

Fluoride Containing Bioactive Glass Composite For Orthodontic Adhesives

Abstract:

Objectives: Dental materials that release calcium, phosphate and fluoride ions could prevent demineralisation and/or enhance remineralisation of enamel. The objective was to develop a novel bioactive glass (BAG) resin and investigate pH changes and ion release in 3 immersion media.

Methods: Quench melt derived BAG (35.25% SiO₂, 6% Na₂O, 43% CaO, 5.75% P₂O₅, and 10% CaF₂) was incorporated into a resin (42.25% BisEMA, 55% TEGDMA, 0.25% DMAEM, 0.5% camphorquinone and 2% 4-Meta), with a filler load of 80% by weight. Ninety composite disks for each BAG loading of 80%, 60%, 50%, 40%, 20%, and 0% were made and each disk was immersed in 10ml of either tris buffer (TB), or artificial saliva at pH=7 (AS7) or pH=4 (AS4), n=30 for each solution. Three disks of each loading were taken from each of the solutions, at ten time points (6 hours-6months), for measurement of pH, fluoride, calcium and phosphate.

Results: The BAG adhesive raised the pH in all the solutions, release Ca, PO₄ and F ions especially in AS4. The rise in pH and the release of Ca and F are directly related to the BAG loading and the time of immersion. The pH and the ion releases were maintained and continued over 6 months.

Significance: Unlike glass ionomer resins, favourable ions F, Ca and PO₄ releases were maintained over a long time period especially in acidic condition for this novel BAG-resin composite. This indicates the resin has the potential to prevent formation and progression of early caries lesions.

1. Introduction:

Bioactive glass (BAG) is a material that degrades and dissolves upon contact with a physiological fluid to allow controlled release of therapeutic ions and the formation of an apatite like surface layer [1, 2]. It was first developed by Hench in 1969 [3] and was called Bioglass® (BAG 45S5), which has been in clinical use since 1985. The BAG 45S5 is composed of 46.1 SiO₂, 2.6 P₂O₅, 24.4 Na₂O and 26.9 CaO (mol %). The composition of BAGs strongly influences their dissolution kinetics and their ability to form apatite (bioactivity), whereby the concentration of the glass formers (silica and phosphate) and the degree of polymerisation (network connectivity) are the main determinants of its bioactivity [4]. The effect of phosphate is attributed to the formation of orthophosphate phase which is more reactive than the silica phase, therefore, an increase in phosphate content allows for a faster apatite deposition and thereby enhances bioactivity [5-7]. It was found that the network connectivity (NC) remains constant when the addition of P₂O₅ is accompanied with the addition of network modifiers to charge balance the orthophosphate in the glass. Furthermore, increasing the P₂O₅ content reduces the rise in pH upon dissolution of bioactive glasses, which is beneficial since optimal activity and apatite formation is around a pH of (7.3). Hence, combining the basicity from the silicate of the bioactive glass with the acidity of the orthophosphate seems to have a good effect in maintaining the pH around neutral [8]. The effect of fluoride in BAGs is to allow for fluoroapatite (FAP) formation, which in turn is more resistant to acid dissolution than hydroxyapatite [9-12]

The incorporation of BAG into the resin based composites is a relatively new approach in dentistry [13, 14]. Partial or full replacement of the inert glass filler particles in composite resins with BAG particles were implemented to develop a composite resin that can release therapeutic ions. Pit and fissure sealants containing BAG 45S5 fillers were studied by Yang and co-workers and found to have a neutralizing effect in acidic environments [14]. These authors also reported similar neutralising effect with three other different BAGs [15]. However, these studies only contained reports on the impact of pH only, with no data on ion release from the BAGs. Incorporating a sol gel BAG in an orthodontic adhesive was shown to result in significantly higher calcium release and buffering ability, with a potential in prevention of white spot lesions (WSL) [16-18]. However, these studies found limited fluoride

release, which is probably attributed to the potential loss of fluorine during the sol-gel glass production route.

Most of the above mentioned studies used the BAG 45S5 or one of its modified versions where the sodium contents was around 20 (mol %). Although sodium is theoretically important for the BAG reaction and dissolution [2], high sodium concentration could lead to excessive water exchange between the glass particles and the media, which in turn might result in swelling of the glass particles and consequent adverse effects on the physical properties, survival and longevity of the composite resin. Decreasing sodium concentration in BAG would enable incorporating more advantageous elements like calcium, phosphate and fluorides which are essential for apatite formation.

No studies was found on the impact of the different BAG filler loadings (especially for those manufactured by the melt quench route) within the resin in ion releases and their potential neutralising effect. Furthermore, apart from deionised water, the effect of immersion on other media was not known. Hence, the aim of the present study was to incorporate a novel BAG (by the melt quench route) with low sodium but high fluoride, phosphate and calcium contents, into a specially designed resin and to characterise the behaviour of the resin with different BAG loadings in three immersion media.

2. Materials and methods:

2.1. Synthesis of BAG:

The strategy of BAG design is to decrease the sodium with an increase in calcium and fluoride content in order to allow for more beneficial therapeutic ion release. Phosphate concentration was also increased, since Eden has stated that “bioactivity increases monotonically with increasing phosphorus content of the BAG” [7]. Therefore, 5 glasses with variable concentrations of phosphate and fluoride were manufactured and tested for the most appropriate BAG as the replacement fillers in the resin. The glasses were characterised using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) followed by ion release tests after immersion in Tris buffer solution (TB). The test revealed that the BAG with the composition listed in Table 1a was the most suitable in terms of bioactivity. The NC of this BAG calculated according to Hill and Brauer [4] was 2.19.

The BAG was manufactured via the melt quench route. A 200g batch of analytical grade SiO₂ (Prince Minerals Ltd., Stoke-on-Trent, UK), Na₂CO₃, CaCO₃, P₂O₅ and CaF₂ (all Sigma-Aldrich, Gillingham, UK) were mixed and transferred into a platinum-rhodium (80/20) crucible for melting in an electric furnace (EHF 17/3, Lenton, Hope Valley, UK) at 1480°C for 1 hour. The melted glass was then quenched quickly in distilled water. The glass frit was milled in a gyro-mil (Gyro mill, Glen Creston, London, UK) and sieved using a 38 micron sieve (Endecotts Ltd., London, UK). The average particle size for the BAG glass powder was approximately 10 microns. To ensure that the experimental composition is similar to the theoretical composition, which is particularly important for the F-containing BAG as some F may be lost on melting, the BAG powder (20 grams of coarse powder, > 38 microns in size) was sent to a commercial laboratory (Lucideon limited, Queens Road, Penkhull, Stoke On Trent, United Kingdom) for chemical analysis using X-ray fluorescence spectrometry (XRF). The results (Table 1b) show that the BAG composition was largely as expected, based on its nominal composition. The decrease of CaF₂ and the corresponding increase in CaO indicated that there was a slight loss of F in the melting process, as shown by Brauer et al. [19].

2.2. Preparation of the resin:

The resin was prepared using 42.25% Bisphenol A ethoxylate dimethacrylate (BisEMA), 55% Tri(ethylene glycol) dimethacrylate (TEGDMA), 0.25% 2-(Dimethylamino)ethyl methacrylate (DMAEM) and 0.5% camphorquinone (all Sigma-Aldrich Gillingham, UK). 2% 4-Meta (First Scientific GmbH, Elmshorn, Germany) was added to enhance the bond with the teeth and the brackets.

2.3. Preparation of adhesive paste and disks:

The adhesive paste was prepared using a glass to resin ratio of 80:20% by weight. The BAG powder load was replaced incrementally by a highly inert glass (CDL Ltd, England) that contains fluoride without calcium or phosphate. The average particle size for the inert glass powder was also 10 microns. Thus, 6 types of adhesive pastes containing 80%, 60%, 50%, 40%, 25% and 0% BAG were made. Disks of these pastes were produced using Teflon moulds of

10mm diameter and 1.2mm thickness. A transparent acetate sheet with a glass slabs was placed below and above the paste filled mould and a 200gm weight was placed to release any air bubbles and remove excess material. Then the disk was cured using 3M ESPE Elipar™ light for 40 seconds.

Ninety disks for each BAG loading were fabricated (total=540). For each loading, they were divided into 3 equal groups (n=30) and each disk was immersed in a 15ml polypropylene centrifuge tube (Fisher Scientific UK Ltd, Leicestershire, UK) containing 10 ml of one of the following solutions: tris buffer pH=7.3 (TB), artificial saliva at pH=7 (AS7) and artificial saliva at pH=4 (AS4). These three media were chosen because TB has a better maintained pH than de-ionised water; AS7 and AS4 were chosen to mimic remineralising and demineralising environments in saliva respectively. At each of the 10 time points (6, 12 and 24 hours, 3, 7, 14, 30, 60, 90 and 180 days), 3 disks from each group of each loading were removed for analysis (Fig. 1). These disks were removed from their solution, washed and dried for investigation using FTIR, XRD and scanning electron microscopy (SEM). Further results for these studies will be presented in a subsequent study. The solutions resulted from the immersion were used for measurement of pH changes using pH meter (Oakton Instruments, Nijkerk, the Netherlands), and fluoride release using ion selective electrode (ISE) (Orion 9609BNWP with Orion pH/ISE meter 710, Thermo Scientific, Waltham, MA, USA). Ca and PO₄ release for BAG loading of 40 and 80% were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Varian Vista-PRO, Varian Ltd., Oxford, UK). The remaining disks were washed and re-immersed in one of the three freshly made solution until the next time point measurement.

3. Results:

3.1. pH changes:

Since fresh solution was used at the beginning of each measuring time point, the duration of the immersion was not constant across all the time point. Hence, only the pH at 3 time points measurements (7, 60 and 180 days) are presented to illustrate the effect of BAG loading on pH (Fig. 2). There was an increase in pH with the presence of the glass-resin disk but a more noticeable increase could be observed with increased BAG loading, especially at the 180 day

time-point. At this final time-point, the pH demonstrated the largest increase (up to 3 pH level) in the AS4 solution, especially with higher loading of BAG in the disk. .

3.2. Fluoride release:

As expected, all disks released F in all the three solutions and the release was continuous even at 180 days (Fig. 3). Cumulatively, more F was released in TB and the least in AS7 (Fig. 4). In TB and AS4, F release increased when the BAG loading was increased. However, in AS7, the amount of F release was highest in 0% BAG loading where the only glass was the inert glass except at the 180 days time point where all BAG loadings released similar amounts. With the presence of BAG in AS7, irrespective of its loading, the F release was nearly 0 at 60 to 90 days time point but increased substantially thereafter.

In all the 3 immersion media, there was a fall in F release from the 6 to 12 hour time-points, followed by an increase in later time points. At 180 days, F release was generally found to be the highest among all the time points, except for the disks with 25-50% BAG loading in AS4.

3.3. Calcium release:

The cumulative Ca concentration graph (Fig. 5) showed that Ca continued to be released through the whole experimental period of 180 days. The highest release was found in the AS4 media. The BAG loading did not seem to have significant effects on Ca release in TB and AS7 media. In AS4, after 30 days, more Ca was found for disks with 80% loading than those with 40%.

3.4. Phosphate release

The cumulative PO₄ concentration graph (Fig. 6) showed that little release was found in TB media. For AS4 and AS7, most PO₄ was released within the first 14 days. The release then slowed down and began to level off after 30 days, except for the BAG 40% loading in AS4 where PO₄ release continued to the end of the experimental period. It was also noted that more PO₄ release occurred in BAG loading of 40% than 80%.

4. Discussion

4.1 pH changes:

The increase of pH in the media was mainly due to the ion exchange of Ca and Na ions for H protons from the dissolution of the glass particles. As the BAG contained a high percentage of Ca ions, the increase of BAG loading in the composite resulted in a greater increase in pH (Fig. 2). The greatest pH rise was observed in AS4, the media with the lowest pH, which was shown to cause faster dissolution of BAG [20], resulting in more Ca being available to replace the H⁺ ion in the solution. This property of BAG has advantageous clinical applications, since the acid arising from either dietary intake or produced by cariogenic bacteria, can be neutralised by the dissolution of BAG to prevent/reduce damage on enamel through demineralisation [21]. In media with neutral pH, the BAG appeared to be stable as observed from the comparative small increase in pH in TB and AS7. Hence, this BAG-resin could be regarded as a smart material clinically as it reacts preferentially when the condition becomes hostile to the enamel, e.g. heavy plaque accumulation around orthodontic brackets.

4.2 Fluoride:

Most currently available resin adhesives have fillers that contain fluoride in order to facilitate the match in the refractive index to enamel and to prevent enamel demineralisation. Hence, in this study, an inert glass was used as a reference/control to investigate the effect of the BAG loading. It is expected that the presence of Ca and PO₄ in the BAG may cause formation of either fluoroapatite (FAP) or calcium fluoride (CaF₂). Thus free F ions would be reduced, resulting in a decrease in the measured F concentration. This effect is only observed for BAG-resin disk in AS7 media initially up to 3 months. At the 6 month timepoint, no observable difference could be found (Fig. 3b). For the other two media, TB and AS4, as F concentration increased with BAG loading, it indicates that FAP and CaF were probably not formed. Therefore, clinically, the high release of F in the high BAG loading composite could have a preventive effect on enamel demineralisation.

The pattern of fluoride release in these composites are different from that observed with glass ionomer cements (GICs) and resin modified glass ionomer cements (RMGICs), where fluoride release has an initial burst in the first 24 hours, decreases substantially in the

following 3 days to 2 weeks, and then often stabilises after 2-4 weeks [22-24]. For the BAG-resin composite, apart from the initial drop at the 12 hour timepoint, the release of F generally increased or remained constant with time, especially for those composites with higher BAG loading in TB and AS4 media (Fig. 3). Although the amount of fluoride release was found to be higher in RMGIC compared to other fluoride releasing resin composites [23-26], our BAG-resin has higher cumulative fluoride release (>2 times; Fig. 4) than the RMGIC after six months of immersion. The BAG-resin in the present study was also found to have higher F release than previous studies [16, 18] which reported a similar pattern to GIC/RMGIC. This might be attributed to previous BAG having: i) low concentration of fluoride (3 mol%) used in the glass, ii) FAP and CaF_2 formation, and/or, iii) the loss of fluorine during sol gel glass synthesis.

Although fluoride release could be affected by the diffusion potential of the solution to dissolve glass particles, fluoride released from the BAG is anticipated to be consumed in the formation of FAP. This might explain the dip in the fluoride release at the early time points (12 hours) as discussed previously with the pH changes. Consequently, when the cumulative fluoride release is plotted against the square root of time (Fig. 4), it deviates negatively from linearity which might reflect the consumption of fluoride in FAP formation compared to GICs which is generally linear reflecting a diffusion driven process uninfluenced by a precipitation of any fluoride containing phases.

4.3 Calcium and phosphate:

Calcium is nearly 20 times more effective than phosphate in preventing dissolution of enamel. It is considered as the major rate limiting mineral component in saliva and a calcium/phosphate ratio of 1.6 would be expected to result in an optimal rate of remineralisation [27, 28]. Hence, one of the potential ways to prevent demineralisation and enhance remineralisation is by delivering additional calcium and phosphate ions.

The concentrations of Ca and PO_4 ions already available in the AS7 and AS4 were 60 ppm and 30 ppm respectively. In TB, the rapid increase of Ca initially (Fig. 5) may be due to diffusion along a concentration gradient from the BAG-resin to the lower concentrated TB media. Once

it became equilibrated, the increase was small and not dependant on BAG loading. This was also found in AS7 media. Hence, this would indicate that in neutral solution, where the pH rises significantly to 8.5, the BAG in the composite becomes stable. It can also be argued that the released Ca was consumed to form calcium phosphate compound as discussed later. In acidic media (AS4), as expected, more Ca was released because dissolution of material is usually faster in lower pH solution. As a result, the pH of the solution was raised (Fig. 2), and clinically it would be beneficial as discussed above. In the AS4 media, the effect of higher Ca in higher BAG loading is only apparent in the long term. This may be attributed to the Ca content in the lower BAG (40%) loaded composite showing early signs of exhaustion. Further experiments are needed to investigate the longer term of release of Ca in the BAG-resin composite.

The concentration of the PO_4 in TB did not show an initial rise (Fig. 6). It is unlikely that Ca was released from the composite but the PO_4 was not. Hence, it could be speculated that the PO_4 that was released was consumed to form calcium phosphate products, possibly apatite, in the presence of high Ca in the solution. Further research is needed to investigate whether any new materials are formed on the surface of the disks. In both the neutral AS7 and the acid AS4 media, there was an initial rise of PO_4 concentration and the increase became minimal (apart from 40% BAG in AS4). The initial rise corresponded to the initial drop of F (Fig. 3). This may indicate that fluoroapatite was formed, rather than hydroxyapatite initially in artificial saliva. For the continuous increase in PO_4 for the 40% BAG loading in AS4 in later timepoints, this also corresponds to the decrease of F (Fig. 3) and the same argument above can be applied. Hence, further investigation is needed to analyse any material formed (if any) on the surface of the disk after immersion.

5. Conclusion:

The incorporation of low Na, high Ca, PO_4 and F containing bioactive glass to the resin resulted in an adhesive composite that can react favourably in acidic condition to release high

and sustained amounts of fluoride and calcium, and raise pH to protect enamel. In neutral conditions, this composite may have ability to form apatite. Hence, this composite could be further develop as a smart material for clinical use to prevent demineralisation around the orthodontic brackets.

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