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Remineralization of early enamel caries lesions induced by bioactive particles: An *in vitro speckle analysis*

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ABSTRACT

This study aimed at evaluating the remineralization effect promoted by different bioactive fillers on simulated early caries lesions in enamel (ECLE). Forty sound bovine incisors were used to prepare buccal enamel specimens ($6 \times 6 \times 2$ mm). The specimens were divided into two areas (3×3 mm^2): control (CTR) and experimental (EXP). All the enamel specimens were then submitted to a specific protocol for a period of 48 h to induce simulated caries lesions. Subsequently, the specimens were treated for 7 days (2 min, twice a day) with a slurry pastes containing different bioactive particles (P/L ratio: 1 g/mL). All the specimens were analyzed by laser speckle before and after treatments. The results showed after the first analysis that the ECLE had very low average intensity (back-scattered light). Conversely, after application of the bioactive pastes, higher average intensity was always detected; this was comparable to CTR sound specimens. In conclusion, innovative pastes/gels developed for enamel remineralization should contain bioactive particles that when applied daily on early caries lesions may "boost" the remineralization process to reestablish a sound enamel.

1. Introduction

The mineral phase of enamel is mainly characterized by complexes of calcium and phosphates (e.g. hydroxyapatite), which can be dissolved by organic acids (e.g. acetic, lactic, propionic and formic acids) produced by cariogenic bacteria [1,2]. At the early stages of enamel caries formation, acids produced by such bacteria can diffuse into the enamel through microscopic channels present between enamel rods, so forming a primary subsurface lesion. Clinically, such lesions appear as a white spots with an intact surface zone of enamel. There is clear clinical evidence that prior to cavitation, the aforementioned partially demineralized subsurface lesion might be totally remineralized, as far as enamel surfaces remain intact, free of plaque, constantly under adequate salivary flow, especially if regularly stimulated by use of sugar-free gum, and treated constantly with topical fluoride [3–5]. The subsurface lesion provides a suitable matrix for the growth of mineral crystals upon passage of calcium and phosphate ions through the salivary acquired pellicle to the enamel surface [6].

The clinical benefit of using fluoride-containing dentifrices to prevent caries cavitation has been clearly established, especially when high fluoride concentration is employed [7]. The main mechanism of action of fluoride can be attributed to its activity to inhibit bacteria growth [8,9] as well as to its aptitude to reduce the solubility of enamel when incorporated into the structure of hydroxyapatite and form fluorapatite and/or fluoride-rich hydroxyapatite [10–13]. Since even small amounts of fluoride provide some degree of cariostatic effect [14,15], a great

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number of fluoride-containing dental materials [e.g. glass ionomer-based materials, composites, adhesives and varnishes) and oral care products (mouthrinse, toothpaste, topical gels), have been developed and commercialized so far [13].

Nevertheless, due to an increasing use of processed sugars, acidic foods and acidic hypotonic beverages in the diet of people of our modern society, the incidence of caries has drastically increased [16–19]. This issue represents the main reason why it is of key importance to develop innovative remineralizing strategies able to deliver suitable amount of calcium and phosphates ions into the caries lesion in order to attain a reliable remineralization of the enamel [20].

Bioactive glasses represent some of the most promising materials with a great remineralizing potential in enamel and dentin. Indeed, such bioactive materials have been incorporated into toothpaste formulations [21], as well as many other dental products [22,23] in order to accomplish such a target.

This *in vitro* study aimed at evaluating the remineralization effects of different bioactive particles applied on the early caries lesions in enamel in form of slurry pastes. This aim was accomplished by using a recent laser speckle imaging system, which represents an innovative non-invasive, non-destructive method based on the evaluation of back-scattered coherent light from enamel and dentin.

The hypothesis tested in this study was that all the tested bioactive particles would remineralize early caries lesions in enamel when compared to non-treatment groups.

2. Material and methods

2.1. Bioactive fillers

The first bioactive powder used in this study was **BAG-*Zn*-*PAA***. It was made by first preparing Bioglass 45S5 (BAG) using a high-temperature melting process as described by Sauro et al. (2013) [24] (nominal composition: 46.1 mol% SiO_2, 26.9 mol% CaO, 24.4 mol% Na_2O, and 2.5 mol% P_2O_5). Subsequently, BAG (80 wt%) was mixed with 20 wt% zinc oxide (ZnO: Sigma-Aldrich) and a polycarboxylic acid solution (10% PAA: Mw 1800; Sigma-Aldrich). The mixture was maintained in a furnace at 40 °C for 12 h and subsequently ground and sieved (particle size < 20 mm) [25,26].

A fluoride-containing bioactive glass **(BAG-F)** with a nominal composition of 42.7 mol% SiO_2, 26.2 mol% CaO, 26.1 mol% Na2O, 4.0 mol% P_2O_5, and 1 mol% CaF_2, was also prepared as described previously [27,28]. The BAG-F powder was milled and finally sieved (< 20 µm). The third bioactive filler (**BAG-F PAA)** was created by mixing BAG-F with a 10% polycarboxylic acid solution (Sigma-Aldrich). The mixture was maintained in a furnace at 40 °C for 12 h and subsequently ground and sieved (particle size < 20 mm).

A polycarboxylated calcium-silicate powder **(CaSi-PAA)** was the fourth powder use in this study. It was prepared by mixing 10% polycarboxylic acid solution (Sigma-Aldrich) with a commercial calcium-silicate cement (ENDOPASS, DEI Italia, Mercallo, VA, Italy) constituted by 70–80 wt% (weight %) of Portland cement and 5–8 wt% of a phyllosilicate, also known as smectite, 1–3 wt% of hydrotalcite (Sigma–Aldrich, Gillingham, UK) and 5–10 wt% zirconium oxide (Sigma–Aldrich). The mixture was maintained in a furnace at 40 °C for 12 h and subsequently ground and sieved as described above (< 20 mm).

2.2. Sample preparation and artificial early caries lesion in enamel formation

Forty enamel blocks (6 × 6 mm^2) were cut from the buccal surface of bovine incisors. These were immediately embedded in each holder (PVC tube) with acrylic resin leaving the enamel exposed. Each specimen was polished using #400, #600, #1000 and #1200 (Buehler, UK) grits SiC abrasive papers under continuous water irrigation (60 s each paper-step). A felt disk with a diamond paste (3 M, USA) was used for final polishing. At this point, the enamel surface was evaluated by visual inspection under stereomicroscopy in order to verify defects; in such a case specimens were replaced with fresh ones. Each specimen was divided into two zones: 1) one protected with adhesive tape (3 × 3 mm^2) and classified as control sound tissue (CTR); ii) the other zone (3 × 3 mm^2) was left exposed and classified as the experimental one (EXP). All the specimens were then immersed into a demineralizing solution (pH 3,5-4,0) to generate a simulated early caries lesion in enamel (ECLE) [29]. In details, the specimens were kept at 37 °C for 48 h in a demineralizing solution (DS; H_2O 2400 ml, $Ca(OH)_2$ 462 mg, CH_3COOH 894 ml, $C_2H_3NaO_2$ 9,30 g, $H_3PO_4$0,39 ml; pH 3,5-4.0). Afterward, all the specimens were rinsed with deionized water for 10 s, dried with absorbent paper and analyzed using speckle laser (first speckle analysis).

2.3. Laser speckle imaging

The surfaces of each specimen were imaged under a coherent light illumination generated by using the HeNe laser (Uniphase, USA) emitting at 633 nm with 40 mW energy. The bean was expanded by a f = 100 mm lens to achieve a circular spot size with 6 mm in diameter. The specimens were then imaged using a Complementary Metal Oxide Semicondutor (CMOS) sensor with 23.7 mm X 15.3 mm in area (4752 × 3168 pixels; pixel pitch = 4.99 µm) (Canon EOS Rebel Tli camera fitted with a macro 100 mm Canon lens, Japan). The

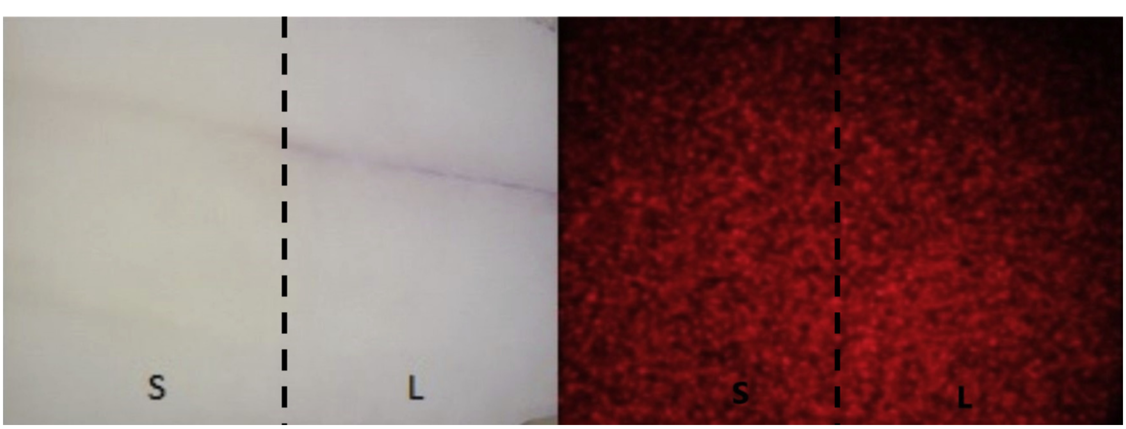

Fig. 1. The figure shows the images under white illumination and RAW laser speckle. The image under white illumination shows that the left side of the specimen was considered as the sound tissue (S) while the right side of the specimen was considered as the subsurface caries lesion in enamel tissue (L). This pattern was applied in all the images captured.

Table 1
Represents experimental groups tested in this study.

Groups	Treatment
Control (CTR)	Only deionized water/ No bioactive glass
BAG-Zn-PAA	BAG-Zn PAA slurry prepared with deionized water (P/L ratio of 1 g/mL)
BAG F-PAA	BAG F-PAA slurry prepared with deionized water (P/L ratio of 1 g/mL)
CaSi-PAA	CaSi-PAA slurry prepared with deionized water (P/L ratio of 1 g/mL)

photometric parameters were: exposure time = 1/50 s; f/29; ISO 100, while the camera was placed at an angle $\Theta < 10°$ with the laser. No data binning was performed by the camera. Each specimen was imaged under white and laser illumination (Fig. 1). The digital images were transferred to a computer and processed numerically using a software program as describe in previous works [32–36].

2.4. Surface treatments with bioactive glasses

After artificial early caries lesion formation, the specimens were divided in four groups based on the experimental bioactive glass powder tested in this study (Table 1).

The specimens of the control group were treated with deionized water. Whereas, in the experimental groups, a slurry of each bioactive glass powder was prepared with distilled water (P/L ratio of 1 g/mL) [30] and applied on the ECLE with a microbrush. This was left undisturbed for 2 min on the enamel and finally rinsed with deionized water for 10 s; this procedure was repeated twice daily for one week [31]. A demineralization/remineralization [DE/RE] cycling challenge was performed during the one-week treatments protocol. After this period, the specimens were analyzed again by laser speckle (Fig. 2).

2.5. Statistical analysis

All values obtained during this study were processed for baseline data subtraction. The data to the time 1 (T1) were considered as the first week subtracted from baseline data, and to the time 2 (T2), the data were considered as the second week subtracted from baseline data.

The data passed the analysis for normal distributions (Shapiro-Wilk; $p > 0.05$), so that the analysis of variance (ANOVA) could be used to analyze the data, followed by Tukey post-hoc and t-paired tests. The statistical analysis was performed by IBM SPSS 23 and the significance level was set at $\alpha = 0.05$.

3. Results

The laser speckle analysis demonstrated that it is possible to gather information from the microstructure of the enamel detecting minimal changes such as early enamel caries lesions. Indeed, it was possible to see under laser illumination a discreet white spot lesion in the experimental zone of each specimens; the images were averaged ($n = 4 \times 4$) and a pseudo-color algorithm was applied for better visualization. The average intensity of the backscattered light values was obtained from a standardized area of the carious and healthy/treated tissue by ImageJ-win64 program. All specimens exhibited lower average intensity of the backscattered light on the -ECLE tissue, compared to sound enamel. After treatments of the ECLEs with different types of bioactive products tested in this study, it was possible to observe an increase of the light scattering again, which was quite similar to that of the sound enamel. Images from white illumination, RAW laser speckle, Gray scale and processed images were qualitatively analyzed (Figs. 3–10).

The contrast ratio carious/healthy was obtained as demonstrated in a previous study [33,34] and it was calculated as ratio between the mean intensity value of the carious region and the mean intensity value of the healthy region for each sample. This data provided a comparison between the sound and caries specimens of every single specimen simplifying the analysis and removing any bias inherent to the variability of the teeth. The data obtained from the average intensity of the backscattered light (ImageJ-win64) are shown in Table 2, where it is possible to see the average and standard deviation of all groups

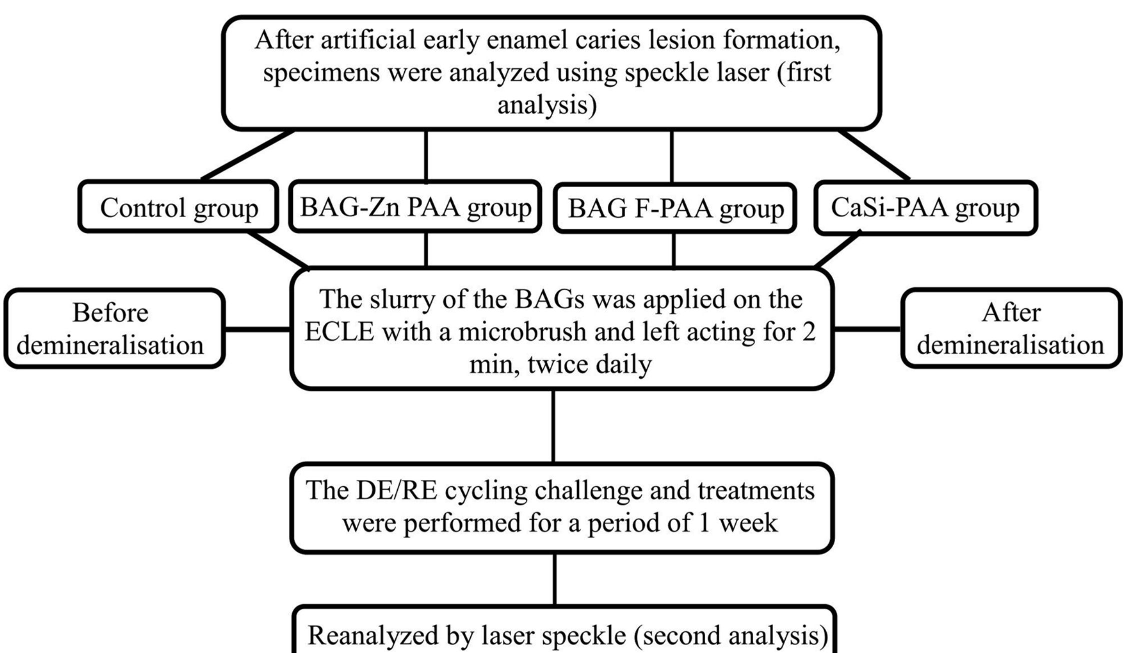

Fig. 2. The figure describes the surface treatments with bioactive glasses after artificial early enamel caries lesion formation and the analysis by Speckle laser.

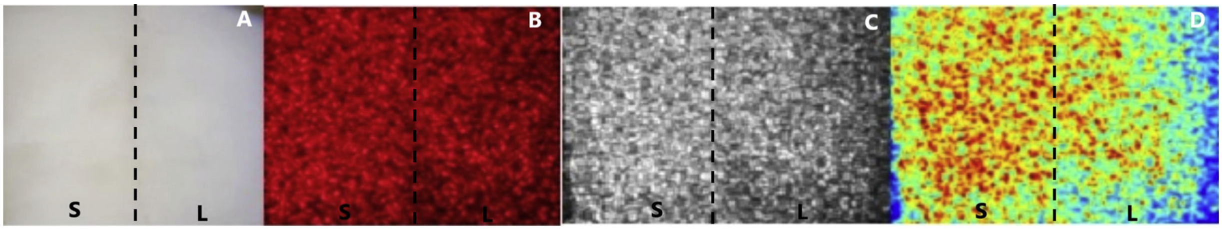

Fig. 3. The figure describes the group CTR after ECLE formation. **A**. the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel (L). **B**. the image is under RAW laser speckle and shows a higher intensity light on the left side (S) and a lower intensity light on the right side (L), which means that there was a discrete demineralization of the enamel surface after ECLE formation. **C**. the image is under a Gray scale and a discrete demineralization can be observed on the right side (L). **D**. the image processed showing a higher amount of red points on the left side (S) when compared to the right side (L).

(baseline, after first week of pH cycling to the enamel demineralization and second week of pH cycling, along with the treatments using the bioactive materials).

The descriptive data after subtraction of the baseline are presented in Table 3, while a multiple comparison for the experimental data is presented in Table 4. In details, after pH cycling (7 days DEM/REM solution), the group treated only with deionized water (no bioactive glass) (CTR) showed a light intensity statistically lower (39.04) when compared to all the treated groups (BAG-Zn-PAA = 46.37; BAG-F-PAA = 47.55; CaSi-PAA = 52.44), although the BAG-*Zn*-PAA was quite similar to CTR ($p = 0.06$). The group BAG-*Zn-P*AA was statistically similar to BAG-F-PAA ($p = 0.319$) and CaSi-PAA ($p = 0.925$); no significant difference was found between the experimental treated groups (Fig. 11).

4. Discussion

This study demonstrated through analysis of speckle pattern that all the different types of bioactive materials used in this study were able to remineralize early enamel caries lesions. Thus, the hypothesis of this study must be accepted.

The use of bioactive materials for dentin and enamel remineralization has been widely studied by several investigators [23,37–40]. For instance, silicate-based materials containing calcium and phosphate (e.g. bioglass) [41] have been shown to react with body fluids to induce the precipitation of hydroxycarbonated apatite (HCAP) [42,43]. Kokubo et al (1990) [44] used different glass compositions to study the growth of apatite in simulated body fluid (SBF). The mechanism responsible for apatite formation seems to be related to the ability of some specific bioactive glasses to release hydrated silica $Si(OH)_4$, which then polymerizes into a porous SiO_2-rich layer and serve as a template for precipitation of amorphous calcium phosphate [41,45]; this converts later into biomimetic nonstoichiometric apatite in an alkaline environment. This latter environment is attained through a rapid exchange of sodium (Na^+) and hydrogen ions (H^+) or hydronium ion (H_3O–) [46].

Moreover, several bioactive particles have been used in operative and preventive dentistry due to their ability to interact with dental hard tissues, inducing calcium-phosphates (Ca/P) deposition in the presence of body fluids or saliva [47,48,37,23]. A previous study of Reynolds (2008) [49] showed that bioactive glasses may induce cell-signaling for regeneration of soft and hard tissues. Bioactive glasses are considered a breakthrough in dental remineralization since the current standard treatment for tooth remineralization and prevention of caries lesion is still a slow-acting process and it depends on adequate salivary calcium and phosphates concentrations [50,21].

The current laser speckle study has demonstrated that it was possible to acquire information on the microstructure of the enamel and detect minimal changes, such as ECLEs [33,34].

According to Goodman [51], the phenomenon of speckle can be statistically described. The Goodman model was developed in first-order statistics, in which optical granulates are described using the mean, variance and standard deviation of the intensities. Indeed, the images obtained in this study were analyzed and interpreted based on information obtained by means of the intensities captured before and after the demineralization and remineralization process. The digital image (RAW format) were defined as a two dimensional matrix, expressed by function f (x,y) of RAW values, where x and y, as spatial coordinates, provided the intensity or gray level of each point of the image that was the smallest element of a digital image (e.g. pixel) [52,53]. By convention, the origin of the image is located in the upper left corner of the image. According to Woods and Gonzales [52], the numerical matrix was used for the processing and development of algorithms. Each image captured by the image acquisition system was stored in the standard RGB camera. In the RGB model, the images consisted of three components of images, one for each primary color: [(R,G,B)]. The colors of a pixel could be represented as an 8-bit integer ranging from 0 to 255, with a value of 0 corresponding to black, and 255 to white. For analytic purposes, the first primary color (Red) was extracted from the RGB model. Thus, the speckle image was associated with the brightness of the digital image, and the image transformed into a numerical matrix [54].

This current study also demonstrated that the use of bioactive materials can modify the enamel surface structures and remineralize early caries lesion in a very short period time (7-day application). Indeed, the results of this study clearly showed that after one week of pH cycling

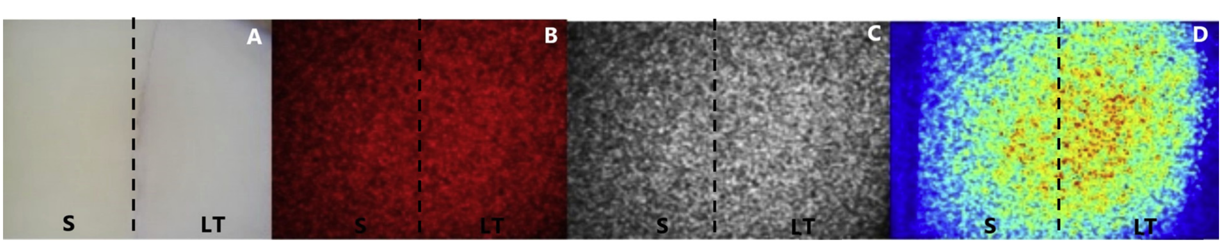

Fig. 4. The figure describes the group CTR after one week of pH cycling treated with deionized water/no bioactive glass. **A**. the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel after treatment with deionized water (LT). **B**. the image is under RAW laser speckle and shows the differences of intensity light on the both sides (S e LT) **C**. the image is under a Gray scale. **D**. the image processed showing a few red points on the right side (LT) which may have occurred a discrete remineralization of the surface after one week of immersion in remineralization solution (pH cycling); however there was no an increase of intensity light statistically significant when compared to the groups treated with bioactive glass.

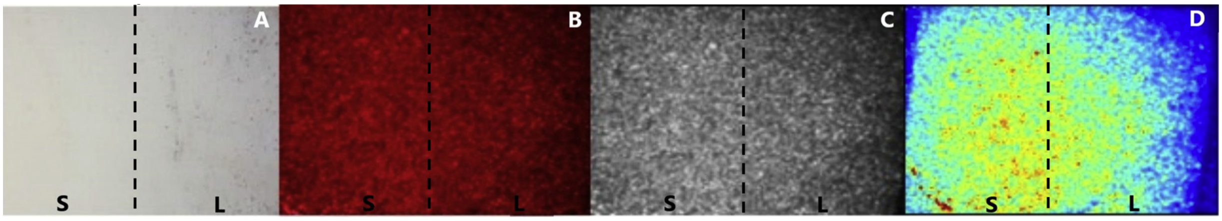

Fig. 5. The figure describes the group BAG-Zn-PAA after ECLE formation and before one week of treatment. **A**. the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel (L). **B**. the image is under RAW laser speckle and shows a higher intensity light on the left side (S) and a lower intensity light on the right side (L), which means that there was a discrete demineralization of the enamel surface after ECLE formation. **C**. the image is under a Gray scale and a discrete demineralization can be observed on the right side (L). **D**. the image processed showing a few red points on the left side (S) when compared to the right side (L).

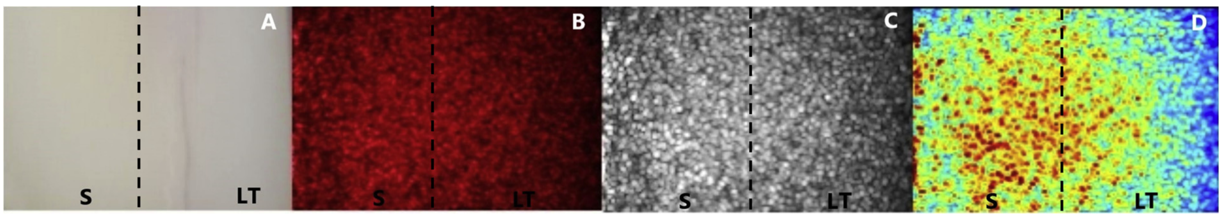

Fig. 6. The figure describes the group BAG-Zn-PAA after one week of pH cycling treated with BAG-Zn-PAA slurry. **A**. the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel after treatment with BAG-Zn-PAA (LT). **B**. the image is under RAW laser speckle and shows similar intensity light on the both sides (S e LT) **C**. the image is under a Gray scale. **D**. the image processed showing an increase of red points on the right side (LT) which might have occurred remineralization of the ECLE surface after one week of treatment with bioactive glass.

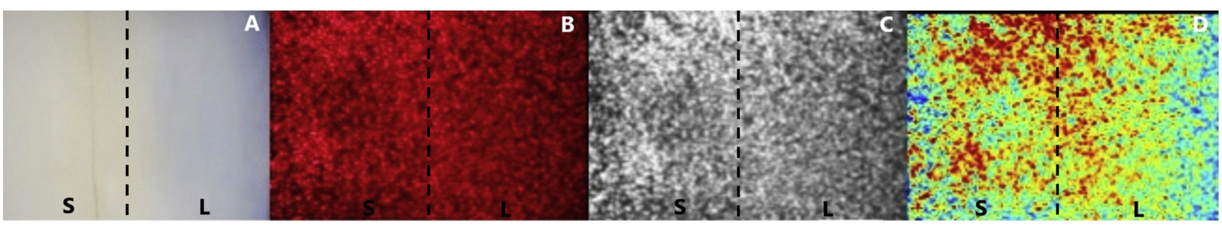

Fig. 7. The figure describes the group BAG-F-PAA after ECLE formation and before one week of treatment. **A**. the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel (L). **B**. the image is under RAW laser speckle and shows a higher intensity light on the left side (S) and a lower intensity light on the right side (L), which means that there was a discrete demineralization of the enamel surface after ECLE formation. **C**. the image is under a Gray scale and a discrete demineralization can be observed on the right side (L). **D**. the image processed showing a few red points on the left side (S) when compared to the right side (L).

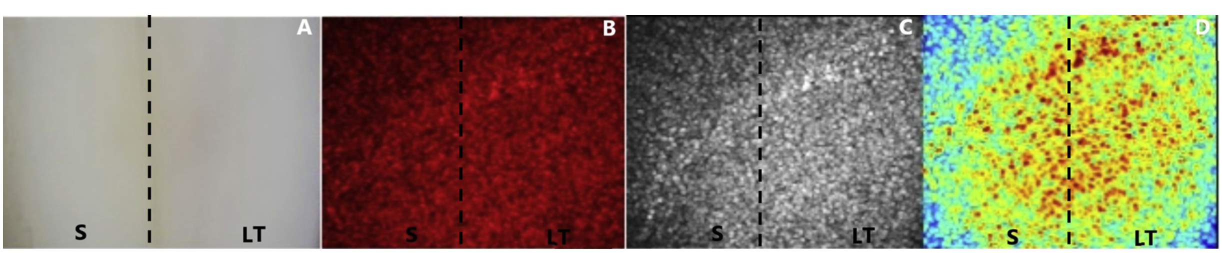

Fig. 8. The figure describes the group BAG-F-PAA after one week of pH cycling treated with BAG-F-PAA slurry. **A**. the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel after treatment with BAG-F-PAA (LT). **B**. the image is under RAW laser speckle and shows similar intensity light on the both sides (S e LT) **C**. the image is under a Gray scale. **D**. the image processed showing an increase of red points on the right side (LT) which might have occurred remineralization of the ECLE surface after one week of treatment with bioactive glass.

(DE/RE solution) for ECLE formation, all the enamel specimens exhibited lower average intensity of the backscattered light in comparison to the sound tissue. Such a change of intensity is related to the heterogeneity present within the microstructure of the demineralized enamel, which increases the interprismatic porosity and light scattering, so reducing the backscattering coefficient of the enamel structure; this was translated into light intensity with lower values in laser speckle imaging [33,34]. Furthermore, it was also demonstrated that it is possible to analyze the microstructure of eroded enamel using patterns and LASCA maps [33]. This latter method was also employed in our study to evaluate the remineralization of the specimens in a non-invasive, non-destructive real-time approach. Indeed, this may allow dentists to detect lesions even in the absence of biofilm or moisture, and it may result an innovative suitable diagnostic method for early detection diagnosis [32]. Moreover, the results of this study also suggest that laser speckle imaging may be used to analyze the remineralization of ECLEs over time. A previous study [39] has shown the ability of different types of BAGs to remineralize the eroded and early enamel caries lesions in enamel and dentin.

Moreover, it has been conveyed that biomimetic remineralization of

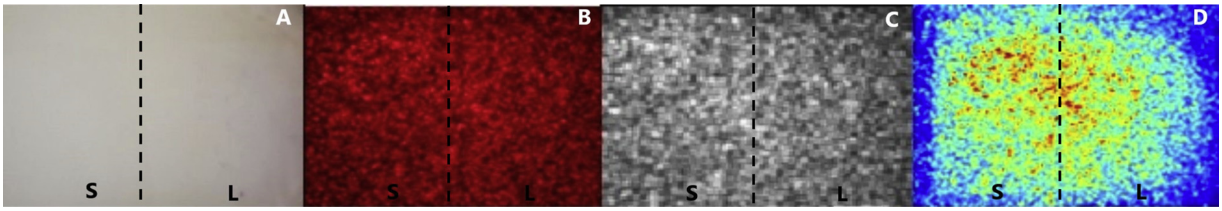

Fig. 9. The figure describes the group CaSi-PAA after ECLE formation and before one week of treatment. **A.** the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel (L). **B.** the image is under RAW laser speckle and shows a higher intensity light on the left side (S) and a lower intensity light on the right side (L), which means that there was a discrete demineralization of the enamel surface after ECLE formation. **C.** the image is under a Gray scale and a discrete demineralization can be observed on the right side (L). **D.** the image processed showing a few red points on the left side (S) when compared to the right side (L).

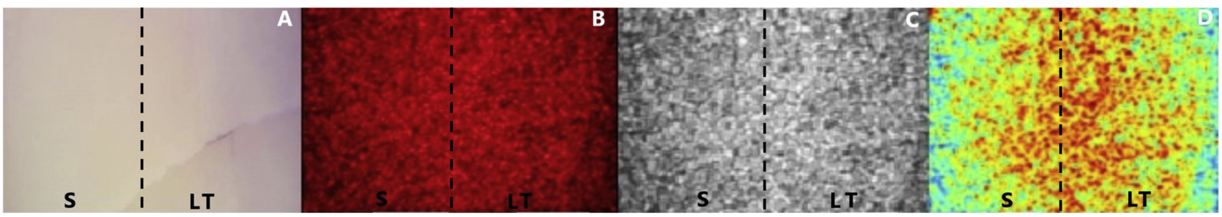

Fig. 10. The figure describes the group CaSi-PAA after one week of pH cycling treated with CaSi-PAA slurry. **A.** the image is under white illumination and shows the sound (S) and subsurface caries lesion in enamel after treatment with CaSi-PAA (LT). **B.** the image is under RAW laser speckle and shows similar intensity light on the both sides (S e LT) **C.** the image is under a Gray scale. **D.** the image processed showing an increase of red points on the right side (LT) which might have occurred remineralization of the ECLE surface after one week of treatment with bioactive glass.

Table 2
Descriptive Statistics of the original data obtained from ImageJ program.

Groups	Baseline		First week		Second week	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
CTR	51.04	4.85	30.56	9.01	39.04	6.16
BAG-Zn-PAA	50.04	5.45	37.52	6.30	46.37	4.47
BAG-F-PAA	44.15	7.05	37.04	4.01	47.55	4.82
CaSi-PAA	45.17	5.25	34.58	4.59	52.44	6.05

Table 3
Descriptive Statistics of the experimental data (First and Second week – baseline) after baseline data subtraction.

	T1 (First week – baseline)		T2 (Second week – baseline)	
Groups	Mean	Std. Deviation	Mean	Std. Deviation
CTR	−20.49	7.85	−12.00	5.49
BAG-Zn-PAA	−12.52	7.74	−3.67	8.92
BAG-F-PAA	−7.11	5.24	3.40	5.40
CaSi-PAA	−10.59	6.65	7.27	6.87

Table 4
Multiple comparison table for the experimental data.

Groups	T1 (First week – baseline)	T2(Second week – baseline)
CTR	$-20.49^{B;a}$	$-12.00^{A;a}$
BAG-Zn-PAA	$-12.52^{B;a,b}$	$-3.67^{A;b}$
BAG-F-PAA	$-7.11^{B;b}$	$3.40^{A;b,c}$
CaSi-PAA	$-10.59^{B;b}$	$7.27^{A;c}$

Capital case letters = Intra group comparison between T1 and T2 (Paired T tests).
Lower case letters = inter groups comparison for each time (one way anova followed by Tukey).

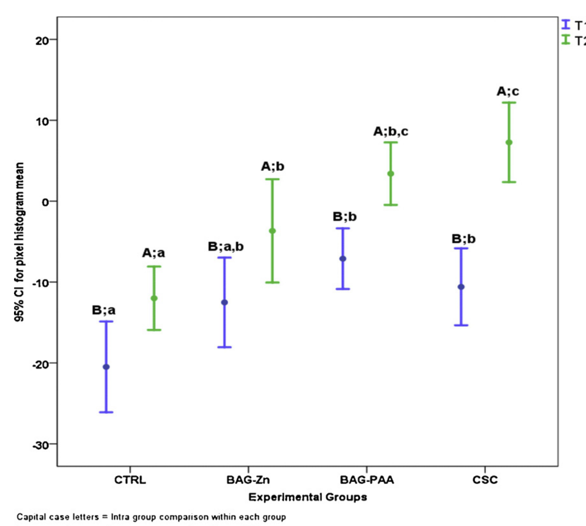

Fig. 11. Difference of the subtracted data from the baseline of light intensity by Speckle in EECL (time 1 - T1) and after treatments (time 2 - T2).

caries-like lesions is also possible when using such a strategy in the presence of a simulated body fluid containing poly-aspartic, poly-acrylic and polyvynil-phosphoic acid PVPA [55]. Liu et al. [56] proposed the use of a polycarboxylic acid (e.g. polyaspartic acid) as the stabilizer for amorphous calcium phosphates. This biomimetic analog of matrix phosphoproteins may participate in the recruitment of pre-nucleation clusters to produce fluidic, polymer-stabilized amorphous calcium phosphate nano-precursors [57,58]. Gower et al. [59] postulated that the formation of liquid nano-precursors sequestered by polyanionic polymers might be a fundamental step in biomineralization. Indeed, these fluidic nano-precursors induce a remineralization process from the bottom of the lesion to the outer surface (Bottom-up remineralization process) [58,59].

It is well known that the use of bioactive glasses in dental research has been proved to favor the remineralization of demineralized dentin and enamel [60–62,23]. Milly et al. [30] showed that mineral deposits were readily detected within BAG and PAA-BAG groups implying that the observed structures firmly attached to lesion surface. These authors concluded that mineral depositions of BAG and PAA-BAG was formed at the lesion surface, and 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 [30]. Our results may corroborate with the aforementioned studies, since all the groups treated in this study with the slurries containing bioactive glasses showed higher average intensity in laser speckle images, which were similar to those images of the sound enamel.

There is a general consensus in the literature that fluoride can accelerate the rate of calcium phosphate precipitation in dentin and enamel by catalyzing the conversion of OCP crystals into apatite crystals and by enhancing the growth rate of apatite crystals [63]. Amjad et al., (1981) [64] suggested that fluoride at levels below 1 ppm could retard or inhibit OCP precipitation process and favor apatite formation. Explanations given for such a retardation effect were that the presence of fluoride may disturb a cascade process involving the formation of kinetically favorable precursor phases, or that fluoride ions might be adsorbed onto an OCP-like precursor phase and block its growth. However, Mura-Galelli et al., (1992) [65] showed that fluoride steadily accelerated the precipitation reaction (i.e., precipitation of fluoridated apatite without OCP) when its concentration increased from 0.05 up to 1 ppm.

Bioactive glasses and fluoride-releasing bioactive glasses are of interest in both orthopedic and dental applications due to their ability to form fluorapatite in body fluids. A number of publications investigate the ability of fluoride-containing bioactive glasses to form apatite in SBF [66–68]. Fujii et al. [66] tested fluoride-containing bioactive glasses in SBF and performed XRD on the SBF-treated specimens. The presence of fluoride changes the reactivity, degradation, pH or apatite formation in bioactive glasses [66–68]. In fact this can be explained by changes in the glass network (network connectivity) due to unsuitable glass design: if CaF_2 is substituted for network modifier oxides such as CaO or Na_2O, it causes cross-linking of the silicate network and an increased NC, as shown previously in structural investigations [13] and molecular dynamics simulations [69]. Gentlemen et al. [70] showed that addition of fluoride results in formation of fluorapatite in simulated body fluid, which is more acid resistant than carbonated hydroxyapatite, and therefore fluoride-containing bioactive glasses are particularly interesting for applications in dentistry.

In conclusion, within the limitations of this in vitro study, it is possible to affirm that the laser speckle imaging technology may be a suitable method for early detection and assessment of remineralization of early caries lesions. This study also showed that it is possible to remineralize enamel early caries lesions in a period relatively short when employing materials containing bioactive particles. Further studies are ongoing to compare the results attained with the laser speckle imaging technology to cross-sectional microhardness tests. Moreover, in vivo studies are necessary to confirm the results of this study in real clinical scenarios.

Declaration of Competing Interest

The authors declare that there was no financial or commercial conflicts of interest in any of the products employed in this investigation.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pdpdt.2019.07.022.

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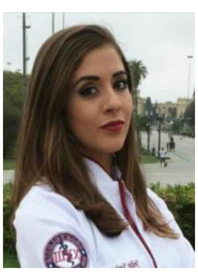

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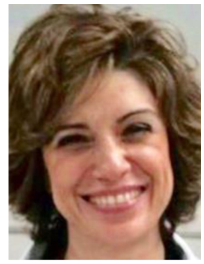

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