Effect of air-abrasion pre-treatment with bioactive glass 45S5 on enamel surface loss after erosion/abrasion challenge

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ABSTRACT

Objectives. The aim of this *in vitro* study was to evaluate the effect of air-abrasion surface pre-treatment with bioactive glass (BAG) 45S5 on enamel surface loss after erosion/abrasion challenge.

Methods. Twenty-four sound bovine incisors were used for this study. Four experimental groups (n = 12) were assigned as follows: Group 1 was the negative control group, Group 2 specimens were treated with a SnF_2 gel (positive control group), Group 3 specimens were air-abraded with BAG 45S5 (ProSylc) and Group 4 received both treatments. The specimens were submitted to erosion/abrasion challenge using a common soft drink. Enamel surface loss was evaluated using an optical profilometer. Additionally, surface roughness (VSI method) and surface microhardness (Vickers method) changes were evaluated, as well as SEM and EDS analyses were performed on enamel surface. The data were statistically analyzed using one-way ANOVA and Tukey's *post-hoc* test at a level of significance $a = 0.05$.

Results. Surface pre-treatment with BAG 45S5 reduced surface loss in comparison with negative control group ($p < 0.001$), which exhibited the highest surface loss of the experimental groups ($p < 0.05$). The positive control group (SnF_2 treatment) and Group 4 specimens, which received both air-abrasion pre-treatment and SnF_2 treatment, presented the lowest surface loss ($p < 0.05$), but did not show significant differences to each other ($p = 0.65$).

Significance. Air-abrasion pre-treatment with BAG 45S5 may be beneficial as an in-office preventive method for the limitation of enamel erosive wear induced by excessive consumption of soft drinks. The clinical significance of the results regarding this preventive method should be confirmed by clinical studies.

1. Introduction

Erosive tooth wear is defined as a chemical–mechanical process which leads to a cumulative loss of hard dental tissues by acids of non-microbiological origin [1]. Erosive agents attack tooth mineral by first softening the surface, and this softening makes the tooth more susceptible to some of the abrasive forces that develop in oral cavity. The mechanism of erosive tooth wear deals with the fact that erosive demineralization on tooth surface takes place not only at the tooth surface/erosive agent interface, but also within a partly demineralized thin layer beneath the tooth surface, during a

process namely near-surface demineralization [2]. These sub-surface lesions present low mechanical properties rendering the tooth tissues vulnerable to the progress of erosive wear and also more susceptible to the caries process [3].

The etiology of erosive tooth wear includes a combination of various chemical, biological and behavioral factors which affect its pathogenesis and in different interactions of these factors are attributed the different clinical expressions in patients [4]. Prevention of erosive tooth wear includes causal therapy and an individually tailored preventive program [5]. Recently, due to changes in lifestyle a rapid increase in daily consumption of soft drinks has been observed. More often, soft drinks are very acidic (pH < 3) and because of their excessive consumption they induce dissolution of tooth surface minerals and reduction of tooth surface volume [6].

Currently, various clinical treatments have been proposed for protection of the tooth structures from erosive tooth wear by increasing their resistance to acidic attacks. Most of those treatments act by modifying the tooth surfaces in a way that hydroxyapatite crystals become less acid soluble [2] or by forming protective layers on the tooth surfaces [7]. The use of fluoride has been investigated and there is evidence that is capable of decreasing erosive tooth wear. Monovalent and polyvalent fluorides have been used for different vehicles such as mouthrinses, toothpastes, gels and varnishes. Most effective formulations were the products containing stannous fluoride (SnF_2) [1,8]. In particular, it has been reported that Sn-containing toothpastes can reduce enamel loss around 33–60% [9]. However, tooth brushing may significantly increase enamel surface loss [10].

Recently, bioactive agents such as bioactive glass (BAG) 45S5 [11], casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) [12] and nano-hydroxyapatite [13] have been introduced in various forms for prevention and remineralization of erosive lesions of the teeth. BAG 45S5 is an inorganic amorphous, calcium, sodium phospho-silicate material which contains fivefold ratio of Ca/P. It interacts with aqueous solutions such as saliva to form a hydroxycarbonate apatite layer, attached chemically to the treated surfaces [14]. BAG application on tooth tissues can release high concentrations of calcium and phosphate ions which may be capable of penetrating and remineralizing the sub-surface demineralized enamel *via* the porous enamel surfaces that was subjected to an acidic attack [15].

Therefore, the aim of this *in vitro* study was to evaluate the effect of an in-office preventive treatment against erosive tooth wear, which involves air-abrasion surface pre-treatment with BAG 45S5, on enamel susceptibility after erosion/abrasion challenge using a common soft drink. For this reason the depth of the formed lesions was estimated by measuring enamel surface loss, as well as the surfaces of the lesions were qualitatively investigated by measuring surface roughness and surface microhardness. Moreover, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) analysis of the mineral composition of the enamel were also performed for deeper analysis of the results. The null hypothesis of the study was that there were no significant differences in enamel surface loss among the experimental groups after erosion/abrasion challenge.

2.   Materials and methods

2.1.   Preparation of enamel specimens

Twenty-four sound bovine incisors were used in this study and were stored in a 0.5% chloramines T solution at 6 °C for up to 1 month prior the experiment. The crowns of the teeth were separated from the roots, and each crown was sectioned into two halves using a water-cooled diamond disc (Isomet, Buehler, Lake Bluff, IL, USA). The dimensions of each enamel specimen were 4 mm long, 4 mm wide and 1.5 mm thick. The 48 enamel specimens were not allowed to be dehydrated and examined by means of optical microscope for any surface structural defection. Subsequently, they were randomly distributed into 4 groups (n = 12) and were embedded in epoxy resin (Epofix resin, Struers Tech A/S, Denmark) with facial or lingual surface facing up. The enamel surfaces were ground and polished on a polishing machine (Jean Wirtz TG 250, Dusseldorf, Germany) with 200 rpm under water cooling (50 ml/min) using up to 1200 grit silicon carbide abrasive papers (Struers, Copenhagen, Denmark) and a 0.4 μm alumina polishing suspension, in order to form parallel planar surfaces. After polishing, the specimens were immersed in an ultrasonic bath (Euronda Spa, Montecchio Precalcino, Vicenza, Italy) for 5 min to remove any impurities and stored in a remineralizing solution for 24 h at 37 °C before the experiment. The composition of the remineralizing solution was as follows [16]: 0.103 g/l of $CaCl_2$, 0.019 g/l $MgCl_2{}^*6H_2O$, 0.544 g/l KH_2PO_4, 2.24 g/l KCl and buffer (TCP-KOH) was added to adjust the pH to 7. The specimens were stored in fresh remineralizing solution during all the experimental period.

2.2.   Formation of erosive enamel surfaces

In order to receive baseline erosive enamel surfaces, the specimens were cycled four times through alternating erosive and remineralizing solutions to induce erosive-like lesions [17]. Each cycle consisted of enamel specimen immersion in 10 ml of a cola drink (Coca Cola, 3E Company, Greece) for 2 min, followed by 2 h in the remineralizing solution. Between each cycle, the specimens were rinsed with deionized water for 10 sec and gently dried with paper towel.

2.3.   Experimental groups of the study

The four experimental groups (n = 12) of the study were submitted to one of the following treatments:

Group 1, was the negative control group of the study and the specimens did not receive any preventive treatment during the experimental period.

Group 2, was the positive control group of the study. The enamel specimens before the first erosive challenge of each day were smeared with Emofluor® gel (Dr. Wild & Co. AG, Muttenz, Switzerland), which contains 0.4% SnF_2 (Table 1) and presents RDA 18. The gel was left for 1 min on the enamel surfaces according to manufacturer's instructions and then the specimens were immersed into the remineralization solution for 1 h before the first erosive challenge of the day.

Product	Active agent (form)	Manufacturer	Composition	Lot number
Table 1 – The technical characteristics of the materials used in the present study according to manufacturers.				
ProSylc	NovaMin® (powder)	Velopex, Harlesden, UK	100% NovaMin®, particle size: 30–60–90 μm, SiO_2: 45%, CaO: 24.4%, Na_2O: 24.6%, P_2O_5: 6%	160316
Emofluor	Stannous fluoride (gel)	Dr. Wild & Co. AG, Muttenz, Switzerland	Aqua, glycerin, propylene glycol, PEG-40-hydrogenated castor oil, cellulose gum, PEG-8, phosphocolamine, aroma, SnF_2 0.4% (1000 ppmF-), sodium saccharin	6190
Colgate® Cavity Protection	Sodium fluoride, sodium monofluorophosphate (paste)	Colgate-Palmolive Co., Guildford, Surrey, UK	Dicalcium phosphate dihydrate, aqua, glycerin, sodium lauryl sulfate, cellulose gum, aroma, MFP 0.76% (1000 ppmF−), tetrasodium pyrophosphate, sodium saccharin, NaF 0.1% (450 ppmF−)	–
Coca Cola	Phosphoric acid, carbonate (solution)	Coca Cola 3E Company, Marousi, Greece	Water, sugar, carbon dioxide, caramel color E 150d, phosphoric acid, natural flavors, caffeine, pH = 2.47	–

An Aquacare™ clinical air-abrasion unit (Velopex, Harlesden, UK) was used to treat the enamel surface of the Group 3 specimens with ProSylc (Velopex, Harlesden, UK), which contains powder of BAG 45S5 (NovaMin®). Each enamel specimen received air-abrasion for 10 s in wet abrasion mode fulfilled by shrouding the air stream with a curtain of de-ionized water. The following operating parameters were selected: air pressure, 20 psi (~1.38 bar); powder flow rate dial, 1 g/min; nozzle angle, 90°; nozzle-surface distance, 5 mm and the internal nozzle diameter, 900 μm [18]. The composition of ProSylc is presented in Table 1. Air-abrasion was carried out only once at the first day, 1 h before the first erosive challenge and then the specimens were immediately immersed in fresh remineralizing solution.

Group 4 specimens received both preventive treatments described for Group 2 and Group 3 in order to investigate if there is any interaction between the two treatments.

2.4. Erosion/abrasion challenge

In the present investigation, a common soft drink (Coca Cola, 3E Company, Greece) was used as erosive agent (Table 1). The pH of the soft drink was measured using a digital pH-meter (Orion Star™ Series ISE Meter, Thermo Scientific, Beverly, USA) and was stable (pH = 2.47 ± 0.09) for at least 15 min at room temperature (23 ± 1°C). The enamel specimens were demineralized 6 times per day for 10 days following the protocol of Schlueter et al. [19]. More specifically, each enamel specimen was removed from the remineralizing solution, rinsed with deionized water for 10 s and air-dried for 5 s. Subsequently, each specimen was immersed into 30 ml of fresh soft drink in a plastic container for 2 min, at 25°C and then rinsed again with deionized water for 20 s, air-dried for 5 s and stored in fresh remineralizing solution at 37°C. After the first and the last erosive challenge of the day an abrasion challenge was followed using a commercial electric toothbrush (Oral-B®, Braun, France). This means that the abrasive challenge was performed twice a day for 10 days. The parameters of the tooth brushing were as follows: load of the toothbrush, standardized at 200 g; medium hardness, round toothbrush head; rotation rate, 7500 rpm and time of brushing for each enamel specimen, 5 s/abrasive challenge resulting in 10 s per day. The electric toothbrush was fixed in a constructed device

that allowed the heads of the brushes to be aligned parallel to the surface of the specimens and to control the pressure by an electric system. The toothbrush head was in direct contact with the specimen and Colgate® Total toothpaste (Table 1), with RDA 70, was used to form slurry that consisted of 1:1 (w/W) deionized water and toothpaste. The specimens were kept in the slurry for 2 min during which time the brushing procedure was performed (5 s each specimen). Subsequently, the specimens were rinsed with deionized water for 20 s, air-dried for 5 s and immersed into remineralizing solution at 37°C [20].

2.5. Surface loss measurements

Before air-abrasion treatment and erosion/abrasion cycles, half of each enamel surface of the specimens was covered with one-sided silver adhesive tape (Wonder® Tape, P.V.C. Electrical Tape). Measurements for surface loss were carried out after air-abrasion treatment and following erosion/abrasion challenge using an optical profilometer (Bruker, ContourGT, Berlin, Germany). After removal of the adhesive tape four images (magnification ×20) were obtained from the centre of each specimen's surface corresponding to a surface of 0.317 × 0.238 mm². The enamel surface loss was calculated after superimposing the baseline and post-treatment profiles. The depth of the lesion for each specimen was calculated based on the subtraction of the two profiles. Five measurements were performed for each image (40 μm apart) and data were averaged and reported in μm.

2.6. Surface roughness measurements

Surface roughness analysis of the enamel specimens was performed according to ISO 25178 (non-contact type) which is related to the analysis of 3D areal surface textures. The measurements were carried out prior and after the erosion/abrasion challenge and additionally in Groups 3 and 4 after air-abrasion treatment using a Vertical Scanning Interference (VSI) microscope (Bruker, ContourGT, Berlin, Germany). Three images were obtained (magnification ×20) from each specimen at the four quadrants of the enamel surface which correspond to a surface of 0.317 × 0.238 mm². Vision64™ software (Bruker, ContourGT, Berlin, Germany) was used to

Table 2 – Means and standard deviations (n = 12) of surface loss (μm) of the experimental groups of the study after erosive/abrasion challenge. Same uppercase superscripts in columns indicate no significant differences among treatments ($p > 0.05$).

Groups	Treatments	Surface loss (μm) after air-abrasion treatment	Surface loss (μm) after erosion/abrasion challenge
1	No treatment (− control)	–	24.8 ± 4.2^A
2	SnF_2 gel (+ control)	–	15.9 ± 2.4^B
3	Bioglass air-abrasion	2.4 ± 0.6	18.6 ± 3.5^C
4	Bioglass air-abrasion + SnF_2 gel	2.6 ± 0.4	15.2 ± 1.7^B

acquire the data and compute the mean surface roughness in S_q units of each image. The values of the twelve images of each specimen were averaged and the mean value was calculated.

2.7. Surface microhardness measurements

Surface microhardness of each enamel specimen was evaluated prior and after the erosion/abrasion challenge. In Groups 3 and 4, surface microhardness was also evaluated after air-abrasion treatment. The method used for the assessment of surface microhardness was the Vickers method with a hardness tester (HMV-2000, Shimadzu, Tokyo, Japan) at a load of 200 g and indentation time of 10 s. Five indentations were made on the top surface of each enamel specimen, one in the centre and one in every quadrant (500 μm apart). Data were independently averaged and reported in Vickers Hardness Numbers (VHN).

2.8. SEM and EDS analysis

Aiming to investigate the modification of enamel surface prior and after the erosion/abrasion cycling, ten specimens of each experimental group were prepared. The first five specimens (n = 5) of each group were observed prior the erosion/abrasion cycling and the other five (n = 5) after the end of the procedure. The specimens were mounted on aluminum stubs, sputter coated with carbon to a thickness of approximately 200 Å in a vacuum evaporator (at low vacuum) and examined under Scanning Electron Microscope (JEOL Ltd, JSM-840, Tokyo, Japan) at accelerated voltage of 20 kV. Four photomicrographs were performed (one in every quadrant) at ×1000 magnification on the surface area of the enamel specimens in order to detect any changes in surface morphology after the treatments. The mineral composition of the enamel surface of each photomicrograph was evaluated using Energy Dispersive X-ray Spectroscopy (EDS).

2.9. Statistical analysis

The sample size of each testing was calculated considering 80% power and a significance level of 0.05. The data were statistically analyzed using SPSS Statistics 20.0 software (IBM Corp, ILL, Chicago, USA). Data were preliminary tested for normality and homogeneity using Shapiro–Wilk test and Levene test, respectively. Surface loss, surface roughness and surface microhardness data of the enamel specimens were statistically analyzed using one-way ANOVA and Tukey's *post-hoc* test was used to detect statistical differences at a level of significance $a = 0.05$. Elemental composition of

the enamel was analyzed using Wilcoxon signed rank and Kruskal–Wallis tests and the level of significance was preset at $a = 0.05$.

3. Results

3.1. Surface loss

Means and standard deviations of surface loss (μm) of the experimental groups of the study after erosion/abrasion challenge are shown in Table 2. In Fig. 1, a representative topographic surface map at magnification ×20 and surface analysis of an enamel specimen is presented. Surface loss was detected in all experimental groups after erosion/abrasion challenge. Surface pre-treatment with ProSylc significantly reduced surface loss in comparison with negative control group ($p < 0.001$), which exhibited the highest surface loss of the experimental groups of the study ($p < 0.05$). The positive control of the study (SnF_2 treatment) and Group 4 specimens, which received both air-abrasion pre-treatment with ProSylc and SnF_2 gel treatment, presented the lowest surface loss ($p < 0.05$), but did not show significant differences to each other ($p = 0.65$). Air-abrasion pre-treatment induced around 2.5 μm enamel surface loss.

3.2. Surface roughness

Means and standard deviations of surface roughness expressed in S_q (μm) of the experimental groups of the study before and after erosion/abrasion challenge are shown in Table 3. The 3D topographic surface map at magnification ×20, the contour histogram and the contour bearing ratio of a representative specimen are illustrated in Fig. 2. Surface roughness was significantly decreased in all experimental groups after erosion/abrasion challenge ($p < 0.05$), except for Group 4, which did not present statistically significant change ($p = 0.81$). This slight decrease in Groups 1–3 was similar and ranged between 5.4% and 6.8%. In Groups 3 and 4, enamel surface roughness was not changed significantly after air-abrasion pre-treatment ($p = 0.45$) and ($p = 0.68$), respectively.

3.3. Surface microhardness

Means and standard deviations of surface microhardness expressed in VHN of the experimental groups of the study before and after erosion/abrasion challenge are shown in Table 4. Surface microhardness was significantly reduced in all experimental groups after erosion/abrasion challenge
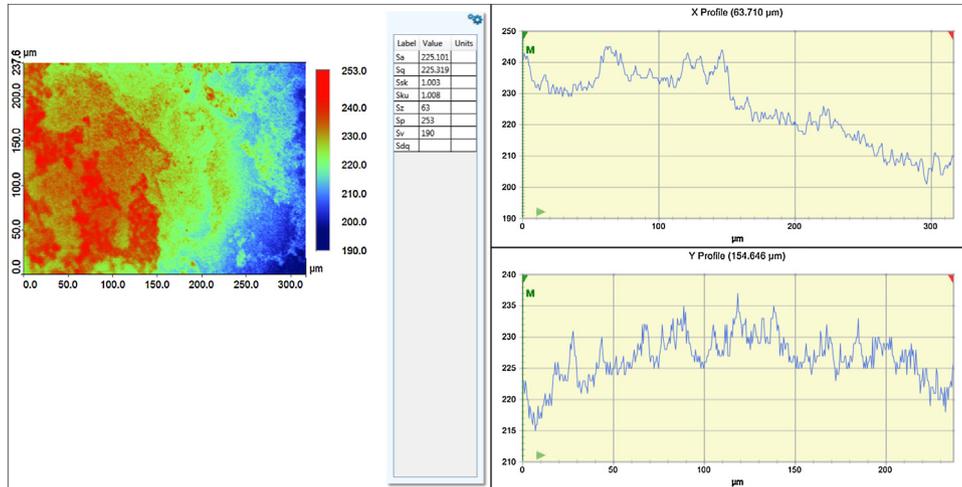
Fig. 1 – Representative topographic surface map (magnification ×20) and surface analysis of an enamel specimen (0.317 × 0.238 mm²) which were used for calculating surface loss. This surface area was in the centre of the specimen in order to observe baseline surface and surface after the abrasion/erosion challenge.

Table 3 – Means and standard deviations (n = 12) of surface roughness (S_q, μm) of the experimental groups of the study before and after erosive/abrasion challenge. Same uppercase superscripts in columns indicate no significant differences among experimental groups ($p > 0.05$). Same lowercase superscripts in rows indicate no significant differences between before and after erosive/abrasion challenge ($p > 0.05$).

| Groups	Treatments	Before air-abrasion treatment	Before erosion/ abrasion challenge	After erosion/ abrasion challenge	Reduction (%) after erosion/ abrasion challenge
1	No treatment (− control)	–	0.221 ± 0.008^{Aa}	0.207 ± 0.010^{Ab}	6.3%
2	SnF$_2$ gel (+ control)	–	0.223 ± 0.002^{Aa}	0.211 ± 0.011^{Ab}	5.4%
3	Bioglass air-abrasion	0.216 ± 0.009^{a}	0.220 ± 0.008^{Aa}	0.205 ± 0.012^{Ab}	6.8%
4	Bioglass air-abrasion + SnF$_2$ gel	0.214 ± 0.011^{a}	0.221 ± 0.006^{Aa}	0.216 ± 0.008^{Aa}	2.3%
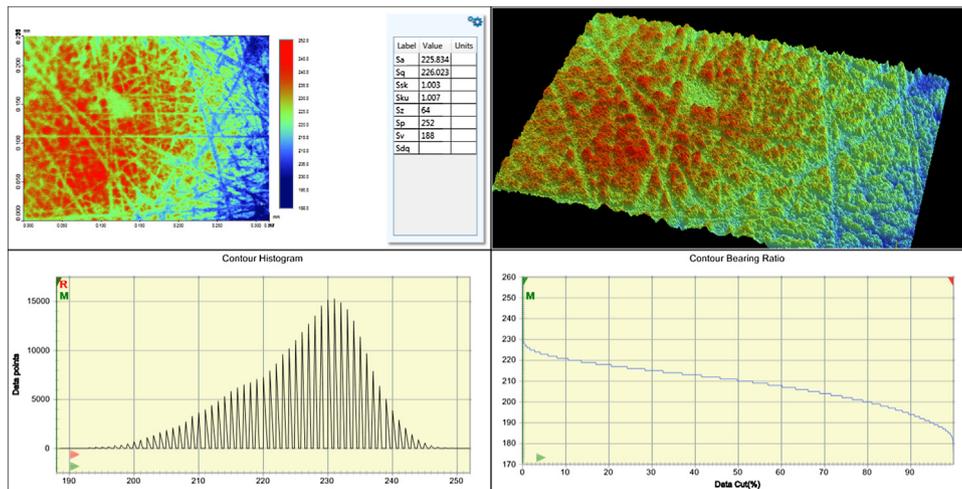
Fig. 2 – Representative topographic surface map (×20 magnification) which correspond to 0.317 × 0.238 mm² surface area of an enamel specimen and was used for calculating surface roughness. Surface roughness units (S_a, S_q, S_{sk}, S_{ku}, S_z, S_p and S_v), 3D surface map, contour histogram and contour bearing ratio of the same surface were also illustrated.

($p < 0.05$). The highest reduction in surface microhardness exhibited the negative control group of the study, while the lowest presented Groups 3 and 4 which received air-abrasion pre-treatment ($p < 0.05$) but did not present significant differences to each other ($p = 0.88$). In Groups 3 and 4, enamel surface microhardness was not changed significantly after air-abrasion pre-treatment ($p = 0.72$) and ($p = 0.54$), respectively.

Table 4 – Means and standard deviations (n = 12) of surface microhardness (VHN) of the experimental groups of the study before and after erosion/abrasion challenge. Same uppercase superscripts in columns indicate no significant differences among experimental groups (p > 0.05). Same lowercase superscripts in rows indicate no significant differences between before and after erosion/abrasion challenge (p > 0.05).

| Groups	Treatments	Before air-abrasion treatment	Before erosion/ abrasion challenge	After erosion/ abrasion challenge	Reduction (%) after erosion/ abrasion challenge
1	No treatment (− control)	–	253.8 ± 30.9^{Aa}	118.6 ± 19.5^{Ab}	53.3%
2	SnF_2 gel (+ control)	–	247.9 ± 19.0^{Aa}	138.3 ± 17.1^{Bb}	44.2%
3	Bioglass air-abrasion	247.4 ± 21.7^{a}	243.6 ± 25.4^{Aa}	160.4 ± 38.1^{Cb}	34.1%
4	Bioglass air-abrasion + SnF_2 gel	227.5 ± 25.2^{a}	225.6 ± 31.1^{Ba}	170.5 ± 36.0^{Cb}	24.4%
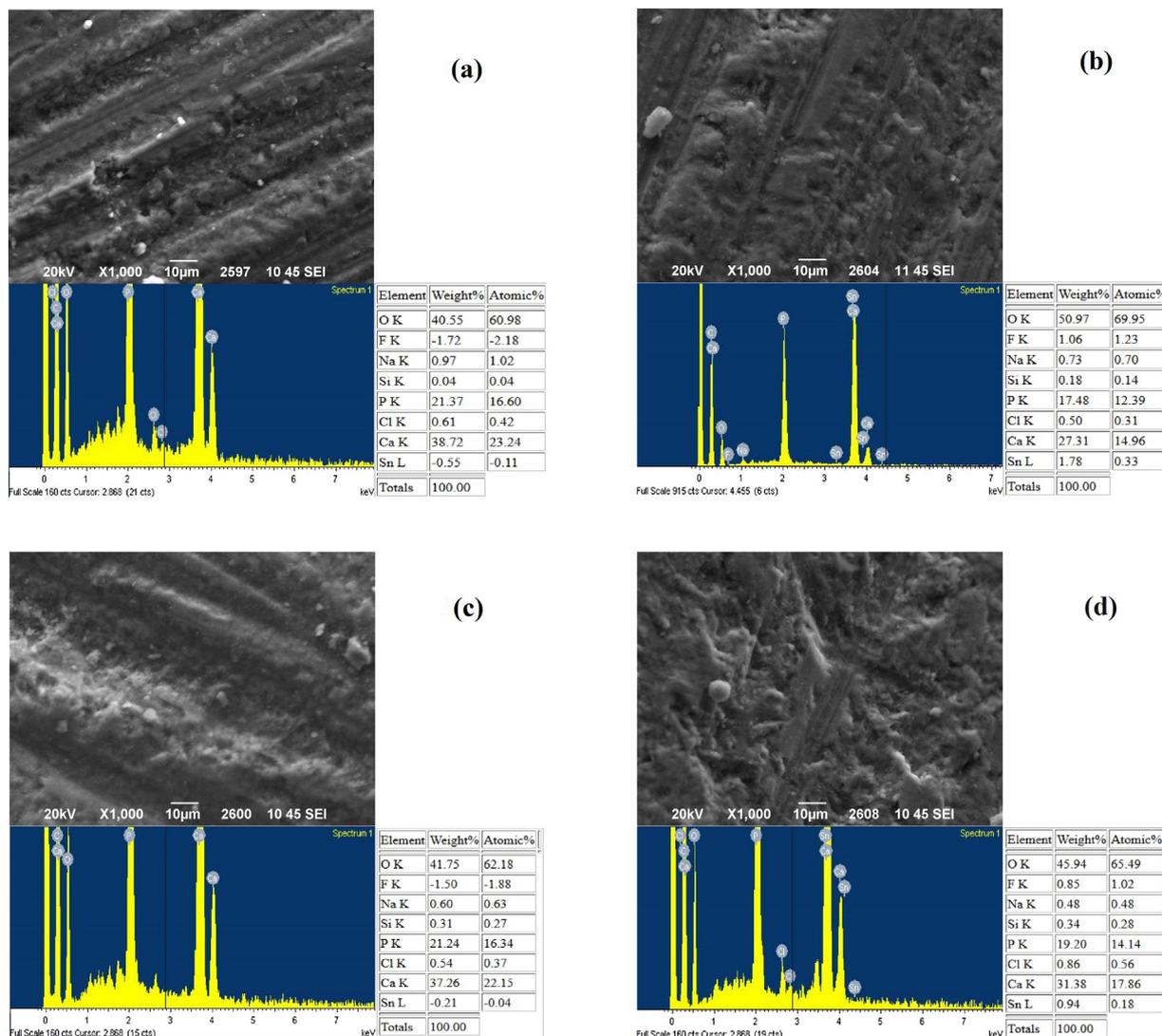
Fig. 3 – (a–d) Representative photomicrographs (magnification ×1000) of enamel surface of each experimental group of the study after the treatments and before erosion/abrasion challenge. EDS spectrum and analytical composition of the elements of each representative photomicrograph also appear below the SEM images. (a) Group 1, (b) Group 2, (c) Group 3 and (d) Group 4.

3.4. SEM observations and EDS analysis

Representative photomicrographs of the enamel surface of the experimental groups of the study before the erosion/abrasion challenge are illustrated in Fig. 3(a–d) and after the ero-sion/abrasion challenge in Fig. 4(a–d). Observations of the SEM images revealed alterations in enamel surface morphology in Groups 3 and 4 after air-abrasion with bioglass particles. More specifically, deposits of inorganic spherical particles were detected. After erosion/abrasion challenge all the exper-
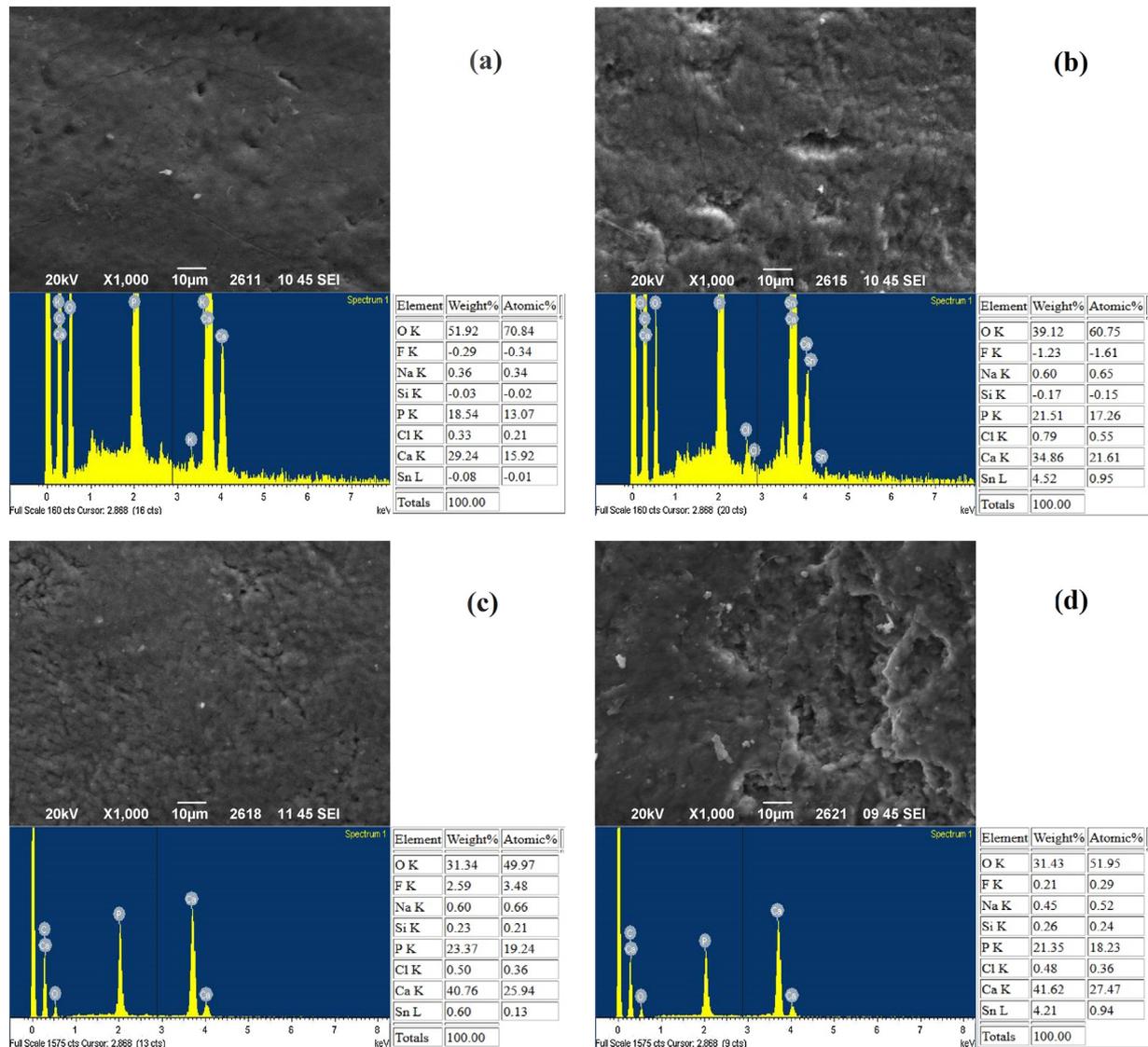
Fig. 4 – (a–d) Representative photomicrographs (magnification ×1000) of enamel surface of each experimental group of the study after erosion/abrasion challenge. EDS spectrum and analytical composition of the elements of each representative photomicrograph also appear below the SEM images. (a) Group 1, (b) Group 2, (c) Group 3 and (d) Group 4.

imental groups presented smoother enamel surfaces where the enamel prisms were visible in most cases. The smoother enamel surfaces confirm the results mentioned above for surface roughness analysis before and after erosion/abrasion challenge.

EDS spectrum and analytical composition of the elements of each representative photomicrograph of the enamel surface of each group also appear below the SEM images. Means and standard deviations of elemental content (%wt) of enamel surface before and after erosion/abrasion challenge for each experimental group are shown in Tables 5 and 6, respectively. EDS analysis revealed increased content of Sn in Groups 2 and 4 which were treated with SnF_2 gel ($p < 0.05$). Moreover, in Groups 3 and 4 increased content of Si was detected ($p < 0.05$), confirming the existence of bioglass particles on enamel surface. After the erosion/abrasion challenge the negative control group presented decreased content of Ca and P compared to

the other experimental groups ($p < 0.05$), which did not show alterations in Ca and P content.

4. Discussion

Based on the results of the present study the null hypothesis, which stated there were no significant differences in surface loss among the experimental groups after erosion/abrasion challenge, was rejected. Surface loss after erosion/abrasion challenge was detected in all experimental groups of the study and ranged between 15.2 ± 2.7 and $24.8 \pm 4.2\,\mu m$. However, the tested anti-erosive treatments of the study reduced significantly surface loss after the erosion/abrasion challenge compared to the negative control group. This is in agreement with previous investigations regarding bioactive glass [11,15] and stannous fluoride [7,9] treatments against enamel erosion.

Table 5 – Means and standard deviations (n = 5) of elemental content (%wt) of enamel surface before erosion/abrasion challenge for each experimental group of the study. Different lowercase superscripts in rows indicate statistically significant difference (p < 0.05).

Elements	Group 1	Group 2	Group 3	Group 4
Ca	38.63 ± 3.37[a]	36.12 ± 7.13[ab]	37.42 ± 3.20[a]	34.91 ± 5.40[b]
P	19.81 ± 3.77[a]	19.74 ± 2.93[a]	20.44 ± 3.81[a]	19.29 ± 3.42[a]
Si	0.02 ± 0.01[a]	0.10 ± 0.04[b]	0.38 ± 0.11[c]	0.41 ± 0.09[c]
Na	0.87 ± 0.12[a]	0.79 ± 0.11[a]	0.64 ± 0.12[b]	0.56 ± 0.13[b]
Sn	0.00 ± 0.00[a]	1.89 ± 0.13[b]	0.00 ± 0.00[a]	1.14 ± 0.21[c]
Cl	0.68 ± 0.11[a]	0.59 ± 0.09[b]	0.56 ± 0.07[b]	0.74 ± 0.13[a]
F	0.12 ± 0.06[a]	1.27 ± 0.19[b]	0.07 ± 0.04[a]	0.82 ± 0.08[c]
O	39.87 ± 2.66[a]	39.62 ± 4.25[a]	40.49 ± 3.02[a]	42.13 ± 4.88[a]

Table 6 – Means and standard deviations (n = 5) of elemental content (%wt) of enamel surface after erosion/abrasion challenge for each experimental group of the study. Different lowercase superscripts in rows indicate statistically significant difference (p < 0.05).

Elements	Group 1	Group 2	Group 3	Group 4
Ca	29.61 ± 3.71[a]	34.21 ± 3.04[b]	40.63 ± 4.44[c]	41.53 ± 3.72[c]
P	18.61 ± 2.16[a]	20.75 ± 2.73[ab]	22.37 ± 3.00[b]	21.16 ± 2.41[b]
Si	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.26 ± 0.05[b]	0.30 ± 0.05[b]
Na	0.40 ± 0.08[a]	0.57 ± 0.10[b]	0.62 ± 0.06[b]	0.45 ± 0.06[a]
Sn	0.00 ± 0.00[a]	4.48 ± 0.31[b]	0.00 ± 0.00[a]	4.27 ± 0.42[b]
Cl	0.36 ± 0.05[a]	0.74 ± 0.08[b]	0.52 ± 0.06[c]	0.49 ± 0.05[c]
F	0.00 ± 0.00[a]	0.26 ± 0.03[b]	2.55 ± 0.09[c]	0.23 ± 0.04[b]
O	51.02 ± 4.15[a]	38.99 ± 3.25[b]	33.05 ± 3.08[c]	31.57 ± 4.69[c]

As Schlueter et al. [19] claim, there is no standard for evaluating anti-erosive/anti-abrasive treatments, making the comparison of study outcomes difficult. In the present study, a very common commercial soft drink was tested as erosive agent, which is broadly used all over the world and has been used in various previous studies [1,12]. Additionally, erosion/abrasion challenge of the current study, which corresponds to excessive consumption of the tested soft drink, was adopted from previous investigations [19,20], as well as the protocol for air-abrasion pre-treatment [18].

In the present study, a remineralizing solution was used as a substitute for human saliva. This formula has been used in previous laboratory studies for the same purpose [16,21]. The use of human saliva is very difficult due to its variable composition and lack of stability outside the oral cavity. The main purpose of using synthetic preparations is to obtain a stable environment and standardized procedures in laboratory tests across a wide range of biological studies [22].

Another limitation of the present in vitro study was the use of bovine teeth. Bovine teeth are more readily available and have a more uniform composition when compared to human teeth, thus providing a less variable response to erosive challenges. Although bovine enamel is more porous than human enamel, which results to faster demineralization and remineralization processes, these differences are mainly quantitative and not qualitative in behavior [23]. Thus, bovine enamel can be considered an acceptable alternative to human enamel and is very commonly used in laboratory studies for evaluating dental erosion [24–27].

Preventive measures against erosive tooth wear are usually suggested when there is early diagnosis of erosive tooth lesions. Different enamel lesion depths may have different susceptibility to demineralization. Carvalho et al. [26], who focused on how dental erosion progress differs between sound teeth and teeth already presenting clinical signs of erosive

tooth wear, reported that the teeth already had erosive signs presented different demineralization patterns than originally sound teeth after further in vitro erosion. In this context, the tested preventive treatments of the present study were applied to erosive enamel surfaces forming according to a protocol of a previous study [17].

In the current investigation, air-abrasion pre-treatment with bioglass particles reduced significantly enamel surface loss after erosion-abrasion challenge compared to the negative control group. Bioactive materials, such as bioactive glass, have been broadly investigated for multiple applications in dentistry in recent years due to their beneficial properties [28]. Bioactive glasses are surface reactive glass-ceramic biomaterials and include BAG 45S5. When it is in contact with tooth surfaces and saliva under acidic conditions, a series of chemical reactions are initiated (involving release of calcium and phosphate ions) and the formation of a hydroxycarbonate apatite layer takes place and chemically bonded to the tooth surfaces [29]. The bioactive cycle of 45S5 bioglass needs at least 2 h to be completed [15] and the forming layer could act as a protective barrier to acidic attacks enhancing the resistance to dissolution of enamel. More specifically, after air-abrasion treatment and during erosion/abrasion cycle calcium, phosphate, and sodium crystals leach out of the bioglass network into the aqueous acidic solution, while calcium and phosphate ions are released from the underlying demineralized enamel. Sodium is washed out by the aqueous solution, whereas the phosphate ions released from enamel and 45S5 bioglass react with the calcium ions to form acidic calcium-phosphate salts (i.e., brushite), which precipitate on the enamel surface [30]. The silica network of the 45S5 bioglass reacts with hydroxyl ions released from aqueous storage media to form silanol compounds [31], which are soluble in water and this may be the explanation why only trace amounts of silica were detected upon elemental analysis by EDS.

The aforementioned protective layer is rich in calcium, phosphorus and silica and attached to the enamel surface after bioglass paste application providing a large reservoir of Ca and P ions ready to form hydroxyapatite in acidic conditions [15]. However, this positive effect of bioglass application may be counteracted when the physical forces from toothbrushing are added and this is the reason of using an erosion/abrasion challenge in the current study. Generally, bioactive glass 45S5 has been introduced in preventive dentistry due to its unique properties such as antimicrobial and remineralizing activities [32], as well as selective removal of softer dISEased/damaged tooth tissues [33]. These activities may contribute to the better behavior of the specimens treated with ProSylc compared to those of negative control group in the present investigation.

Milly et al. [18] reported that enamel air-abrasion using polyacrylic acid-BAG abrasive powder (2-8-17 μm), which contains 60 wt% BAG 45S5 powder and 40 wt% polyacrylic acid powder, removed approximately 5 μm enamel tissue. Although this surface loss was not clinically significant [34], air-abrasion pre-treatment increased the average surface roughness. Nevertheless, in the present study the same preventive technique removed approximately 2.5 μm enamel tissue and did not change enamel surface roughness significantly, which may be attributed to different composition of the bioglass powder used (i.e., wt% bioglass, size and distribution of particles), different enamel substrate or other differences in the experimental parameters. It is interesting to mention that in the current study, the removal of 2.5 μm enamel tissue from the surface was included in the total surface loss of air-abrasion pre-treatment (Groups 3 and 4) and as a result, the surface loss attributed only to erosion/abrasion challenge might be less.

Notwithstanding enamel specimens pre-treated with bioglass presented less surface loss in comparison with those of negative control group, they also exhibited higher surface loss than those treated with SnF$_2$ gel (positive control). Application of SnF$_2$ gel daily by the patient is currently considered as the most effective preventive method for erosive tooth wear [9] and for this reason was selected as positive control of the present study. Stannous fluoride has been reported to impart significant resistance to enamel mineral against acidic attack [35]. This anti-erosive activity is believed to be driven by the strong affinity of Sn ions for the mineral surface on enamel [36]. Faller et al. [37] found that after an aggressive erosive challenge, SnF$_2$ deposits a barrier layer onto the pellicle-coated enamel surface, which attaches onto acid challenged tooth surfaces. Furthermore, it has been demonstrated that the bond between F and Ca resulting from treatment with other F salts was weaker than that of the SnF$_2$-treated enamel and that the bond between the stabilized SnF$_2$ and the enamel surface is more than simple attachment between F and exposed Ca sites on the tooth surface [37]. In the present investigation, SnF$_2$-treated specimens exhibited the lowest enamel surface loss which may be attributed to the above findings. Sakae et al. [27] reported that viscosity and frequency of application of fluoride/tin solutions affect the progression of enamel erosion. In the present study, a highly-viscous gel that contains 0.4% SnF$_2$ was selected as a positive control and was applied once a day according to manufacturer's instructions.

Air-abrasion with bioglass particles is an in-office treatment which has been recommended for protecting and remineralizing enamel surface from acidic attacks [11,15,18]. As an in-office treatment provides advantages because it is applied exclusively by the dentist and lasts much shorter time compared to SnF$_2$ treatment; its effectiveness strictly depends on patient's discipline during treatment. Therefore, a possible therapeutic scheme should be a combination of the two treatments. Nevertheless, in the present study this combination did not yield better results compared to SnF$_2$ treatment alone regarding enamel surface loss, which means that for patients that show consistency to SnF$_2$ treatment they may not need an additional in-office preventive treatment.

The experimental groups of the study presented a slight reduction in enamel surface roughness after erosion/abrasion challenge, except for Group 4 which did not show any change. Previous studies have described various morphological changes of enamel surface following acidic attack resulting from the collapse of demineralized apatite crystals which lead to changes in surface roughness [38]. These changes depend mainly on the type of erosive challenge used and probably they do not influence the progress of erosive tooth wear because there is no such evidence in literature.

In the current investigation after erosion/abrasion challenge all the experimental groups of the study exhibited lower microhardness than that observed initially. Reduction in surface microhardness may be due to loss of minerals of the enamel surface, which is attributed to the erosive action of the soft drink. This evidence coincides with the results of previous reports [12,34]. The tested preventive treatments reduced surface microhardness drop compared to the negative control group. Most effective was the combination of the two tested preventive treatments, followed by air-abrasion pre-treatment with BAG and then by SnF$_2$ treatment. These discrepancies may be attributed to the remineralizing effect and protecting layer that may form BAG particles which were deposited on enamel surface [39]. Bakry et al. [15] found that the tested 45S5 bioglass paste can efficiently improve microhardness of the eroded enamel surface and agree with the outcomes of previous studies [39–41]. In contrast, Wang et al. [42] reported that the use of a slurry containing 7.5% W/W Novamin® was not advantageous for enamel surface nanohardness after erosive challenge.

SEM observations revealed alterations on enamel surface after air-abrasion pre-treatment compared to the negative control. In particular, a layer of BAG particles was observed and identified by EDS method (increased Si content) in Groups 3 and 4, implying that an amount of BAG particles remains on enamel surface. Previous studies also reported morphological changes on enamel surface after BAG paste application [15,42]. More specifically, Bakry et al. [15] who focused on the effect of a BAG paste on eroded enamel surface, observed a newly formed crystalline layer which was rich in calcium and phosphate with the presence of trace amounts of silica covering the whole surface of enamel. Taha et al. [39] reported that following BAG air-abrasion, mineral deposits were detected on the remineralized enamel surfaces, forming a protective layer which was rich in calcium, phosphate, and fluoride, while using ^{19}F MAS-NMR confirmed the formation of fluorapatite, which is more chemically stable than hydroxyapatite and has

greater resistance to acidic attacks. Additionally, in Groups 2 and 4 specimens, which were treated with SnF_2 gel, increased content of Sn was detected, which means that an amount of Sn ions was also retained on enamel surface after the treatment. This finding agrees with the results of previous studies [7,36].

Following the erosion/abrasion challenge, the content of Ca and P was reduced in negative control group. This evidence may be explained by the demineralization of enamel surface due to the erosive activity of the components of the soft drink. In the other groups this reduction was not observed may be due to the protective and remineralizing effects of BAG 45S5 and SnF_2. This was also supported by the results of surface microhardness measurements of the study, which revealed higher surface microhardness drop in negative control group than the other groups after the erosion/abrasion challenge. The mentioned changes in morphology and composition of enamel surface confirm the findings of the study which indicate different behavior of the experimental groups during the erosion/abrasion challenge.

5. Conclusion

Within the limitations of this *in vitro* study it could be concluded that air-abrasion pre-treatment with BAG 45S5 may be beneficial as an in-office preventive technique for the limitation of erosive tooth wear induced by excessive consumption of soft drinks. However, currently none of the available preventive treatments provided complete protection against the development of erosive tooth wear, which means that they should be suggested as a part of an individually tailored preventive program including measures such as diet modification, instructions of oral hygiene, and regular dental supervision. The main efforts for the prevention of erosive tooth wear should be focused on the identification and elimination of the causal factors. Nevertheless, this is not always possible and thus, recommendation of preventive treatments such as those tested in the current study should be valuable. Further studies, mainly in clinical conditions, are necessary to clarify whether this protective effect of the tested preventive treatment is clinically significant and if it is possible to improve its effectiveness.

Acknowledgments

REFERENCES

[1] Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, et al. Consensus report of the European Federation of Conservative Dentistry: erosive tooth wear—diagnosis and management. Clin Oral Investig 2015;19:1557–61.

[2] Shellis RP, Barbour ME, Jesani A, Lussi A. Effects of buffering properties and undissociated acid concentration on dissolution of dental enamel in relation to pH and acid type. Caries Res 2013;47:601–11.

[3] Amaechi BT, Higham SM. Eroded enamel lesion remineralization by saliva as a possible factor in the site-specificity of human dental erosion. Arch Oral Biol 2001;46:697–703.

[4] Lussi A, Hellwig E, Ganns C, Jaeggi T. Buonocore memorial lecture. Dent Erosion Oper Dent 2009;34:251–62.

[5] Esteves-Oliveira M, Wollgarten S, Liebegall S, Jansen P, Bilandzic M, Meyer-Lueckel H, et al. A new laser-processing strategy for improving enamel erosion resistance. J Dent Res 2017;96:1168–75.

[6] Poggio C, Lombardini M, Dagna A, Chiesa M, Bianchi S. Protective effect on enamel demineralization of a CPP-ACP paste: an AFM in vitro study. J Dent 2009;37:949–54.

[7] Ganss C, von Hinckeldey J, Tolle A, Schulze K, Klimek J, Schlueter N. Efficacy of the stannous ion and a biopolymer in toothpastes on enamel erosion/abrasion. J Dent 2012;40:1036–43.

[8] Huysmans MC, Young A, Ganns C. The role of fluoride in erosion therapy. Monogr Oral Sci 2014;25:230–43.

[9] Ganss C, Marten J, Hara AT, Schlueter N. Toothpastes and enamel erosion/abrasion-impact of active ingredients and the particulate fraction. J Dent 2016;54:62–7.

[10] Magalhaes AC, Wiegand A, Buzalaf MA. Use of dentifrices to prevent erosive tooth wear: harmful or helpful? Braz Oral Res 2014;28(Spec No):1–6.

[11] Johnson King O, Milly H, Boyes V, Austin R, Festy F, Banerjee A. The effect of air-abrasion on the susceptibility of sound enamel to acid challenge. J Dent 2016;46:36–41.

[12] Wang CP, Huang SB, Liu Y, Li JY, Yu HY. The CPP-ACP relieved enamel erosion from a carbonated soft beverage: an in vitro AFM and XRD study. Arch Oral Biol 2014;59:277–82.

[13] Min JH, Kwon HK, Kim BI. Prevention of dental erosion of a sports drink by nano-sized hydroxyapatite in situ study. Int J Paediatr Dent 2015;25:61–9.

[14] Thompson ID, Hench LL. Mechanical properties of bioactive glasses, glass–ceramics and composites. Proc Inst Mech Eng H 1998;212:127–36.

[15] Bakry AS, Marghalani HY, Amin OA, Tagami J. The effect of a bioglass paste on enamel exposed to erosive challenge. J Dent 2014;42:1458–63.

[16] da Costa Soares MU, Aaújo NC, Sales WS, Sobrar AP. Impact of remineralizing agents on enamel microhardness recovery after in-office tooth whitening therapies. Acta Odontol Scand 2013;71:343–8.

[17] Rocha CT, Turssi CP, Rodrigues-Junior AL, Corona SAM. Impact of CO_2 laser and stannous fluoride on primary tooth erosion. Lasers Med Sci 2016;31:567–71.

[18] Milly H, Festy F, Andiappan M, Watson TF, Thompson I, Banerjee A. Surface pre-conditioning with bioactive glass air-abrasion can enhance enamel white spot lesion remineralization. Dent Mater 2015;31:522–33.

[19] Schlueter N, Lussi A, Tolle A, Ganns C. Effects of erosion protocol design on erosion/abrasion study outcome and on active agent (NaF and SnF2) efficacy. Caries Res 2016;50:170–9.

[20] Gyurkovics M, Baumann T, Carvalho TS, Assuncao CM, Lussi A. In vitro evaluation of modified surface microhardness measurement, focus variation 3D microscopy and contact stylus profilometry to assess enamel surface loss after erosive/abrasive challenges. PLoS One 2017;12:e0175027.

[21] Dionysopoulos D, Strakas D, Koliniotou-Koumpia E, Koumpia E. Effect of Er,Cr:YSGG laser irradiation on bovine enamel surface during in-office tooth bleaching ex vivo. Odontology 2017;105:320–8.

[22] Pytko-Polonczyk J, Jakubik A, Przeklasa-Bierowiec A, Muszynska B. Artificial saliva and its use in biological experiments. J Physiol Pharmacol 2017;68:807–13.

[23] Buzalaf MAR, Hannas AR, Magalhaes AC, Rios D, Honorio HM, Delbem ACB. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. J Appl Oral Sci 2010;18:316–34.

[24] Aykut-Yetkiner A, Attin T, Wiegand A. Prevention of dentine erosion by brushing with anti-erosive toothpastes. J Dent 2014;42:856–61.

[25] Dionysopoulos D, Tolidis K, Strakas D, Sfeikos T. Evaluation of a clinical preventive treatment using Er,Cr:YSGG (2780 nm) laser on the susceptibility of enamel to erosive challenge. Lasers Med Sci 2018, http://dx.doi.org/10.1007/s10103-018-2679-2 [Epub ahead of print].

[26] Carvalho TS, Baumann T, Lussi A. Does erosion progress differently on teeth already presenting clinical signs of erosive tooth wear than on sound teeth? An in vitro pilot trial. BMC Oral Health 2017;17:14.

[27] Sakae LO, Bezerra SJC, Joao-Souza SH, Borges AB, Aoki IV, Aranha ACC, et al. An in vitro study on the influence of viscosity and frequency of application of fluoride/tin solutions on the progression of erosion of bovine enamel. Arch Oral Biol 2018;89:26–30.

[28] Deb S, Chana S. Biomaterials in relation to dentistry. Front Oral Biol 2017;17:1–12.

[29] Hench LL. The story of Bioglass. J Mater Sci Mater Med 2006;17:967–78.

[30] Bakry AS, Takahashi H, Otsuki M, Tagami J. Evaluation of new treatment for incipient enamel demineralization using 45S5 bioglass. Dent Mater 2014;30:314–20.

[31] Beletskii BI, Sventskaya NV. Silicon in living organisms and new-generation biocomposite materials (review). Glass Ceram 2009;66:104–8.

[32] Hu S, Chang J, Liu MQ, Ning CQ. Study on antibacterial effect of 45S5 bioglass. J Mater Sci Mater Med 2009;20:281–6.

[33] Banerjee A, Pabari H, Paolinelis G, Thompson ID, Watson TF. An in vitro evaluation of selective demineralised enamel removal using bio-active glass air abrasion. Clin Oral Investig 2011;15:895–900.

[34] de Carvalho FG, Brasil VLM, Filho TJS, Carlo HL, Santos RL, Lima BASG. Protective effect of calcium nanophosphate and CPP-ACP agents on enamel erosion. Braz Oral Res 2013;27:462–70.

[35] Baig AA, Faller RV, Yan J, Ji N, Lawless M, Eversole SL. Protective effects of SnF2 — part I. Mineral solubilisation studies on powdered apatite. Int Dent J 2014;64(Suppl. 1):4–10.

[36] Faller RV, Eversole SL. Protective effects of SnF2 — part III. Mechanism of barrier layer attachment. Int Dent J 2014;64(Suppl. 1):16–21.

[37] Faller RV, Eversole SL, Tzeghai GE. Enamel protection: a comparison of marketed dentifrice performance against dental erosion. Am J Dent 2011;24:205–10.

[38] Hara AT, Livengood SV, Lippert F, Eckert GJ, Ungar PS. Dental surface texture characterization based on erosive tooth wear processes. J Dent Res 2016;95:537–42.

[39] Taha AA, Fleming PS, Hill RG, Patel MP. Enamel remineralization with novel bioactive glass air abrasion. J Dent Res 2018;97:1438–44, http://dx.doi.org/10.1177/0022034518792048.

[40] Burwell AK, Litkowski LJ, Greenspan DC. Calcium sodiumphosphosilicate (NovaMin): remineralization potential. Adv Dent Res 2009;21:35–9.

[41] Dong ZH, Chang JA, Zhou Y, Lin KL. In vitro remineralization of human dental enamel by bioactive glasses. J Mater Sci 2011;46:1591–6.

[42] Wang X, Megert B, Hellwig E, Neukaus KW, Lussi A. Preventing erosion with novel agents. J Dent 2011;39:163–70.