Enamel white spot lesions can remineralise using bio-active glass and polyacrylic acid-modified bio-active glass powders

Hussam Milly¹, Frederic Festy¹, Timothy F. Watson¹,², Ian Thompson¹, Avijit Banerjee¹,²,*

¹Biomaterials, Biomimetics & Biophotonics Research Group, King’s College London Dental Institute at Guy’s Hospital, King’s Health Partners, London, UK
²Unit of Conservative Dentistry, King’s College London Dental Institute at Guy’s Hospital, King’s Health Partners, London, UK

ARTICLE INFO

Article history:
Received 16 September 2013
Received in revised form
11 November 2013
Accepted 18 November 2013

Keywords:
Bio-active glass (BAG)
Polyacrylic acid (PAA)
Enamel white spot lesion (WSL)
Remineralisation
Microhardness
Micro-Raman spectroscopy

ABSTRACT

Objective: To evaluate the potential of bio-active glass (BAG) powder and BAG containing polyacrylic acid (PAA-BAG) to remineralise enamel white spot lesions (WSL).
Methods: 32 human enamel samples with artificial WSLs were assigned to 4 experimental groups (n = 8); (a) BAG slurry, (b) PAA-BAG slurry, (c) “standardised” remineralisation solution (positive control) and (d) de-ionised water (negative control). Mechanical properties of enamel were assessed using surface and cross-section Knoop microhardness. Micro-Raman spectroscopy in StreamLine™ scan mode was used to scan lesion cross-sections. The intensity of the Raman phosphate peak at 959 cm⁻¹ was fitted and measured producing depth profiles analysed using a double-step fitting function. A further 20 samples (n = 5) were used to obtain 3D images of surfaces using non-contact white light profilometry permitting measurement of lesion step height in relation to the sound enamel reference level, and to scan the lesion surface using scanning electron microscopy (SEM). Data were analysed statistically using one-way ANOVA with Tukey’s HSD post-hoc tests.
Results: BAG, PAA-BAG and the remineralisation solution exhibited statistically significantly higher surface and cross-section Knoop microhardness compared to the negative control. Micro-Raman spectroscopy detected significantly higher phosphate content within the treated groups compared to the negative control group. Lesions’ depth was not significantly reduced. SEM images revealed mineral depositions, with different sizes and shapes, within BAG, PAA-BAG and the positive control groups.
Conclusion: BAG and PAA-BAG surface treatments enhance enamel WSL remineralisation, assessed by the resultant improved mechanical properties, higher phosphate content and morphological changes within the artificial lesions.

© 2013 Elsevier Ltd. All rights reserved.

* Corresponding author at: Unit of Conservative Dentistry, King’s College London Dental Institute, Floor 26, Tower Wing, Guy’s Dental Hospital, London. SE1 9RT, UK. Tel.: +44 207 188 1577 / 7486; fax: +44 207 188 1577 / 7486.
E-mail address: avijit.banerjee@kcl.ac.uk (A. Banerjee).
0300-5712/ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jdent.2013.11.012
1. Introduction

Minimally invasive dentistry encompasses the philosophy of preservation of the maximum quantity of repairable dental tissues and utilising preventive, remineralisation approaches in incipient carious lesion management. The enamel white spot lesion (WSL) is the earliest clinically evident manifestation of the caries process, exhibiting subsurface porosity caused by an imbalance between the biological and chemical processes of demineralisation and remineralisation. In the minimally invasive restorative dentistry paradigm, incipient enamel carious lesions should not be managed with surgical intervention, but with non-invasive remineralisation strategies wherever possible.

Bioactive glass (BAG) can act as a source of a large amount of CaO and P2O5 in a Na2O–SiO2 matrix with a rapid dissolution rate and high ionic concentration. The bioactivity index determines the rate at which a bioactive material produces a chemical bond with a natural tissue. BAG 45S5 exhibits a high bioactivity index (β = 12.5) compared to other bioactive materials such as hydroxyapatite (β = 3), and therefore it has the potential to remineralise enamel white spot lesions with an increased rate of HA formation. BAG has been introduced clinically as an air-abrasion abrasive powder to be used under the auspices of minimally invasive dentistry and has showed promising results for the controlled, selective removal of an enamel analogue substrate, demineralised enamel and resin composite restoration/cements, particularly using specific operating parameters. Polyacrylic acid (PAA) has been added to bioactive materials in order to mimic the functional role of non-collagenous proteins in binding the calcium and phosphate ions to form nano-precurors, including amorphous calcium phosphate, small enough to penetrate the carious lesion more effectively. Using BAG powder containing 40 wt% PAA to treat the dentine using air-abrasion technology reduced the micropermeability between the dentine and the adhesive layer in vitro, and might be a suitable strategy to enhance bond durability. To the authors' knowledge, there is no information published to ascertain the potential role of using PAA-BAG on enamel white spot lesion remineralisation.

Micro-Raman spectroscopy is used as a quantitative chemical assessment methodology for biological samples in conjunction with the fact that the Raman peak intensity is proportional to the number of molecules within the volume of scanned area. The Raman phosphate peak at 959 cm⁻¹ characterises tetrahedral PO4 group (P–O bond) within HA. Monitoring the intensity of this peak has been used to assess the degree of demineralisation within enamel caries. The present study utilised this measurement to assess the potential increase in phosphate content within the lesion as a result of remineralisation treatments. Depth profiles of phosphate peak intensity along the cross-sections of the samples were created and fitted using a double-step function. To date, the use of Raman phosphate peak intensity measurement and high-speed line scanning to detect a potential increase in the phosphate content within the incipient lesion as a result of a remineralisation treatment has not been reported in the dental literature.

Hardness measurements provide information about the mineral density and mechanical properties of hard tissue surfaces, and are a reliable, objective method to study demineralised enamel and dentine lesions. The aim of the present study was to evaluate the effect of BAG and PAA-BAG powders on artificial enamel WSL remineralisation through morphological, mechanical and chemical assessments using a “standard” remineralisation solution as a positive control and de-ionised water as a negative control. The morphological changes of the lesion surface were assessed using optical white light confocal profilometry and scanning electron microscopy (SEM). The null hypothesis investigated was that treating enamel WSL with BAG or PAA-BAG slurries has no beneficial effect on enamel WSL remineralisation when compared to the controls.

2. Materials and methods

2.1. Samples preparation and remineralisation treatment

Fifty-two enamel slabs (4 mm × 4 mm × 2 mm) were cut from the buccal surfaces of caries-free human extracted lower molars, collected using an ethics protocol reviewed and approved by the East Central London Research Ethics Committee (Reference 10/H0721/55). The teeth were refrigerated during storage, used within a month from the extraction and sectioned using a diamond wafering blade (XL 12205, Benetec Ltd., London, UK) obtaining one specimen from each tooth. The slabs’ surface integrity was inspected using microscopy at 40× magnification and the samples were then included face down in acrylic resin using a hard-anodised aluminium and brass sample former (Syndical Ingenieurbüro, München, Germany). The superficial enamel layer was removed using a water-cooled rotating polishing machine (Meta-Serv 3000 Grinder-Polisher, Buehler, Lake Bluff, IL, USA) using a sequential polishing protocol; 600-grit silica carbide disc for 10 s, 1200-grit for 20 s, 2400-grit for 30 and 4000-grit for 45 s, followed by 3 min of ultrasonication. This created more consistent, reproducible artificial enamel lesions, and improved the reliability of the profilometry assessment.

Melted dental wax was applied to protect part of the enamel leaving an exposed window of 3 mm × 1 mm in the central area. WSLs were created using a previously reported bi-layer demineralisation protocol of 8% m ethylcellulose gel buffered with a lactic acid layer (0.1 mol/L, pH 4.6) for 14 days at 37 °C.

The ultra-morphology of enamel surface was checked after polishing procedures and after producing artificial WSLs using a confocal tandem scanning microscope (TSM) (Noran Instruments, Middleton, WI, USA), with a ×100/1.4 NA oil-immersion objective in reflection scanning mode, to ascertain the presence of cross-sectional enamel prisms in the intact and demineralised enamel surfaces. Samples were assigned randomly into four experimental groups, with the composition of the applied materials detailed in Table 1. Microhardness measurements of intact sound enamel in each sample were recorded to calculate any statistical differences between specimens in each group prior to any remineralisation treatment. BAG and PAA-BAG were prepared as slurries (LP...
ratio of 1 g/m), and applied without any mechanical agitation. The surface remineralisation treatments were conducted for 7 days at 37 °C and refreshed daily. Samples within each of the four test groups (n = 13) were rinsed thoroughly after treatment with de-ionised water and assigned for profilometric and scanning electron microscopic (SEM) assessments (n = 5), and for microhardness and Raman analyses (n = 8).

2.2. SEM scanning

A scanning electron microscope (FEI Co., Ltd., Cambridge, UK) was utilised to examine the ultra-structure of the lesion surface (accelerating voltage of 10 kV, working distance of 10 mm). The samples were gold sputter-coated before SEM analysis (Emitech K550, UK). Further two samples from PAA-BAG and negative control groups were sectioned, gold sputter-coated and scanned using the same parameters. The scan area included both the lesion cross section and part of its surface.

2.2.1. Surface and cross-sectional microhardness measurements

A Struers Duramin microhardness tester (Struers Ltd., Denmark) with a Knoop diamond indenter was used. A pilot study was conducted to figure out the proper parameters to assess the microhardness of the lesion; 50 g load for 10 s. The indentations were imaged with a 40/0.65 NA objective and the Knoop values were calculated using the manufacturer’s software supplied. Five measurements, 200 μm apart, were recorded and then averaged to measure the lesion surface microhardness of each sample. The samples were then hemi-sectioned using a diamond wafering blade. Each cross-sectioned surface was hand-polished up to 1200 grit to produce a flat surface. The integrity of the lesion and the flatness of the cross-sections were examined using a 40/0.65 NA objective prior to any further experimental analyses. For cross-sectional microhardness testing, three measurements, 100 μm apart and 30 μm away from the outer lesion surface were recorded and averaged within each sample.

2.3. Micro-Raman spectroscopy

A Renishaw inVia Raman microscope (Renishaw Plc, Wotton-under-Edge, UK) running in Streamline™ scanning mode was used to scan the cross-sectioned surfaces using a 785-nm diode laser (100% laser power) focused using a 20/0.45 air objective. The signal was acquired using a 600 lines/mm diffraction grating centred at 800 cm⁻¹ and a CCD exposure time of 2 s. The microscope was calibrated using an internal silicon sample with a characteristic band at 520 cm⁻¹. For each sample, a Raman map of the air/lesion/enamel interface was recorded at the middle part of the lesion. The Raman map was started at 125 μm on the outer side of the lesion (air) and extended to approximately 400 μm within the sound enamel, covering an area of 525 × 350 μm² and containing 1740 spectra acquired with a 2.7 μm resolution across the air/lesion/enamel interface. Raman maps were exported into in-house curve-fitting software to fit the spectra and to generate grey-scale images (Fig. 1A) and depth profiles of phosphate peak intensity at 959 cm⁻¹ (PO₄³⁻). The demineralised enamel produced a small amount of autofluorescence, as do most biological samples (Fig. 1B). To take this slowly varying background into consideration, the PO peak was fitted with a linear combination of a Gaussian function and a first order polynomial, as it is routinely done in Raman analysis. The fitting function was therefore the following:

\[ F(X) = AX + B + C \exp \left( \frac{-(X-D)^2}{2E^2} \right) \]

The intensity of the PO peak was given by the fitting parameter C from the above equation. The Raman analysis in the current study was based on peak ratio analysis, namely the ratio between the mineral peak within the lesion and the mineral peak within healthy enamel. This ratio was analysed by fitting the depth profiles of phosphate peak intensity objectively using a double-step function, by the means of written software (Fig. 1C), to obtain: the phosphate peak intensity percentage within the lesion to that of the deeper sound enamel (the distance between lesion and sound steps in the vertical direction), and the lesion depth (the distance between lesion and sound enamel steps in the horizontal direction).

2.4. Profilometric analysis

A standard scan area of 3 mm × 2 mm was chosen over the WSL to include the lesion in the centre (1 mm) surrounded by flat sound enamel on each side (1 mm), acting as a reference area. The sample surface was scanned before and after treatment using optical white light confocal profilometry.
Fig. 1 – (A) Representative grey-scale image of Raman phosphate peak intensity at 959 cm\(^{-1}\) including the demineralised (L) and sound enamel (S) areas of the scanned map. (B) Raman spectra of demineralised and deep sound enamel areas within the same sample. (C) Depth profile of phosphate peak intensity (broken line) fitted using double-step function (solid line).

Fig. 2 – Representative SEM images of lesion surface according to the treatment (at 50,000\(\times\) magnification). (A) Lesion surface within the negative control group exhibits porosity (arrow) with no mineral depositions. (B) Mineral precipitations with large plate-shape (star) and small cubic-shape (arrow) structures in BAG group. (C) Small plate- (star) and flake-like (arrow) structure covers and blocked the surface porosity in PAA-BAG group. (D) Small rounded-shaped particles (arrow) within the positive control group.
(Xyris™ 4000 WL, TaiCaan™, Southampton, UK) with a 10 μm step-over distance and a 10 nm vertical resolution. The resulting 3D images were analysed by levelling the sound enamel areas to a best-fit (zero plane). The step height measurement of the lesion surface in relation to the sound enamel level, which was protected by a tape throughout the treatment, was obtained by averaging five measurements within each sample.

2.5. Statistical analysis

Statistical analysis was conducted using SPSS statistical package (version 20; SPSS Inc., IBM, Chicago, IL, USA). Data were tested for normality using Q–Q plots and Shapiro–Wilk tests, and using one-way analysis of variance (ANOVA) and Tukey’s HSD post hoc tests to calculate the significant factors at $p = 0.05$.

3. Results

3.1. SEM analysis

Representative SEM images of samples from each of the four experimental groups are shown in Fig. 2. Variance was detected between the negative control and the remaining experimental groups. The lesion surface in the negative control exhibited porosity resulting from the demineralisation process, with no evidence of mineral deposition (Fig. 2A). SEM images of BAG exhibited mineral depositions with large two-dimensional, plate-like structures and small three-dimensional, cubic structures (Fig. 2B). The plate-like structures were smaller within the PAA-BAG compared to BAG group with small flake-like structures blocking completely the porous lesion surface (Fig. 2C). Small rounded particles covering the lesion surface were observed in the remineralisation solution group (Fig. 2D). The cross-sectional views of PAA-BAG (Fig. 3B) showed a layer of mineral covering the lesion surface, whilst no evidence of remineralisation was detected within the negative control group (Fig. 3A). The high magnification images of the cross-sections showed mineral structures firmly attached and embedded to the lesion surface within PAA-BAG group (Fig. 3B).

3.1.1. Surface and cross-sectional microhardness measurements

Knoop microhardness measurements of sound enamel showed consistent values within the experimental groups prior to commencing the remineralisation treatments. The sound enamel Knoop microhardness was $329.5 \pm 23.1$ KHN (mean ± SE) within the BAG group, $307.5 \pm 20.9$ KHN in the PAA-BAG group, $299.9 \pm 22.0$ KHN in remineralisation solution group and $326.1 \pm 21.4$ KHN for the negative control group.

Fig. 3 – SEM images of cross-sections within the negative control and PAA-BAG groups at $800 \times$ (left) and $10,000 \times$ (right) magnifications. The broken line determines the border between the cross-sectional view and the lesion surface (top). The lesion surface within PAA-BAG (B) covered with a layer of minerals in contrast to that of the negative control which showed no mineral precipitations (A). Higher magnification of the outer edge of lesion showed the mineral structures firmly attached to the lesion surface within PAA-BAG.
Knoop microhardness $138.3 \pm 4.7$ KHN, but with no statistically significant difference compared to PAA-BAG and remineralisation groups. The cross-sectional Knoop microhardness within the negative control group was statistically less than those in the BAG: ($p = 0.001$), PAA-BAG: ($p = 0.002$) and remineralisation solution ($p < 0.001$) groups. The highest cross-sectional Knoop microhardness was found within the remineralisation solution group ($77.3 \pm 10.6$ KHN), but with no statistically significant differences to BAG ($64.2 \pm 2.7$ KHN) and PAA-BAG ($62.2 \pm 5.1$ KHN) groups.

3.2. Micro-Raman spectroscopy

Representative Raman spectra of sound and demineralised enamel within the same sample are presented in Fig. 1B. The four internal vibration modes of phosphate ion ($\text{PO}_4^{3-}$) within the enamel were observed as peaks at $433$ cm$^{-1}$ (symmetric bending vibrational mode – $\text{PO}_4^{3-} v_3$), $579$ cm$^{-1}$ (asymmetric bending vibrational mode – $\text{PO}_4^{3-} v_4$), $959$ cm$^{-1}$ (symmetric stretching vibrational mode – $\text{PO}_4^{3-} v_1$) and $1043$ cm$^{-1}$ (asymmetric stretching vibrational mode – $\text{PO}_4^{3-} v_3$). All those peaks were observed within sound and demineralised enamel spectra with no difference in their positions, but with a considerable reduction in the peaks' intensity within the demineralised enamel compared to the sound (Fig. 1B). The strongest peak along sound and demineralised enamel spectra was that of $v_1$, $\text{PO}_4^{3-}$ at $959$ cm$^{-1}$.

The percentage of phosphate peak intensity within the lesion varied statistically significantly according to the treatments ($p = 0.01$). The means and their standard errors of lesion phosphate peak intensity percentage are presented in...
Fig. 5. The phosphate peak intensity percentage within the lesion compared to that of the deeper sound enamel in the negative control group was $38.18 \pm 1.7\%$ (mean $\pm$ SE), statistically significantly less than that of BAG group ($48.93 \pm 2.7\%$) ($p = 0.04$), PAA-BAG ($49.1 \pm 2.6\%$) ($p = 0.04$) and remineralisation solution ($50.19 \pm 3.5\%$) ($p = 0.02$). However, the treatment did not reduce the lesion depth statistically compared to that of the negative control group. The lesion depth was $(81.4 \pm 3.2\, \mu m)$ (mean $\pm$ SE) within the negative control group, $(66.7 \pm 3\, \mu m)$ in the BAG group, $(74.1 \pm 7.1\, \mu m)$ in PAA-BAG group and $(67.6 \pm 4.8\, \mu m)$ for remineralisation group. Representative grey-scale images and depth profiles of $\text{PO}_4^{2-} \nu_3$ peak intensity are presented in Fig. 5. Overall, there was a considerable drop in the depth profile in all groups within the lesion area $(125-200\, \mu m)$ compared to the deep sound enamel area $(\geq 200\, \mu m)$, which, in turn, presented similar intensity profiles within all the samples tested. The depth profiles within the negative control group exhibited larger distances between the lesion and deeper enamel steps, in the vertical direction, compared to the other groups implying that less phosphate content was present within the lesion. The depth profiles of BAG and remineralisation solution showed a sharp peak within the lesion step, whilst within PAA-BAG depth profiles the phosphate peak intensity increased along the whole lesion depth.

3.3. Profilometric analysis

Using BAG and PAA-BAG as a slurry did not damage the surface layer of the lesion as the profilometry step height difference measurement of lesion surface before and after treatment showed no statistically significant difference within all experimental groups; $(0.64 \pm 0.29\, \mu m)$ (mean $\pm$ SE) within the BAG group, $(0.78 \pm 0.24\, \mu m)$ for the PAA-BAG group, $(0.56 \pm 0.24\, \mu m)$ or within remineralisation solution group and $(0.46 \pm 0.20\, \mu m)$ in the control group.

4. Discussion

The key approach in enamel WSL remineralisation is to utilise insoluble materials containing ions required to deposit minerals similar to those of enamel and at the same time which can diffuse through the lesion.24 Biomimetic remineralisation of carious lesions has been reported using bio-active materials in the presence of protein analogues such as PAA to promote remineralisation through the lesion depth.25 BAG may enhance the remineralisation of demineralised dentine and inhibit the demineralisation of enamel.26–29 In the current study, PAA was not included in the BAG processing procedure, and therefore it was not released from BAG particles, but interacted with reacted BAG agglomerates. The concentration of PAA was selected to reduce the abrasiveness of BAG particles for further utilisation with air-abrasion technology in future studies, as well as to benefit from the potential role of PAA in regulating mineral growth.30,31

Even though the lesion surface was thoroughly rinsed prior SEM analysis, mineral deposits were readily detected within BAG and PAA-BAG groups implying that the observed structures firmly attached to lesion surface, and this attachment was detected in the cross-sectional SEM images. The plate- and cubic-like structures observed in the SEM images of BAG and PAA-BAG groups are comparable to the apatite crystals shapes of reacted BAG described in the literature.30,31 Mineral precipitations formed using PAA-BAG slurry were significantly smaller than those of BAG group and completely blocked the porosity of the lesion surface concurring with a previous study which revealed smaller structures could be monitored when PAA was used with Portland cement.31 These smaller mineral structures have a potential to penetrate the lesion surface and enhance the remineralisation along the whole lesion depth. The formation of the small structures within PAA-BAG group may be explained depending on non-classical crystallisation pathway concepts where Ca and P ions are sequestered by biomimetic analogues such as PAA to form amorphous calcium phosphate nano-precursors which in turn transform into small crystalline apatite minerals.32

Lesion surface microhardness was considerably higher than the equivalent cross-sectional measurement implying that much of the new mineral was formed and deposited in the superficial part of the lesion rather than the lesion body.33 BAG, PAA-BAG and the positive control groups exhibited higher surface and cross-sectional Knoop microhardness compared to the negative control group. Previous studies reported an increase in the mechanical properties of acid-etch enamel and demineralised dentine treated by BAG paste.34–36 This mechanical improvement could be caused as a result of “new” mineral deposition within the lesion,18 resulting from BAG 4555 bioactivity process that forms HA layers at the interface level.

The StreamLine™ Raman scanning is a high-speed line scanning system that allows faster and better excitation intensity distribution across the sample surface as it utilises the Raman microscope optics to illuminate a moving line across the sample and to read the data continuously.35 The phosphate Raman peaks were observed within Raman spectra at the same positions detected in the literature.14,36 Peak intensity evaluation has been reported as a suitable parameter to detect a difference between sound and demineralised enamel regions.37 In the present study, the phosphate peak intensity within the demineralised enamel was compared to that of the deeper sound enamel within the same sample, acting as a reference area. The lesion presented 40% phosphate peak intensity compared to the deeper sound enamel in the control group. This drop in the depth profile extended to approximately $80\, \mu m$ depth. These depth profile features, of phosphate peak intensity within artificial enamel white spot lesions, are consistent with those described in a previous study.17

BAG and PAA-BAG were applied as slurry, without mechanical agitation, to avoid any damage to the lesion structure, and this was confirmed by profilometric analysis. The profilometric results imply that the improvement in the mechanical and chemical measurements of the treated lesions occurred within the structure of the lesion and not as a result of damaging histologically, the lesion morphology and exposing the deeper intact tissue. Using BAG, PAA-BAG and remineralisation solution in the present study did not reduce the lesion depth. This result may be explained as the
calcium and phosphate ions’ diffusion/precipitation may be restricted to the superficial area of the lesion inhibiting whole lesion remineralisation. This feature has been reported in the literature when different remineralisation agents were applied to treat enamel carious lesions.\textsuperscript{38–40} To overcome this limitation, altering/modifying the lesion surface to improve mineral diffusion may still be required or even desirable.\textsuperscript{41–43}

Treating BAG particles with an aqueous solution such as saliva causes a leaching and exchanging of BAG ions with those in the solution and that in turn increases the interfacial pH followed by breaking Si–O–Si bridge to form a Si(OH)\textsubscript{2} layer. Calcium and phosphate ions are released from BAG, at this stage, to form an amorphous CaP layer, which is crystallised to a mixed hydroxyapatite apatite layer.\textsuperscript{54} Rama phosphate peak intensity percentages were significantly higher within BAG, PAA–BAG groups compared to that of the negative control group implying that more phosphate ions were presented as a result of remineralisation treatment. The bioactive process of BAG and the precipitation of minerals at the lesion surface, observed within SEM images, may explain the higher Raman phosphate peak intensity monitored in the present study.

The beneficial effect of utilising bio-active glass and polyacrylic acid-modified bio-active glass powders in enamel white spot remineralisation paves the way for further investigation into the clinical application of such materials in the remineralisation of enamel in vivo under the auspices of minimally invasive reparative dentistry which advocates the preservation of repairable enamel structure and the use of remineralisation strategies to “heal” early lesions.\textsuperscript{1}

5. Conclusions

The original null hypothesis was rejected as enamel WSLs treated with BAG and PAA–BAG exhibited improved mechanical properties and higher phosphate content compared to the negative control and presented mineral depositions formed at the lesion surface. Smaller particle precipitations were detected within PAA–BAG compared to the BAG, and therefore this modification has a potential to promote entire mineral gain of treated lesions.

5. Conclusions

The original null hypothesis was rejected as enamel WSLs treated with BAG and PAA–BAG exhibited improved mechanical properties and higher phosphate content compared to the negative control and presented mineral depositions formed at the lesion surface. Smaller particle precipitations were detected within PAA–BAG compared to the BAG, and therefore this modification has a potential to promote entire mineral gain of treated lesions.

R E F E R E N C E S


