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Enamel alteration following tooth bleaching and remineralization

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Key words. Bioactive glasses, CPP–ACP, fluoride, hydroxyapatite, remineralization, tooth whitening.

Summary

The purpose of this study was to compare the effects of professional tooth whitening agents containing highly concentrated hydrogen peroxide (with and without laser activation), on the enamel surface; and the potential of four different toothpastes to remineralize any alterations.

The study was performed on 50 human molars, divided in two groups: treated with Opalescence® Boost and Mirawhite® Laser Bleaching. Furthermore, each group was divided into five subgroups, a control one and 4 subgroups remineralized with: Mirasensitive® hap+, Mirawhite® Gelleè, GC Tooth Mousse™ and Mirafluor® C. The samples were analysed by SEM/3D-SEM-micrographs, SEM/EDX-qualitative analysis and SEM/EDX-semiquantitative analysis.

The microphotographs show that both types of bleaching cause alterations: emphasized perikymata, erosions, loss of interprizmatic substance; the laser treatment is more aggressive and loss of integrity of the enamel is determined by shearing off the enamel rods. In all samples undergoing remineralization deposits were observed, those of toothpastes based on calcium phosphate technologies seem to merge with each other and cover almost the entire surface of the enamel. Loss of integrity and minerals were detected only in the line-scans of the sample remineralized with GC Tooth Mousse™. The semiquantitative EDX analysis of individual elements in the surface layer of the enamel indicates that during tooth-bleaching with HP statistically significant loss of Na and Mg occurs, whereas the bleaching in combination with a laser leads to statistically significant loss of Ca and P.

The results undoubtedly confirm that teeth whitening procedures lead to enamel alterations. In this context, it must be noted that laser bleaching is more aggressive for dental substances. However, these changes are reversible and can be repaired by application of remineralization toothpastes.

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Introduction

Contemporary bleaching agents are mainly based on hydrogen peroxide (HP) or carbamide peroxide (CP). The mechanism of action of bleaching agents is based on the oxidation of large chomophore molecules responsible for enamel and dentin discoloration. The decomposition of HP results in oxygen and perhydroxyl free radicals, which then oxidize the staining molecules, breaking them down into smaller uncoloured fragments which are then converted into carbon dioxide and water (Haywood, 1990).The permeability of dental structures and the low molecular weight of the bleaching agents provide free access of hydrogen peroxide through the enamel and dentin organic matrix (Haywood, 1990; Chen *et al.*, 1993).

A number of studies suggest that bleaching leads to changes in the structure of the enamel surface and these in turn alter the biomechanical properties of the enamel. Some studies report morphological changes, suggesting that bleaching is actually an erosive process (Miranda *et al.*, 2005). Bleaching directly affects the organic protein components of the teeth, which also leads to changes in the mineral phase, resulting in visible morphological changes of the tooth surface (Bitter, 1992).

Proponents of power bleaching claim that the use of light sources such as lasers and plasma lights reduces the time necessary for whitening. In theory, the advantage of light-activated agents is the ability of laser light to heat the HP, thereby increasing the rate of formation of oxygen free radicals and enhancing the decomposition of the staining molecules (Rosenstiel & Gegauff, 1991).

Laser photoactivation for teeth bleaching purposes was introduced in the early 1990s (Jiménez *et al.*, 2007). In 1996, two laser wavelengths were accepted by the FDA to be used in tooth-bleaching procedures: that is, the argon laser

Table 1. Composition of the remineralizing agents.

Toothpaste	Manufacturer	Active ingredient
Mirafluor® C	Miradent, Hager & Werken GmbH & Co. KG	1250 ppm Olaflur (decaflur), aminfluoride
GC Tooth Mouse™	GC International, Itabashiku, Tokyo, Japan	Recaldent™ (CPP–ACP), phosphopeptide–amorphous calcium phosphate 10.0%
Mirawhite® Gellèe	Miradent, Hager & Werken GmbH & Co. KG	5.5% calcium sodium phosphosilicate (NovaMin®), (Bioglass® 45S5 particle size 90 μm)
Mirasensitive® hap+	Miradent, Hager & Werken GmbH & Co. KG	30% hydroxyapatite, xylitol, 1450 ppm fluoride

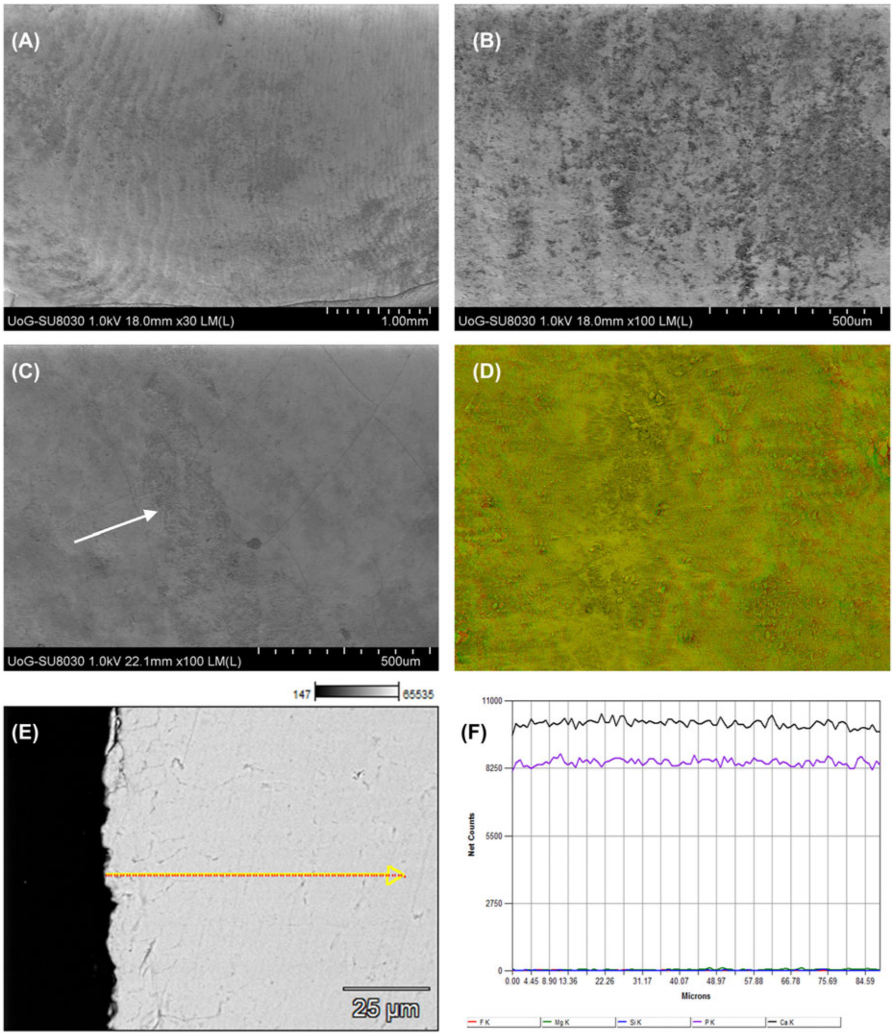

Fig. 1. Microphotographs of enamel bleached with 40% hydrogen peroxide Opalescence® Boost: (A) enamel with emphasized perykimata; (B), (C) eroded enamel surface (arrow), areas of preserved enamel are present; (D) 3D-stereomicrographic images (anaglyphs) of the enamel treated with Opalescence® Boost-preserved enamel surface (field of view 127-μm wide, nominal magnification ×1000); (E) longitudinal section with visible loss of enamel integrity; (F) x-ray line-scan: loss of minerals not detected.

(488/514 nm) and the CO_2 laser (10 600 nm) (Sun, 2000). In 2004, the most commonly used lasers in external tooth bleaching were the diode laser, argon laser, the frequency doubled Nd:YAG or KTP laser and a combination of the CO_2 laser (to heat the mixture) and the argon laser (to accelerate the process of decomposition of hydrogen peroxide). FDA approval for the 980 nm diode laser was also granted in 2007, and currently, diode lasers in the range 790–980 nm are approved for use in this application (Arce *et al.*, 2013). The rationale of the powerful laser bleaching is to achieve

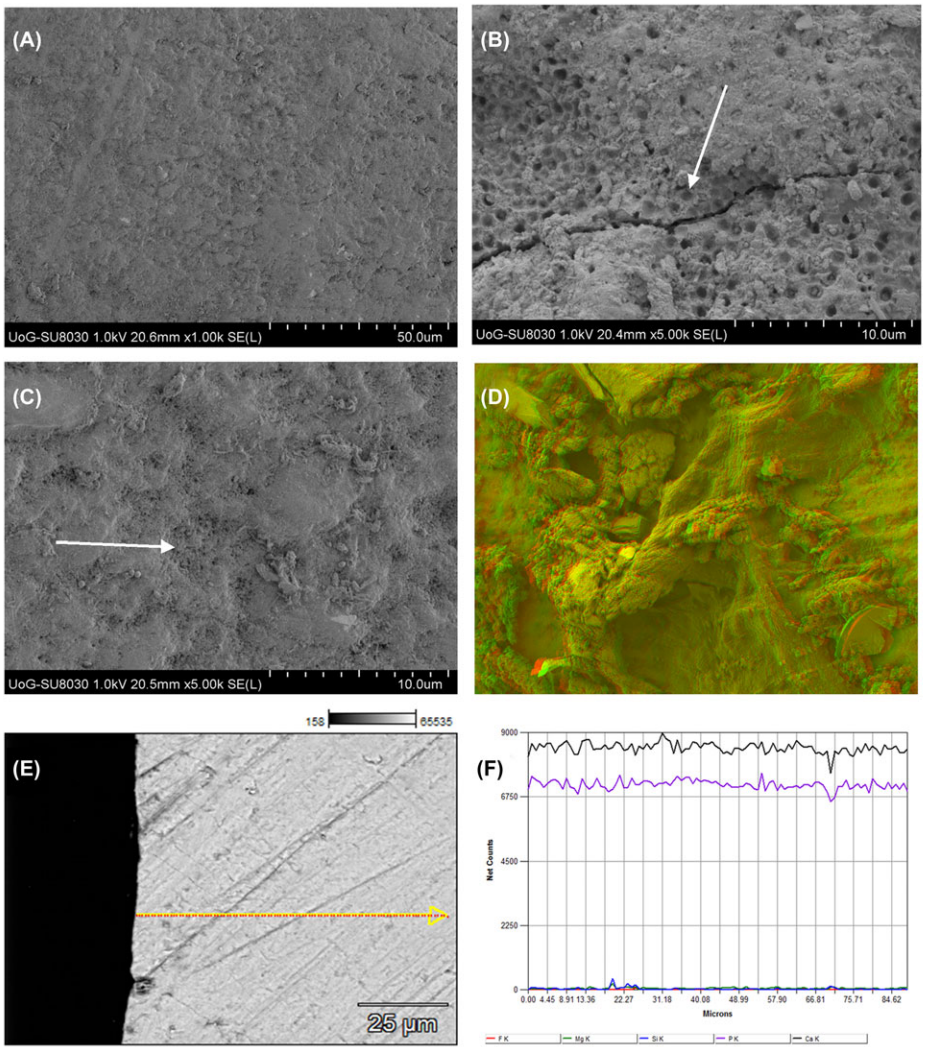

Fig. 2. Enamel micrographs after bleaching with Mirawhite® Laser Bleaching activated with diode laser; (A) loss of enamel surface integrity; (B) enamel erosion with fractured enamel rods; (C) arrow: predominant destruction of interprismatic spaces and loss of the interprismatic substance; (D) 3D-stereomicrographic images (anaglyphs) of the enamel treated with Mirawhite® Laser Bleaching (field of view 127-μm wide, nominal magnification $\times$1000); (E) longitudinal section: minimal loss of surface integrity; (F) x-ray line-scan: loss of minerals not detected.

significant effects *via* a controlled increase in temperature, whilst minimizing morphological and chemical changes to the enamel (Chang & Wilder-Smith, 1998).

Morphological defects introduced by bleaching can be repaired by application of remineralizing agents. Fluorides remain the best established agents to promote remineralization. This is attributed to fluoride-enhanced precipitation of calcium phosphates and formation of fluorhydroxyapatite in dental tissues (ten Cate, 1999). Fluorapatite is less soluble in acid solutions than hydroxyapatite, which in turn is less soluble than carbonated apatite (Shellis & Wilson, 2004).

Other agents are also capable of promoting remineralization of enamel. For example, casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) has been shown to be capable of remineralizing teeth *in vitro* (Gjorgievska & Nicholson, 2010). The rate of calcium loss from plaque during cariogenic attack in the presence of CPP–ACP decreases, and permits a rapid return to resting calcium concentrations (Yamaguchi *et al.*, 2006). Casein is also able to buffer plaque acid directly by releasing amino acids, or indirectly through bacterial catabolism (Rose, 2000).

Bioactive glasses (BGs) developed by Hench in the late 1960s are another possible remineralizing agent (Hench & Anderson, 1996). There is some experimental evidence that BGs can be used after tooth whitening to promote remineralization (Hassanein & El-Brolossy, 2006; Gjorgievska & Nicholson, 2011). In particular BG (45S5) with a particle size of <90 μm has been shown to create a protective layer on the surface of the artificially demineralized enamel (Gjorgievska *et al.*, 2013).

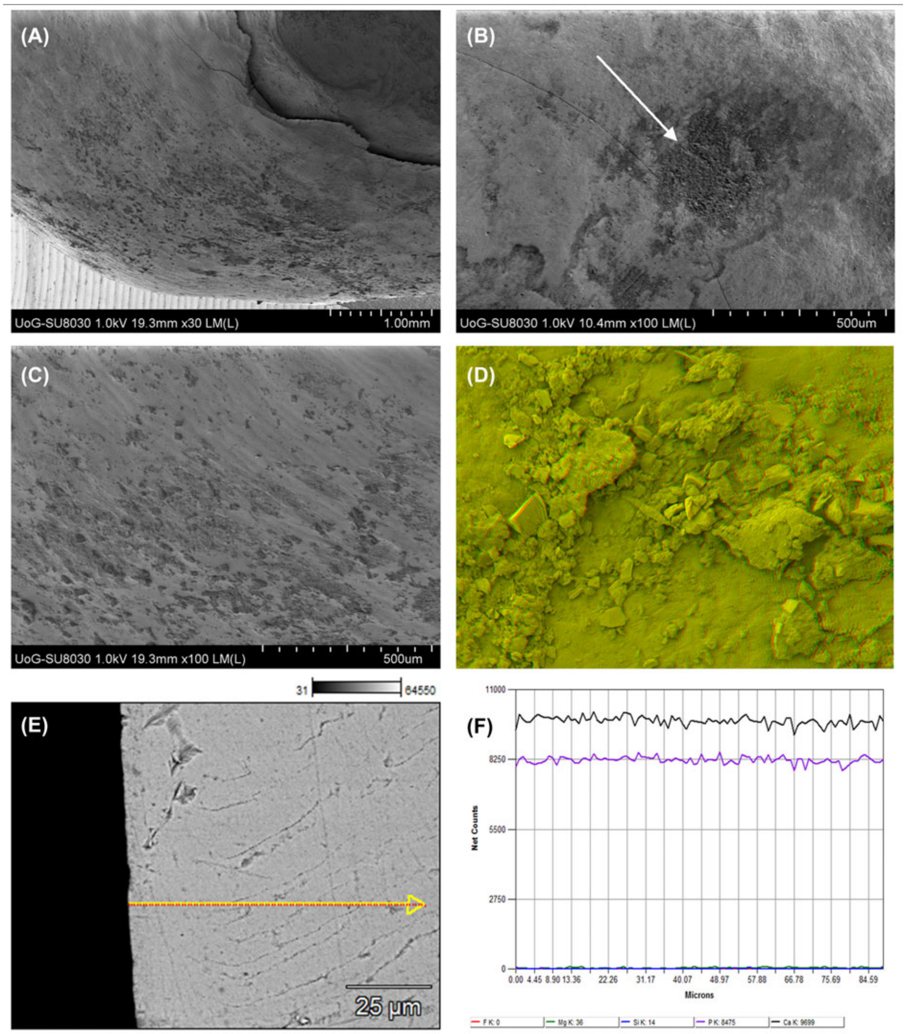

Fig. 3. Enamel treated with Opalescence® Boost, subsequent application of a remineralizing agent Mirafluor® C: (A), (B), (C) altered enamel surface with erosions and depressions (arrow); (D) 3D-stereomicrograph (anaglyph): increased enamel porosity, presence of deposits on the surface (field of view 114-μm wide, nominal magnification $\times$1000); (E) longitudinal section: normal enamel; (F) x-ray line-scan: absence of mineral loss in the enamel surface.

Recently, tooth whitening and remineralization using hydroxyapatite (HA) toothpastes has also attracted attention in the field of dentistry (Jeong, 2006). Tschoppe *et al.* (2011) established that HA toothpastes show stronger remineralization effects than amine fluoride (AF), and Niwa *et al.* (2001) showed that HA toothpastes are able to prevent caries, treat hypersensitivity and cure periodontal diseases. However, their capacity to remineralize whitened teeth has not yet been investigated.

The purpose of this study was to determine the occurrence, nature and potential reversibility of morphological alterations and changes in the elemental composition of the enamel following HP tooth-bleaching and laser-activated HP tooth-bleaching procedures. The ability of four different commercial toothpastes containing AF, CPP–ACP, BG or HA to diminish the detrimental effects of the bleaching protocols were also investigated.

Materials and methods

Sample preparation

The study was performed on 50 human permanent molars, extracted for orthodontic reasons and was approved by the Internal Review Board. The roots were cut with a diamond bur with a high-speed dental handpiece at the cementoenamel junction, and remnants of the pulp tissue were discarded. The coronal segments were thoroughly ultrasonicated and polished with pumice and polishing toothpaste. Excess toothpaste was cleaned by water spray for 3 min. Then, teeth were divided randomly into two groups. The samples of the first group were treated with Opalescence® Boost (Ultradent Products, Inc., St Louis MO, USA) bleaching agent, containing 40% HP, according to the manufacturer's instructions: the procedure was conducted in three sessions, in each session

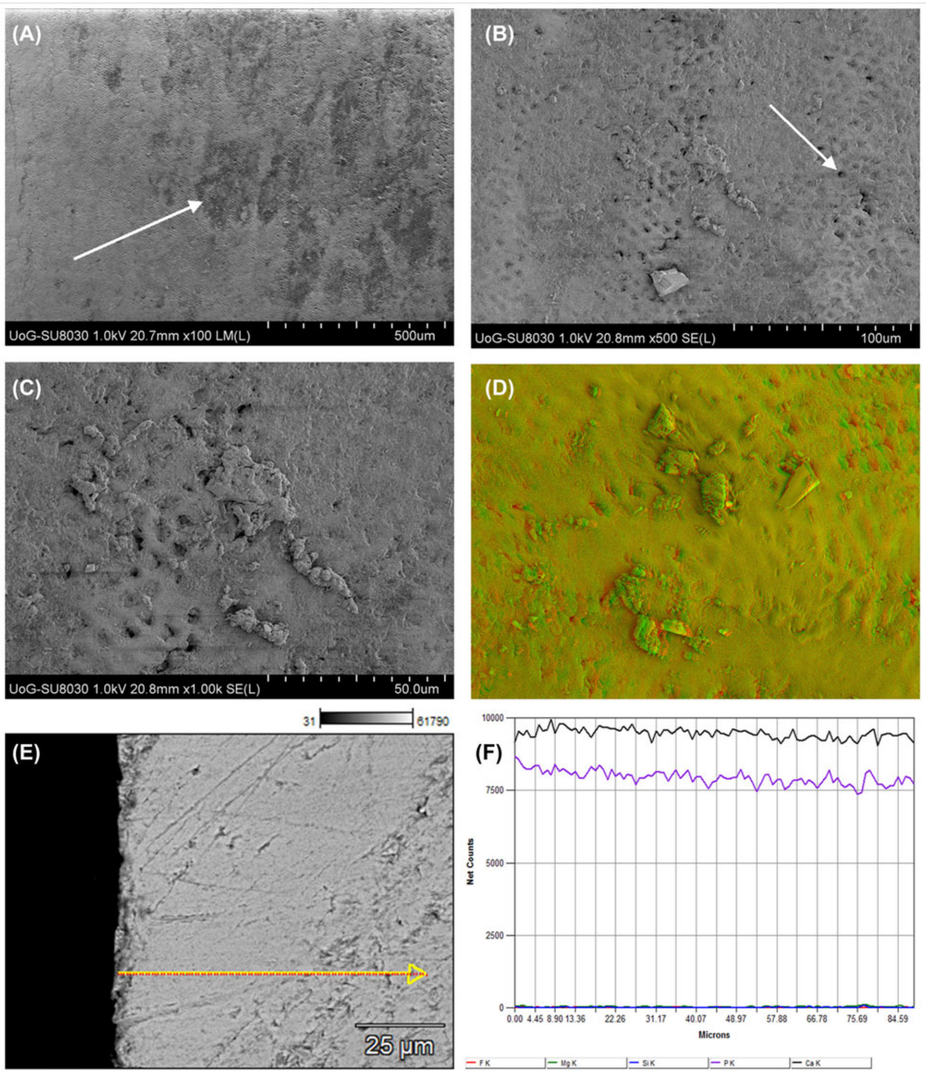

Fig. 4. Enamel treated with Mirawhite® Laser Bleaching and diode laser, subsequent application of a remineralizing agent Mirafluor® C: (A), (B), (C) altered, significantly porous enamel surface with erosions and depressions (arrows); (D) 3D-stereomicrograph (anaglyph): increased enamel porosity, rare deposits on the surface (field of view 127-μm wide, nominal magnification $\times 1000$); (E) longitudinal section: loss of enamel integrity; (F) x-ray line-scan: absence of mineral loss in the enamel surface.

the samples were exposed to the agent twice for 20 min, with a 15 min pause between the sessions. The second group was treated with 30% HP Mirawhite® Laser Bleaching (Hager & Werken GmbH & Co. KG, Duisburg, Germany), activated by a diode laser (Laser® HF, Hager & Werken GmbH & Co. KG) equipped with a special bleaching tip, conducted in 3 sessions of 4 min duration (laser activation 30 s), twice in each session.

Each group was divided into 5 subgroups: the first one served as a control (bleached only), whereas the other four were treated with Mirafluor® C, (Miradent, Hager & Werken GmbH & Co. KG); GC Tooth Mousse, (GC Comp, Japan); Mirawhite® Gelleè, (Miradent, Hager & Werken GmbH & Co. KG); and Mirasensitive® hap+, (Miradent, Hager & Werken GmbH & Co. KG). Each sample was rubbed for 3 min with the designated toothpaste, and then cleaned with copious water spray to eliminate the possible leftovers. Between the sessions the teeth were kept in physiological saline. The composition of the remineralizing toothpastes is given in Table 1.

Scanning electron microscopy, electron microscopy/three-dimensional stereo-photographs (Anaglyphs)

After the bleaching and remineralization procedures, the teeth were cut in half along the longitudinal axis in a bucco-lingual direction. One half of each sample was imaged uncoated using a cold cathode field-emission gun scanning electron microscope (FEG-SEM Hitachi SU 8030, Tokyo,

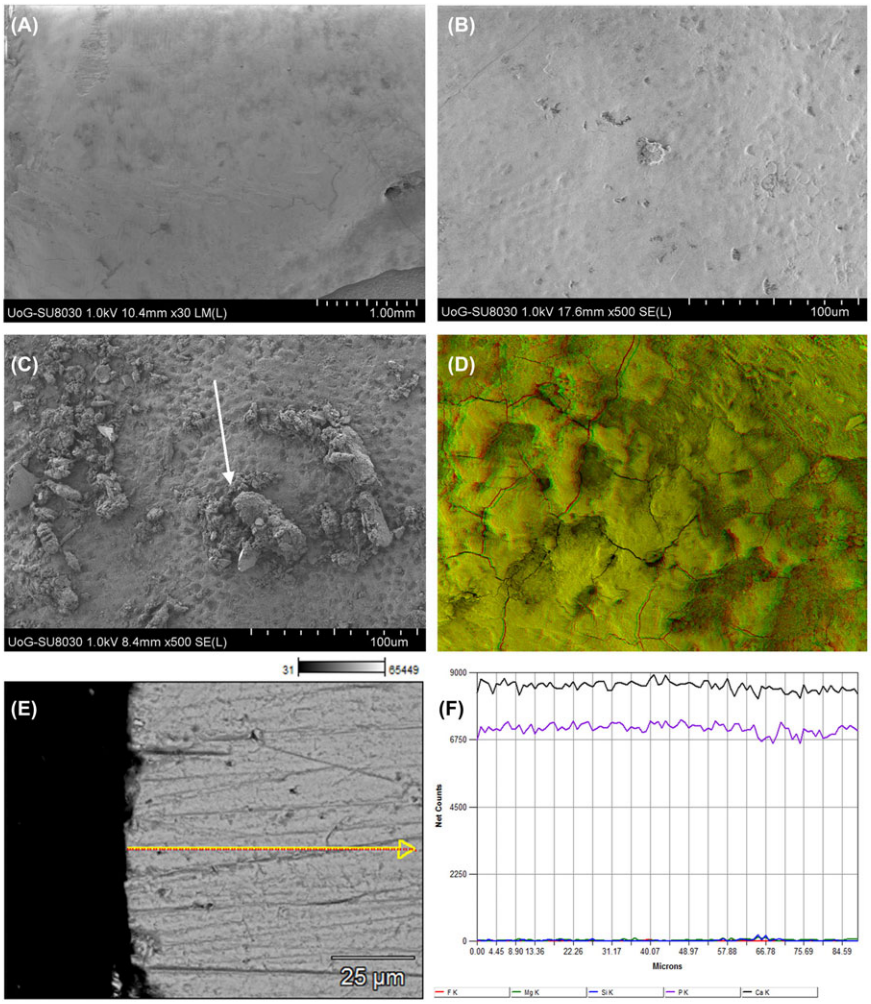

Fig. 5. Enamel treated with Opalescence® Boost, subsequent application of a CPP–ACP-containing remineralizing agent GC Tooth Mousse™: (A), (B), (C) increased enamel porosity, presence of amorphous deposits in the surface (arrow); (D) 3D-stereomicrograph (anaglyph): increased enamel porosity, loss of interprismatic substance (field of view 424-μm wide, nominal magnification $\times$300); (E) longitudinal section: slight loss of surface integrity; (F) x-ray line-scan: absence of mineral loss in the enamel surface.

Japan). The samples were previously pumped down in a vacuum desiccator until sufficient vacuum was achieved to obtain an image by FEG-SEM. The three-dimensional (3D) stereo-photographs (anaglyphs) were created by taking two stereo-pair photographs with a separation of 7°, the first at –8 and the second with a +8 tilt. A red filter was used for the lower angle (left eye), and a green filter was used for the higher angle (right eye). The microphotographs were superimposed and visualized with 3D-anaglyphic glasses.

Scanning electron microscope/energy dispersive x-ray analysis

The second half of each specimen was cast in a plastic mold with 32 mm internal diameter, with the cut surface facing the bottom of the mold. The molds were filled with Epo-Thin resin (Buehler, Lake Bluff, IL, USA, Batch No. 20-8140-032) which was than cured in a vacuum desiccator for 24 h. The surface of each block was ground flat with water-cooled carborundum discs (320, 600 and 1200 grit alumina papers; Buehler) and polished with diamond polishing paper (3M TM Polishing Paper 1Micron 8000 Grit Color Light Green; 3M Dental Products, St. Paul, MN, USA), then carbon-coated (Model S105, Edwards Co., UK). The prepared samples were analysed with a cold cathode FEG-SEM (Hitachi SU 8030) in backscattered electron mode (SEM-BEI). Experimental conditions were set at 15 kV accelerating voltage. Qualitative energy dispersive x-ray (EDX) analysis was performed using a Thermo Noran NSS System 7 (Thermo Scientific, Rockford, IL, USA) with an UltraDry detector (30 mm^2 window) by collecting x-ray line-scans along a line from the resin into the enamel in order to determine the elemental distribution in the surface enamel, as well as in deposits on the surface, if present.

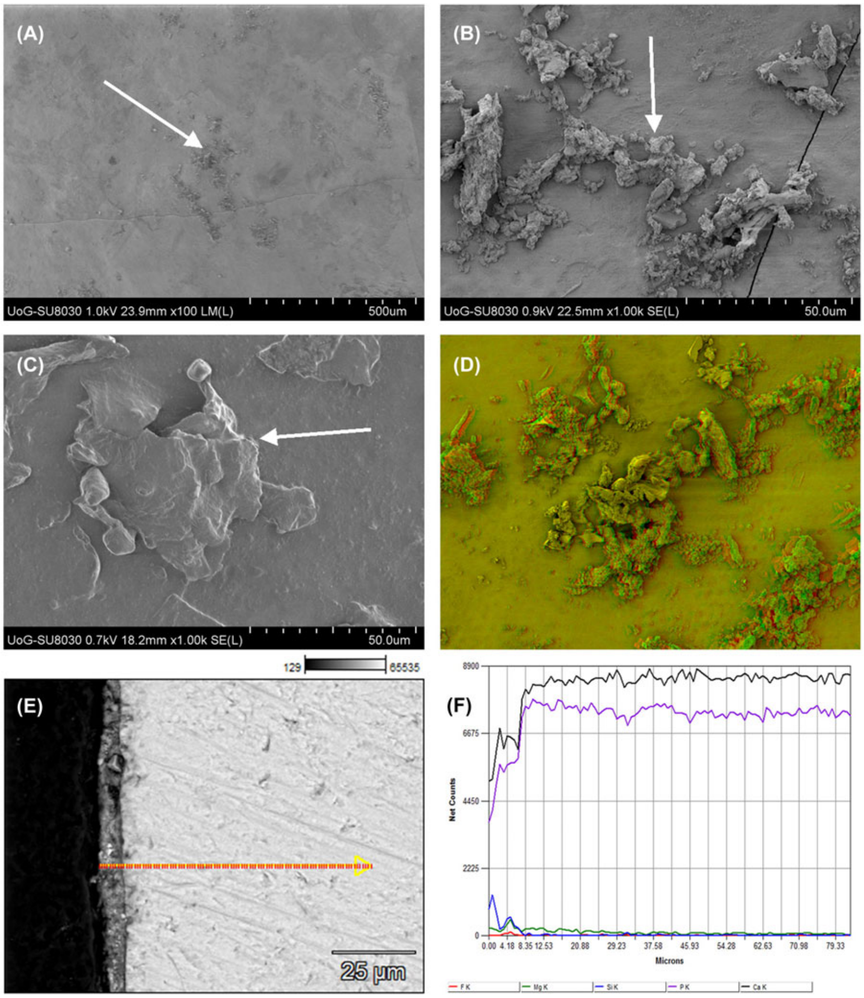

Fig. 6. Enamel treated with Mirawhite® Laser Bleaching and diode laser, subsequent application of a CPP–ACP-containing remineralizing agent GC Tooth Mousse™: (A), (B), (C) presence of rare amorphous deposits in the surface (arrow); (D) 3D-stereomicrograph (anaglyph): increased enamel porosity, loss of interprismatic substance (field of view 228-μm wide, nominal magnification $\times 500$); (E) longitudinal section: protective layer created on the enamel surface; (F) x-ray line-scan: the layer consists of Ca and P.

Finally, semiquantitative EDX point analysis was performed on the bleached enamel surface to determine the percentage elemental levels of sodium (Na), magnesium (Mg), aluminium (Al), phosphorus (P) and calcium (Ca). For each sample, ten points were randomly selected and the mean values calculated.

Statistical analysis was performed by one-way ANOVA. When statistically significant differences appeared (at the level of significance $p < 0.05$), the *post hoc* Tukey's Honest Significance Difference (HSD) test was applied.

In order to avoid the possible misinterpretation of the results, the preparation of the samples was done by one operator (EC), whereas the SEM photography was performed by another operator (IS). The evaluation and the selection of the micrographs was done independently by two coauthors (JWN and NJC) and finally the equivalent micrographs allocated in each group were selected by EG.

Results

The SEM micrographs of the first group treated with 40% HP only (Figs. 1A–C) show partial damage of the enamel surface, while areas of preserved enamel are also present. The enamel has emphasized perikymata spaces and eroded regions.

Unlike the first group, samples subjected to laser-activated bleaching (Figs. 2A–C), demonstrate noticeable loss of integrity of the enamel surface. Additionally, there are erosions of the enamel indicated by the shearing off of the enamel rods. In these samples, the interprismatic spaces are predominantly damaged and loss of the interprismatic substance is evident.

In the samples treated with HP bleaching agent and subsequently with the potential remineralizing agent containing AF (i.e. Mirafluor® C), enamel defects caused by bleaching are still apparent. In Figures 3A–C, the bleached enamel shows depressions and erosions, with emphasized interprismatic spaces.

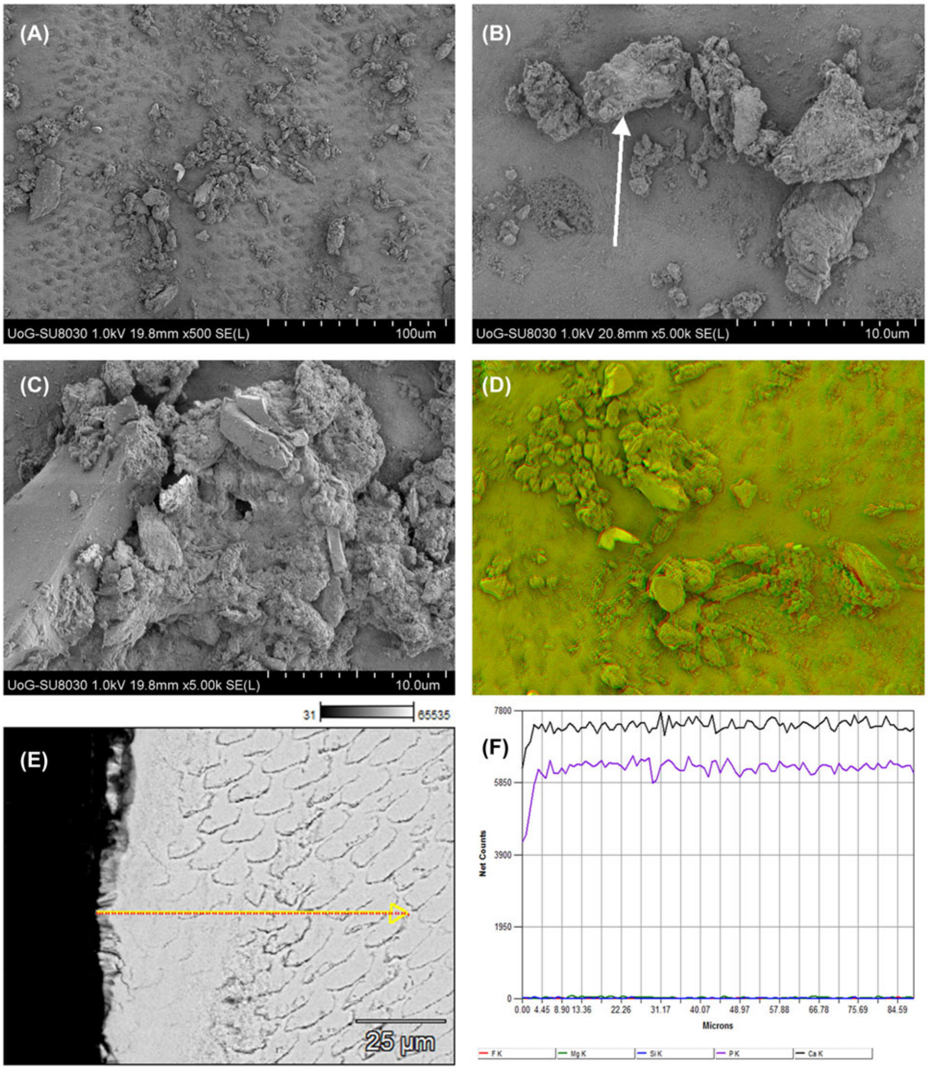

Fig. 7. Enamel treated with Opalescence® Boost, subsequent application of a bioactive glass-containing remineralizing agent Mirawhite® Gelleè: (A), (B), (C) increased enamel porosity, presence of angular deposits on the surface (arrow); (D) 3D-stereomicrograph (anaglyph): increased enamel porosity, loss of interprismatic substance, presence of deposits on the surface (field of view 127-μm wide, nominal magnification $\times 1000$); (E) longitudinal section: slight loss of surface integrity; (F) x-ray line-scan: decreased levels of Ca and P in the enamel surface.

However, the presence of rare deposits on the surface can be observed. Unlike the previously described subgroup, the samples treated by laser-assisted bleaching (Figs. 4A–C), followed by Mirafluor® C toothpaste present numerous erosions and depressions, with no signs of remineralization.

All samples treated with GC Tooth Mouse™ (containing CPP–ACP) showed signs of remineralization. On the samples whitened with the bleaching agent only (Figs. 5A–C), damaged enamel, loss of integrity and erosions are visible. There is increased porosity, mainly due to the loss of inter-rod spaces, but also, the presence of amorphous deposits is observed. The group which was bleached with laser-activation and subsequently treated with GC Tooth Mouse™ (Figs. 6A–C) has a relatively normal enamel surface, with no signs of damage; and again, there is evidence of firmly attached amorphous CPP–ACP deposits.

Unlike the specimens treated with CPP–ACP, both whitened subgroups treated with Mirawhite® Gellèe toothpaste (containing BG) have relatively well preserved enamel, with incremental zones of porosity (Figs. 7A–C and 8A–C). Isolated and aggregated deposits of angular bioactive glass fragments are also present on the enamel surface. Additionally, the enamel treated with the diode laser presents a precipitated layer of BG particles covering almost the entire surface of the enamel.

The SEM micrographs of samples treated with bleaching agent and HA-containing remineralizing agent (Mirasensitive® hap+), denote the presence of normal, even hypermineralized enamel, and a precipitated layer on the

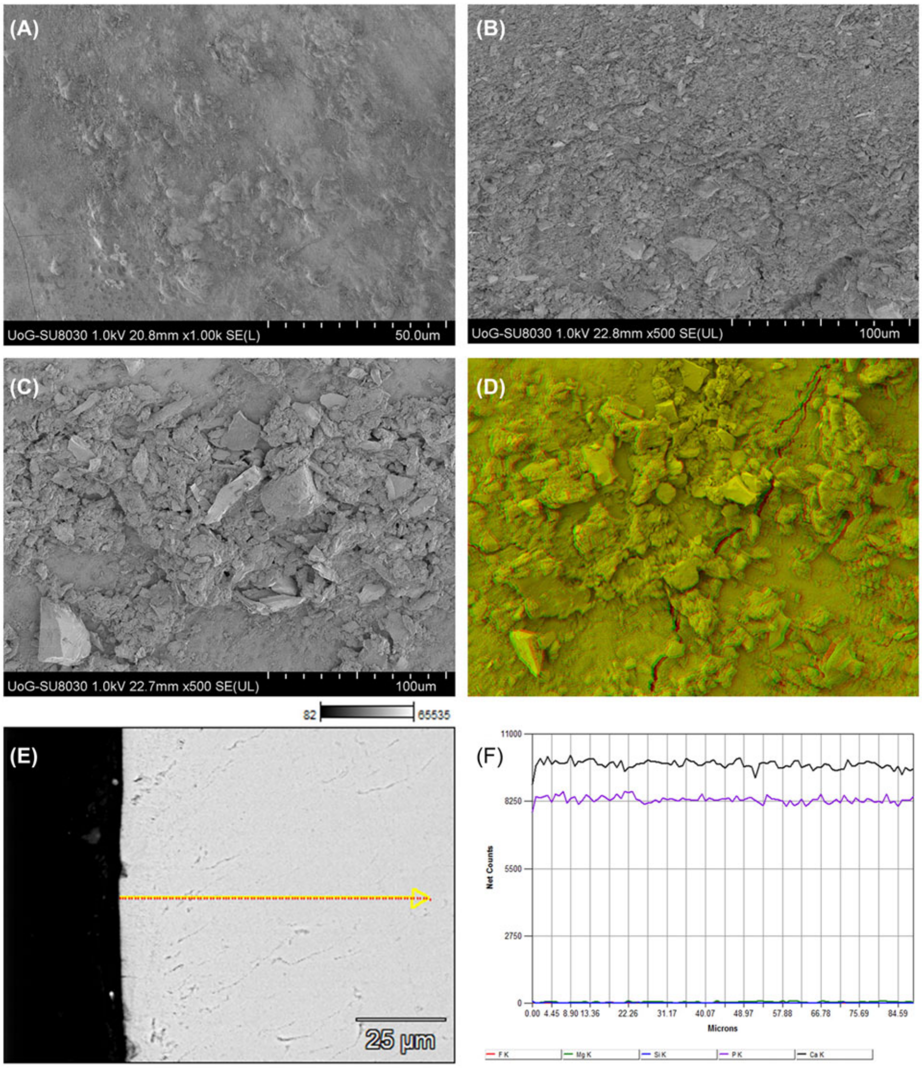

Fig. 8. Enamel treated with Mirawhite® Laser Bleaching and diode laser, with subsequent application of a bioactive glass-containing remineralizing agent Mirawhite® Gelleè: (A), (B) precipitated layer of bioactive glass particles on the surface; (C) presence of conglomerated deposits of angular bioactive glass particles; (D) 3D-stereomicrograph (anaglyph): increased enamel porosity, presence of angular deposits on the surface (field of view 127-μm wide, nominal magnification $\times$1000); (E) longitudinal section: normal appearance of the enamel surface; (F) x-ray line-scan: mineral loss not detected.

surface which resembles artificial enamel. The samples treated with the laser-catalysed bleaching agent show a modest increase in the porosity of the surface enamel and emphasized perikymata, with deposits embedded precisely in the damaged enamel areas (Figs. 10A–C).

The three-dimensional stereomicrographs (anaglyphs) confirm the previous findings in the nonremineralized samples, where more extensive damage is observed in the laser-treated samples (Fig. 1D), as opposed to those treated with nonlaser-activated bleaching (Fig. 2D).

The samples treated with potentially remineralizing toothpastes show discrete signs of damage, but on the stereomicrographs there is a predominance of deposits which differs depending on the type of remineralizing agent used. Namely, samples treated with the agent based on amine fluoride show rare deposits (Figs. 3D and 4D); whereas those remineralized with the CPP–ACP (Figs. 5D and 6D) show individual and slightly confluent deposits attached to the porous enamel surface. The samples treated with BG (Figs. 7D and 8D) reveal angular deposits which originate from the BG particles. These BG deposits are more pronounced on the laser-bleached teeth and cover the entire enamel surface; whereas fewer deposits and a demineralized, porous enamel surface are observed for the teeth bleached only with HP. Treatment with the toothpaste based on HA (Figs. 9D and 10D), leaves a normal enamel surface with the presence of HA deposits.

Qualitative analysis by x-ray line-scans show no loss of minerals in both groups, although in the group where the samples

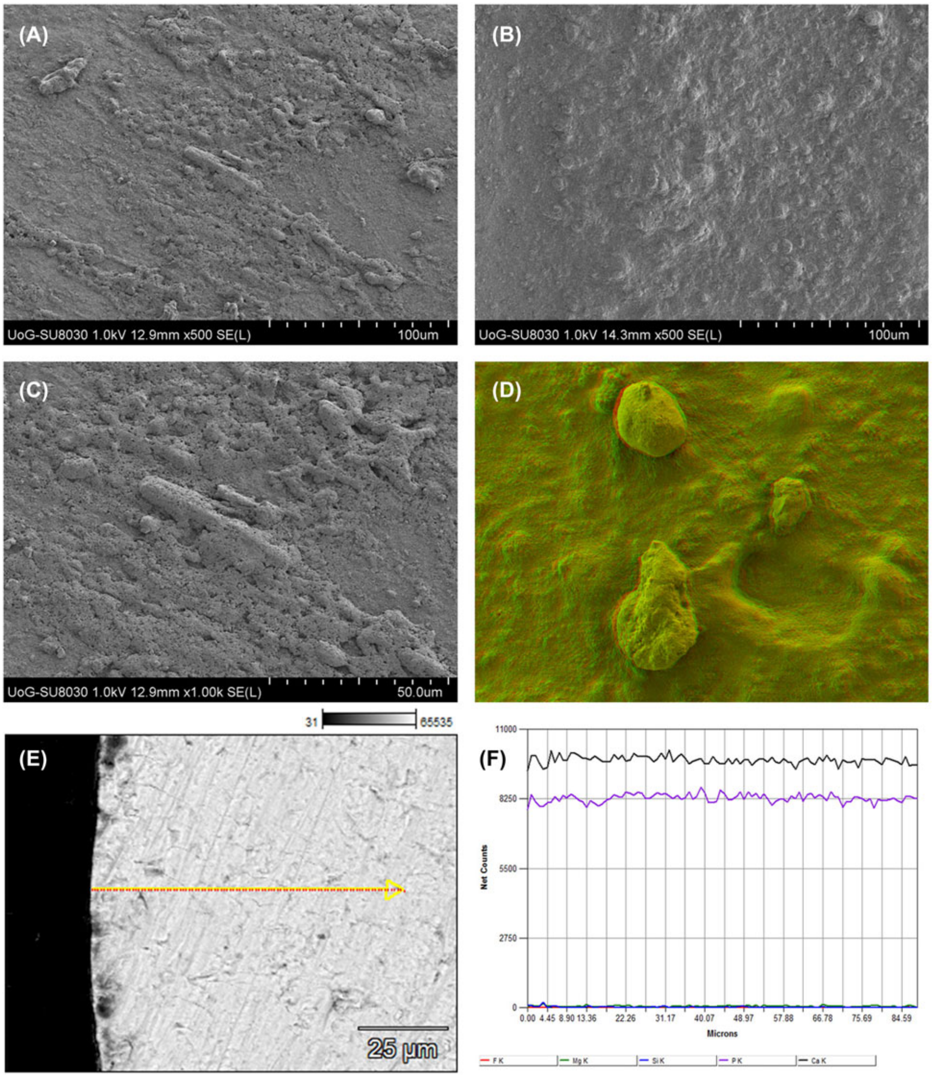

Fig. 9. Enamel treated with Opalescence® Boost, subsequent application of a HA-containing remineralizing agent Mirasensitive® hca+: (A), (B), (C) presence of precipitated artificial-enamel-like layer on the surface; (D) 3D-stereomicrograph (anaglyph): enamel without porosity, presence of deposits on the surface (field of view 127-μm wide, nominal magnification $\times$1000); (E) longitudinal section: normal enamel; (F) x-ray line-scan: absence of mineral loss.

were bleached without laser activation, loss of integrity of the enamel on the longitudinal section was observed.

X-ray line-scans of the samples after the bleaching processes and remineralization with toothpaste containing AF (Figs. 3F and 4F) show no loss of minerals in either group, or any loss of integrity of the enamel (Figs. 3E and 4E). The x-ray line-scans of the sample after the bleaching process and remineralization with toothpaste containing CPP–ACP (Fig. 5F), shows loss of minerals from the enamel, whereas the longitudinal section shows slight loss of integrity of the enamel (Fig. 5E). By contrast, in the samples treated by laser (Fig. 6F), a thin layer of deposits on the surface of the enamel which is composed of Ca, P, Mg and Si is observed. X-ray line-scans of the samples after the bleaching process and remineralization with toothpaste containing BG (Figs. 7F and 8F), show no loss of minerals from the enamel after bleaching and the longitudinal cross-section does not show any loss of integrity of the enamel (Figs. 7E and 8E). Finally, x-ray line-scans of the samples after the bleaching process and remineralization with toothpaste containing HA (Figs. 9F and 10F) do not show loss of minerals from the enamel after bleaching without laser activation, whereas the use of diode laser as a catalyst, causes minimal loss of minerals from the enamel after bleaching. The longitudinal cross-section shows no loss of integrity of the enamel (Figs. 9E and 10E).

The EDX analysis (Table 2) of the individual elements in the surface layer of enamel after different bleaching protocols, indicates that in the teeth bleached with HP without laser

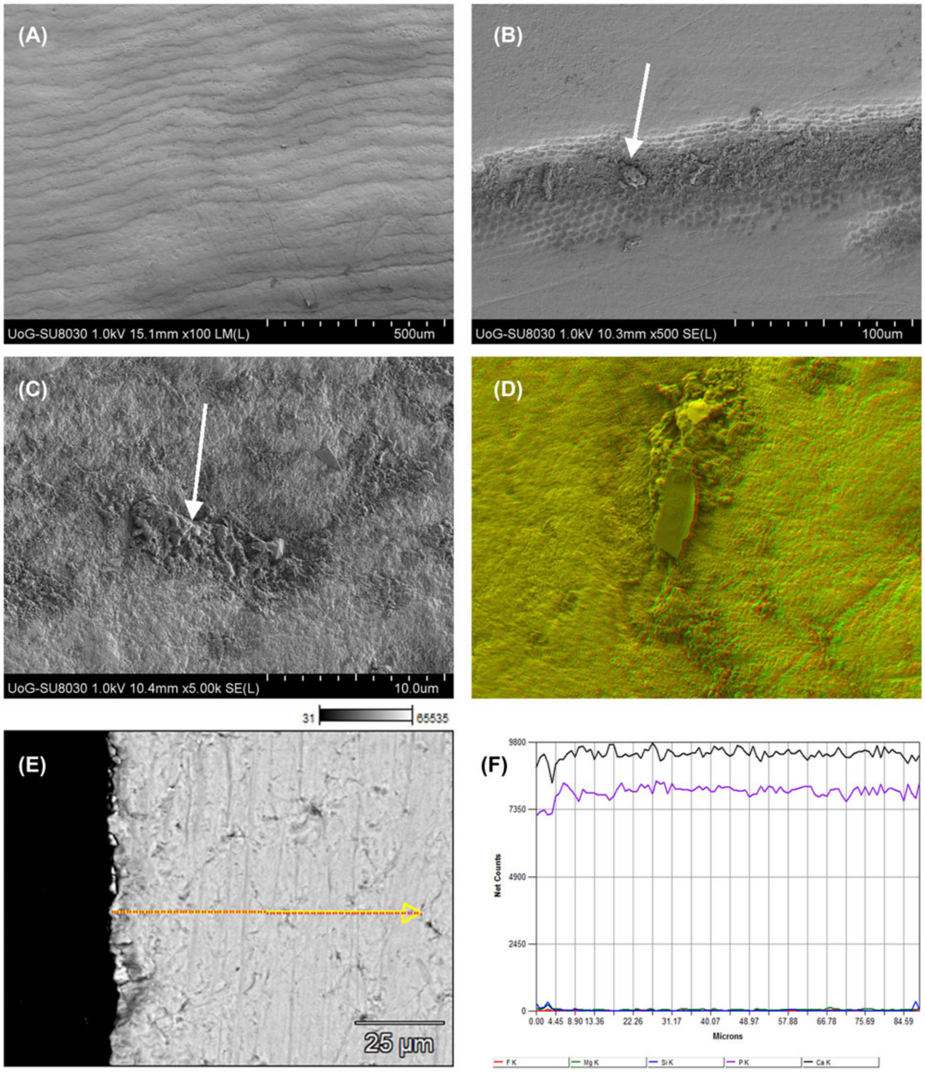

Fig. 10. Enamel treated with Mirawhite® Laser Bleaching and diode laser, with subsequent application of a HA-containing remineralizing agent Mirasensitive® hap+: (A) normal enamel with slightly emphasized perikymata; (B), (C) porosity in the interprismatic regions and presence of conglomerated deposits of HA particles in the porous regions; (D) 3D-stereomicrograph (anaglyph): increased enamel porosity, presence of angular deposits in the depressed areas; (E) minimal loss of enamel integrity (field of view 25-μm wide, nominal magnification $\times 5000$); (F) x-ray line-scan: decreased levels of Ca and P.

activation statistically significant loss of Na and Mg occurs, whereas bleaching in combination with the laser leads to a statistically significant loss of Ca and P. After the remineralization, there isn't a statistically significant increase in the level of P in any of the subgroups, whereas bleaching with HP combined with diode laser leads to statistically significant increase in the postremineralization level of Ca.

Discussion

Although quite widespread, the procedure of teeth whitening is still regarded as controversial. Morphological changes have been observed in the enamel, with erosion, craters and porosity being reported by various authors (Josey *et al.*, 1996; McCracken & Haywood, 1996; Hegedüs *et al.*, 1999)

Our results are consistent with these findings. Micrographs of enamel specimens subjected to HP bleaching only suggest that there is partial damage of the enamel surface with emphasized perikymata and eroded surfaces, whereas the longitudinal section shows loss of enamel integrity. With the specimens subjected to laser-activated bleaching, some loss of the enamel surface also occurred and there was evidence of erosion and shearing off of the enamel rods.

Our results from semi-quantitative EDX point analysis showed statistically significant loss of Na and Mg in the samples bleached without laser activation. Results from specimens

Table 2. Elemental distribution of Na, Mg, P and Ca in the surface enamel by EDX semiquantitative point analysis: OB (Opalescence® Boost), MLB (Mirawhite® Laser Bleaching + diode laser), control (bleaching only), MC (Mirafluor® C), GCTM (GC Tooth Mousse™), MG (Mirawhite® Gelleè), MS (Mirasensitive® hap+).

Element %	Na	Mg	P	Ca
Opalescence® Boost	0.18 (0.02)	0.03 (0.02)	25.71 (1.57)	53.10 (1.38)
Opalescence® Boost + Mirafluor® C	0.29 (0.09)	0.07 (0.03)	22.25 (3.90)	46.23 (7.19)
Opalescence® Boost + GC Tooth Mousse™	0.20 (0.04)	/	25.71 (2.07)	51.12 (3.95)
Opalescence® Boost + Mirawhite® Gelleè	0.32 (0.06)	/	22.99 (2.96)	44.83 (5.22)
Opalescence® Boost + Mirasensitive® hap+	0.21 (0.03)	0.07 (0.06)	22.75 (1.42)	50.78 (1.92)
Mirawhite® Laser Bleaching	0.61 (0.05)	0.33 (0.13)	19.53 (2.28)	37.80 (3.17)
Mirawhite® Laser Bleaching + Mirafluor® C	0.21 (0.04)	0.11 (0.06)	23.44 (1.57)	50.95 (1.79)
Mirawhite® LaserBleaching + GC Tooth Mousse™	0.33 (0.07)	0.05 (0.03)	26.43 (2.31)	47.84 (2.64)
Mirawhite® Laser Bleaching + Mirawhite® Gelleè	0.29 (0.02)	0.19 (0.10)	22.96 (1.49)	47.65 (2.23)
Mirawhite® Laser Bleaching + Mirasensitive® hap+	0.26 (0.08)	0.07 (0.03)	20.61 (1.72)	46.75 (5.55)
p(ANOVA)	3.82^{-30}	1.29^{-11}	2.33^{-09}	7.37^{-12}

bleached with a laser showed statistically significant loss of Ca and P. These changes indicate loss of mineralized tissue which is consistent with findings from previous studies.

The observations of the enamel in this study, following application of amine fluoride, show that there is a degree of remineralization. However, morpholgical defects are still apparent in the enamel. Enamel bleached with laser activation, and then treated with amine fluoride, reveals mainly erosions and depression, without any sign of remineralization; however, representative x-ray line scans show that there has been no loss of mineral phase. Previous studies have also confirmed that the topical application of fluoride immediately after bleaching is able to achieve remineralization of the affected enamel (White & Featherstone, 1987).

Our data revealed that toothpaste based on CPP–ACP is capable of remineralizing damaged enamel. The samples in the first group bleached without laser activation and consecutive application of CPP–ACP toothpaste, still have damaged areas, loss of integrity and appearance of enamel erosions. Enhanced porosity (primarily loss of interprismatic substance) is present, but amorphous deposits implying remineralization are noticeable. The group, subjected to laser-assisted bleaching and CPP–ACP toothpaste displays a relatively normal enamel surface with no signs of damage; and again, as with the previous samples, existence of amorphous deposits firmly attached to the surface of the enamel are found. Loss of mineral occurred after nonlaser bleaching and treatment with CPP–ACP toothpaste; by contrast, the samples treated with laser-activated bleaching and CPP–ACP toothpaste have surface deposits composed of Ca, P, Mg and Si, which can play a protective role in case of further acid attack. These findings confirm previous observations of Yamaguchi *et al.* (Yamaguchi *et al.*, 2006).

The remineralization potential of toothpastes containing BG in the form of 45S5 Bioglass® shown in this study confirms the findings of previous research (Anderson & Kangasniemi, 1991; Burwell & Greenspan, 2007). Unlike the specimens treated with CPP–ACP, all samples treated with bioactive glass toothpaste, showed preserved enamel with zones of porosity; but emphasizing the presence of single and aggregated angular deposits of BG. Additionally, the presence of a precipitated layer of BG is seen on the enamel treated with laser-bleaching. The qualitative analysis by x-ray line-scans showed an absence of mineral loss in both groups, and a loss of integrity of the enamel was noticed in the longitudinal section. The increased level of Ca was significant in samples bleached with diode laser.

The HA-containing toothpastes have several clinical advantages: efficiency in the prevention and treatment of caries, periodontal diseases, elimination of odour from the oral cavity and, finally, teeth whitening effect. The HA component in the paste has a role in removing dental plaque, promoting remineralization and inhibiting the formation of an initial carious lesion. Furthermore the previously demineralized surface becomes smoother and shinier after remineralization with HA (White & Featherstone, 1987).

Overall, our results confirm that tooth whitening procedures damage the enamel surface with laser bleaching being more aggressive than simple peroxide treatment. However, these changes are reversible and can be repaired by the application of remineralizing toothpastes. Although fluoride has been widely studied for this purpose, it has limitations. The various calcium phosphate systems (CPP–ACP, BG and HA) incorporated into toothpastes proved more efficacious in enamel remineralization. It thus seems that the damage done by bleaching procedures can be repaired by subsequent use of toothpastes containing remineralizing agents. In a clinical situation, the clinician should apply a remineralizing agent after each bleaching cycle on the bleached surfaces in order to promote the remineralization of the calcium and phosphate depleted surfaces by rubbing the surfaces with a calcium phosphate containing toothpaste for 3 min.

This approach has the potential to rebuild the enamel completely and to return it to its baseline condition.

Conclusions

Tooth bleaching leads to significant changes to the morphology of the enamel (increased porosity, depressions and erosions) and to reductions in its mineral content. Laser-activated tooth bleaching has been shown to be especially damaging. These changes can be reversed by the application of remineralizing toothpastes, especially those containing calcium and phosphate.

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