of BAG release.

The indirect bacterial growth testing in our study showed that \(S. \text{mutans}\) growth was significantly higher only for the 45S5 experimental BAG adhesive compared to the \(pH\) 5 control (Fig. 5). On the other hand, \(S. \text{sanguinis}\) growth was significantly abundant among all three experimental BAG adhesives compared to the \(pH\) 5 control (Fig. 6). A possible explanation would be that the BAG adhesives significantly raised the initial \(pH\) from 5 to 7 (Fig. 4), where the more neutral environment favors the growth of both bacterial species. This would also agree with the significant moderate positive correlation \((r=0.42, \ p<0.05)\) between the \(pH\) value and \(S. \text{mutans}\) growth in the extracted medium at 24 h. From Fig. 9, the values of the 45S5F experimental adhesive can be seen to be lying beyond the regression line. Hence, it can be reasoned that the growth of \(S. \text{mutans}\) is affected not only by \(pH\), but directly by the adhesive material too. At a constant \(pH\), the antibacterial properties of 45S5F towards \(S. \text{mutans}\) have been shown to be significantly higher than 45S5\(^\text{31}\). On the contrary, a stronger significant positive correlation \((r=0.72, \ p<0.05)\) between the \(pH\) value and growth of \(S. \text{sanguinis}\) was found, suggesting a direct association between the two variables.

The results from the direct bacterial growth testing were similar with the indirect technique. The BAG experimental adhesives displayed higher bacterial growth than the Transbond XT control. Our results also demonstrated the biofilm formation of \(S. \text{mutans}\) and \(S. \text{sanguinis}\) on the surfaces of all adhesive materials. The different amount of biofilms might be due to the surface properties of the test materials, i.e., surface roughness, surface wettability. It should be noted, however, that this in vitro study was conducted on a single-species model, and that the results may differ clinically. A previous study\(^\text{30}\) found that \(S. \text{mutans}\) and \(S. \text{sanguinis}\) display an inverse relationship, where \(S. \text{mutans}\) colonization was reduced in the presence of \(S. \text{sanguinis}\). Besides, early colonization by \(S. \text{sanguinis}\) was significantly correlated with the delayed colonization of \(S. \text{mutans}\). Hence, it has been proposed that \(S. \text{sanguinis}\) is associated with a healthy tooth surface and oral biofilm\(^\text{35}\) by conferring a protective mechanism against colonization by \(S. \text{mutans}\)\(^\text{36}\).

In addition to bacterial species, other factors contributing to the buffering capacity and antibacterial properties of BAG adhesives include BAG composition, concentration, particle size, and manufacturing process\(^\text{15,37-39}\). For our research, the BAG particles with a size of 5.81 µm were produced by the melt-quenching process. An earlier study reported BAG production by the sol-gel process with a particle size of 0.04–0.3 µm\(^\text{40}\). The authors noted that BAG particles made from the sol-gel process released higher amounts of ions than the melt-quenching process. The amount and type of ion release could influence the changes in \(pH\) values and the antibacterial mechanism especially in the presence of calcium ions\(^\text{38,40}\). This ion release is an area of interest that needs to be explored. Also, the use of nanoparticle BAGs is another area to be developed as it has been suggested that BAG nanoparticles exhibit more alkalinity due to their increased surface area, which in turn would provide more substantial antimicrobial effects than micron-sized BAG particles\(^\text{14}\).

To improve on the buffering and antibacterial properties, increasing the concentration of BAGs in our experimental orthodontic adhesive could be considered, however the effects on the mechanical properties need to be properly evaluated as well. Another method would be the incorporation of metallic ions such as \(\text{Ag}_2\text{O}\)\(^\text{41,42}\) and \(\text{ZnO}\)\(^\text{41,43}\) which have also shown promising bactericidal properties.

There are a variety of aspects to yet be further studied, including ion release, long-term buffering capacity, effects on other bacterial species, multiple-species models and many more in order to obtain a thorough understanding before an ideal composition of the BAG orthodontic adhesive can be achieved.

**CONCLUSION**

Experimental 15 wt% BAG-containing (45S5, 45S5F, and S53P4) orthodontic resin adhesives display a significant buffering capacity towards \(S. \text{mutans}\)-produced acids at 24 and 48 h. However, these 15 wt% BAG-containing experimental adhesives were unable to demonstrate antibacterial properties towards either \(S. \text{mutans}\) or \(S. \text{sanguinis}\).

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