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Influence of Bioactive Materials Used on the Dentin Surface Whitened with Carbamide Peroxide 16%

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This study investigated the influence of bioactive materials on the dentin surface whitened. Material and Methods: Three bovine teeth were shaped into three dentin wafers. Each wafer was then sectioned, into six dentin slices. One slice from each tooth was distributed into one of 6 groups: 1.CG = control group (distilled water); 2.WT = whitening treatment; 3.WT + MI Paste Plus, applied once a day; 4.WT + Relief ACP30, applied once a day for 30 mintes; 5.WT + Relief ACP60, applied once a day for 60 minutes; 6.WT + Biosilicate®, applied once a week. All groups were treated over 14 days. Results: CG presented all dentinal tubules occluded by smear layer; WT group was observed all dentinal tubules opened. In the groups 3, 4 and 6, tubules were occluded. Group 5, dentinal tubules were completely occluded by mineral deposits. Conclusion: The use of bioactive materials immediately after whitening treatment can reduce or even avoid the demineralization effect of whitening and avoid exposing dentinal tubules.

Keywords: *dentin, dental whitening, remineralization*

1. Introduction

Dentin hypersensitivity is considered to be partly due to the presence of dentinal tubules at the exposed dentin surface where the tubules are wider and more numerous than in nonsensitive areas[1]. The hydrodynamic theory assumes that a stimulus applied on the dentin surface causes movement of tubular fluid, which activates mechanoreceptor nerves, eliciting pain[2]. According to this theory, if the functional radius of opened dentinal tubules decreases, then the permeability is also decreased, reducing dentin sensitivity[3].

Whitening gels can cause demineralization when in contact with dentin, for example in areas of gingival recession and non-carious lesions, and lead to unwanted side effects, such as the opening of the dentinal tubules[4]. Numerous research studies[4-8] show that whitening agents can lead to alterations in the dental structure, perhaps due to the low pH of the gels and the presence of chelating agents[5].

The resultant dental sensitivity can be a nuisance to most patients, while the prevention and treatment of the sensitivity is a challenge to most dentists[6]. The concern over the sensitivity brought by the whitening treatments has led the dental industry to develop whitening gels enhanced with desensitizers such as potassium nitrate and fluorides[7,8]. Nevertheless, these substances do not solve the issue completely, and do not reduce the demineralization process[6].

Recently, the use of bioactive materials ACP (Amorphous Calcium Phosphate), CCP-ACP (Casein Phosphopeptite and Amorphous Calcium Phosphate), bioglass and bio-glass ceramics as desensitizers and remineralization agents have been used, but there is limited research on this area[9-13]. The main goal of these materials is to promote remineralization of the dental structures by the formation of a new layer of amorphous calcium phosphate on the surface where the product is applied. Latter on, the reaction with hydroxyl ions, carbonate and fluoride, which are available in the oral cavity, initiates the crystallization of a new surface layer into apatite[14]. This remineralization process seems to be much more effective than potassium nitrate or fluoride alone[15,16], because it does not only act to relieve pain but prevents its onset.

This new therapy seems to be a viable alternative as the use of bio-active substances have been proven to be effective in preventing cavities and can be found in a wide variety of toothpastes, desensitizers, composites, sealants and chewing gum[17,18].

Respected authors[19,20] use microscope images in high resolution to study the demineralization and remineralization phenomenon, both in dentin and enamel. The observation of structural and ultra-structural changes can enhance the understanding of the modifications caused by the use of whitening gels, therefore the use of high resolution and higher magnification images is recommended.

Perhaps the combined use of whitening gel and bio-active materials can contribute to less demineralization on the dental structures during the whitening treatment and consequently to reduced sensitivity during and after treatment. Nevertheless, there is little information in the literature regarding the use of these materials combined with whitening agents. Therefore, the main purpose of this study is to investigate the structure effects of 3 bio-active materials on whitened dentin using FEG-SEM (Field Emission Gum Scanning Electron Microscopy) images. The evaluation criteria used was the amount of dentinal tubules occluded or not.

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2. Method

2.1. The dentin specimens were morphologicaly analyzed using a FEG-SEM.

Samples: 3 bovine teeth were cleaned with ultrasound, pumice stone and water. All samples were stored in distilled water at 37 °C.

Sample preparation: The incisal and cervical third of the crown portion of three bovine incisive were removed. Each tooth was then sectioned into six slices and divided into 6 groups. The samples were embedded in self-curing acrylic resin, serially gritted and polished using a polishing device (Knuth Rotor, Struers, Denmark) with aluminum oxide abrasive papers (600; 800; 1,200; 1,500; 2,000; 2,500 and 4,000 grit). The slices were ultrasonically cleansed in distilled water for 20 minutes to remove polishing residues. Then, were stored in distilled water at 37 °C for 24 hours. For each tooth, a slice was obtained for each of the treatment groups. This methodology allowed for standardization in relation to quantity, size and orientation of the dentinal tubules, facilitating the posterior morphologic comparison of the groups.

Materials used: The various products (Table 1) were applied to the surface of the samples according to the different groups.

Composition of the 3 bioactive materials used in this study: MI Paste Plus: 2% casein phosphopeptides (CPP)-amorphous calcium phosphate (ACP).

Relief ACP: 5% potassium nitrate; 0,22% sodium fluoride and 0,75% amorphous calcium phosphate (ACP).

Biosilicate®: >99.5% crystalline glass-ceramic powder of the P_2O_5–Na_2O–CaO–SiO_2 system having a particle size distribution between 0.1 and 10 µm.

pH of the whitening gels: Before applying the whitening gels onto the samples the pH of the gels was measured using a digital pH measuring device (DIGIMED, model DM–20), which was calibrated with standard solutions with a pH = 7 and pH = 4.

Product application: The control group was stored in distilled water (37 °C). In the other groups was applied a 16% carbamide peroxide whitening gel (Perfect Whiteness-FGM) for 8 hours daily. After each application of whitening gel specimens were washed with distilled water.

In the groups WT + remineralizing products, the specimens were treated daily with the whitening gel for 8 hours, washed and then the remineralizing product was applied, according with the time application present in the Table 1. After this application period, the specimens were washed to be stored until the next day.

All products were applied during 14 consecutive days, except for Biosilicate® - Vitrovita, which was applied once a week. The application of all products studied followed manufacturer's instructions regarding technique and time of contact, except for the group WT + Relief ACP 60, where the product was applied for 60 minutes, twice as long as manufacturer's instructions (Table 1).

All specimens were stored in distilled water at 37 °C. When treatment was completed at day 14, samples were fixed in a glutaraldehyde solution, dehydrated in ethanol solutions and hexametildisilazane. After platinum coating, the dentinal surfaces were analyzed using FEG-SEM.

Ultra-structural analyzes: 18 specimens were divided into 6 groups (Figure 1). A FEG (JEOL JSM-7600F, Japan) was used to obtain five (5) representative images at magnification of 20,000 and 50,000x from each specimen. All images were randomly presented to 3 calibrated examiners for evaluation of structural changes in the tubular dentin. All examiners used the same scoring system of 0-4, in ascending order, from the best to worst situation. If dentinal tubules were completely occluded, score 0 was assigned to the specimen. Score 1 was given to specimens exhibiting 75% of the dentinal tubules occluded; score 2 for 50% and score 3 for 25% of the dentinal tubules occluded. If dentinal tubules were not occluded, score 4 was given.

Statistical analysis was performed using the Kruskal-Wallis test at a significance level of 95%. The Kendall coefficient of concordance was used to measure the agreement between examiners.

3. Results

The pH of the whitening gel used in this study (Whiteness Perfect – FGM) was fond to be slightly acidic, at pH 5.9. Figure 1 shows that the untreated control group showed intact dentinal tubules, as expected (Figure 2a), the whitening treatment only group (WT) exhibit mostly open dentinal tubules (Figure 2b). For treatment groups WT + MI Paste Plus, WT + Relief ACP 30 e WT + Biosilicate®, many of the dentinal tubes were obliterated (Figures 2c, d and f respectively). For the group WT + Relief ACP 60 the dentinal tubules were completely obliterated (Figure 2e) and similar to untreated, intact dentin controls.

Table 2 presents the evaluators' scores of the changes observed in the specimens. WT group (whitening treatment only) was statistically different from all treated groups and untreated control. The Kruskal-Wallis test did not demonstrate statistically significant differences among untreated control group and any of the groups treated with the four different bioactive materials.

4. Discussion

Opening of the dentinal tubules is a problem that may occur when using a whitening gel, which has the potential to cause demineralization. If the gel is in contact with areas of exposed dentin, such as gingival recession and non-carious lesions[21], the dentinal

Table 1. Materials used in the study, manufacturer, basic composition, and treatment protocol.

Groups	Whitening treatment	Whitening product/ manufacturer	Remineralizing treatment	Remineralizing product/ manufacturer	Basic-composition	Application time per day	Total number of applications
CG (Control Group)	no	–	no	–	–	–	–
WT (Whitening Treatment)	yes	Whiteness Perfect / FGM, Brazil	no	–	Carbamide Peroxide 16% + Potassium Nitrate + Fluoride	8 hours	14
WT + MI Paste Plus	yes	Whiteness Perfect / FGM, Brazil	yes	MI Paste / GC, Japan	CPP-ACP + Fluoride	5 minutes	14
WT + Relief ACP 30	yes	Whiteness Perfect / FGM, Brazil	yes	Relief ACP / Discus Dental, USA	Potassium nitrate + Fluoride + ACP	30 minutes	14
WT + Relief ACP 60	yes	Whiteness Perfect / FGM, Brazil	yes	Relief ACP / Discus Dental, USA	Potassium nitrate + Fluoride + ACP	60 minutes	14
WT + Biosilicate®	yes	Whiteness Perfect / FGM, Brazil	yes	Biosilicate® / Vitrovita, Brazil	Glass-ceramic crystallized glass P_2O_5–Na_2O–CaO–SiO_2	5 minutes	2

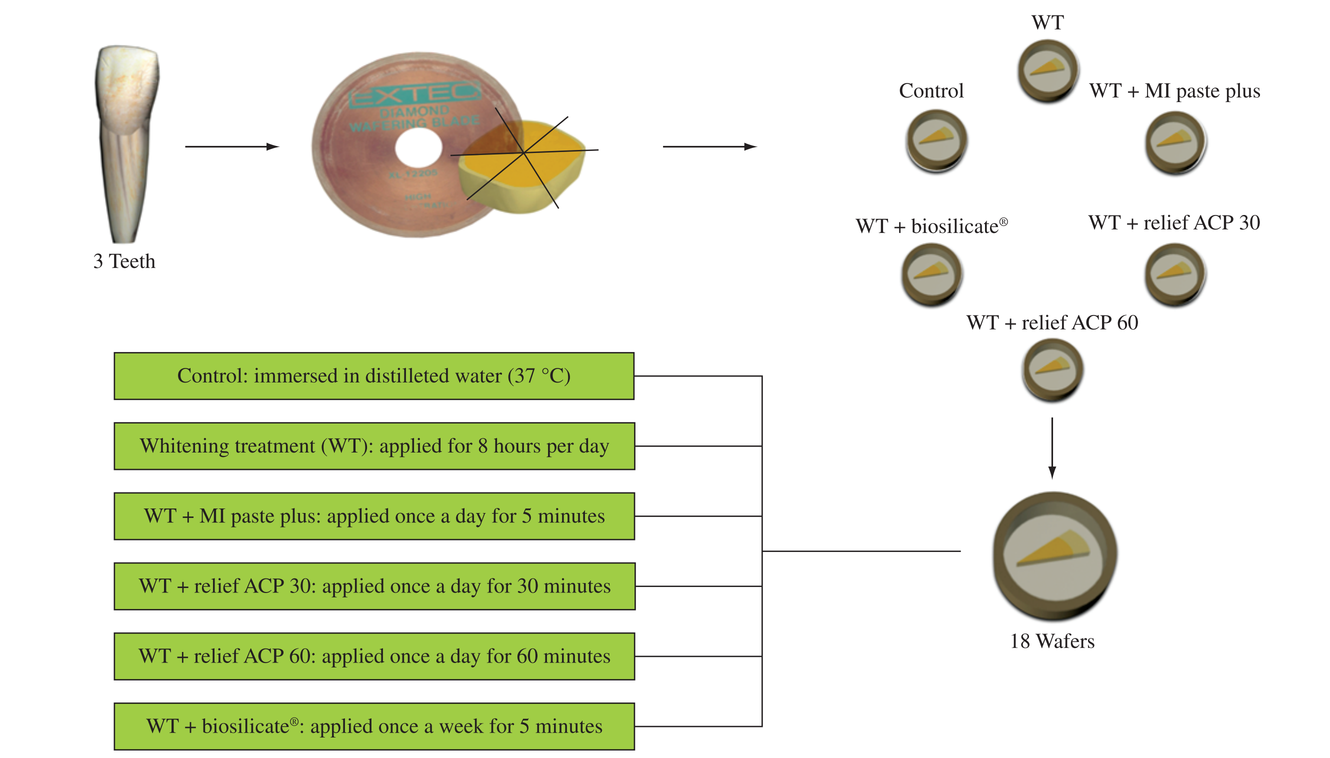

Figure 1. Study outline: preparing, cutting and separating into groups.

tubules will open, causing trans and post treatment sensitivity. Clinical studies have shown that approximately 55% of all patients evaluated after a whitening treatment with 10% CP reported sensitivity. Of these patients, about two-thirds had gingival recession, flawed restorations or defects in the cement-enamel union[22]. Another study showed that patients with pre-existing sensitivity, as a consequence of root exposure, presented higher levels of trans- and post- whitening treatment sensitivity when compared to patients that did not present root exposure[23].

In the present study, the changes in the dental structure have been evaluated using scanning electronic microscope images.

There are several studies in the literature[7, 8, 15, 24-27] that show the influence of conventional bleaching agents (with potassium nitrate and fluoride) on tooth structure.

Hydrogen peroxide is also able to cause changes in the intertubular dentin and a significant decrease in microhardness values of dentin surface[25]. These changes resulted from the oxidative effect of peroxide that acts mainly on the organic components of dentin and the low pH of the gel[6, 25]. However, as it is a recent development of the dental industry, the association of bioactive materials (ACP, CCP-ACP, bioglass and bio glass-ceramics) and bleaching agents lack on studies available in the literature.

Only two clinical studies[28, 29] were found that examined the effectiveness of the addition of amorphous calcium phosphate (ACP) to a whitening gel. These studies showed that the addition of ACP was a viable strategy to reduce trans- and post- bleaching sensitivity.

The variability of the results obtained in different research can be explained by the different methodology used, which varies according to the teeth used, pH and composition of the whitening gel studied, the solution used for sample storage and contact time of the whitening gels[(6)] and also because most studies do not consider the differences in the ultra-structure topography, using microscopic images of low magnification[30, 31].

One of the most significant contributions of this study relies on the method used to obtain the specimens, where a sample was obtained from each tooth for all experimental groups. This facilitates the comparison between groups, as a significant degree of morphology variation can be found from one tooth to the next and that can influence the micro-structural analysis[31]. Most studies[32-34] do not take this into consideration and use specimens for control group and experimental groups deriving from different teeth, furthermore SEM images obtained are frequently used only to illustrate the results. In this study, with the use of images in high magnification, it was possible to observe that the specimens in the control group presented obliterated dental tubules (Figure 2a). Nevertheless, the samples treated with the whitening gel (group WT) presented tubules that were wide open (Figure 2b), showing decalcification, which was confirmed by statistical analysis of the micrographs. This is most likely the effect of the acidic character and chelating agents in the gel. Some studies show that there is loss of calcium[35, 36] and phosphate[25] on the surface of teeth structures in contact with whitening gels, which leads to dental demineralization. The peroxide can cause modifications in the dentin and significantly reduce its hardness. These changes are in general due to the low pH of the gels and the oxidation process of the peroxides, which mostly act on the organic components of dentin[25].

The acidic whitening gel used here opened most dentinal tubules (Figure 2b), but they were closed on the specimens treated with MI Paste Plus, Relief ACP, and Biosilicate® (Figures 2c, 2d and 2f). This observation suggests that the combination of remineralizing products and whitening treatment can drastically reduce the number of opened dentin tubules. These remineralizing products may facilitate the deposition of Ca and PO_4 ions lost during the whitening treatment, which precipitate in the form of amorphous calcium phosphate (ACP), forming an initial layer of new superficial amorphous calcium phosphates which eventually crystallize by the reaction with hydroxyl, carbonate and fluoride from the oral cavity. A recent clinical study

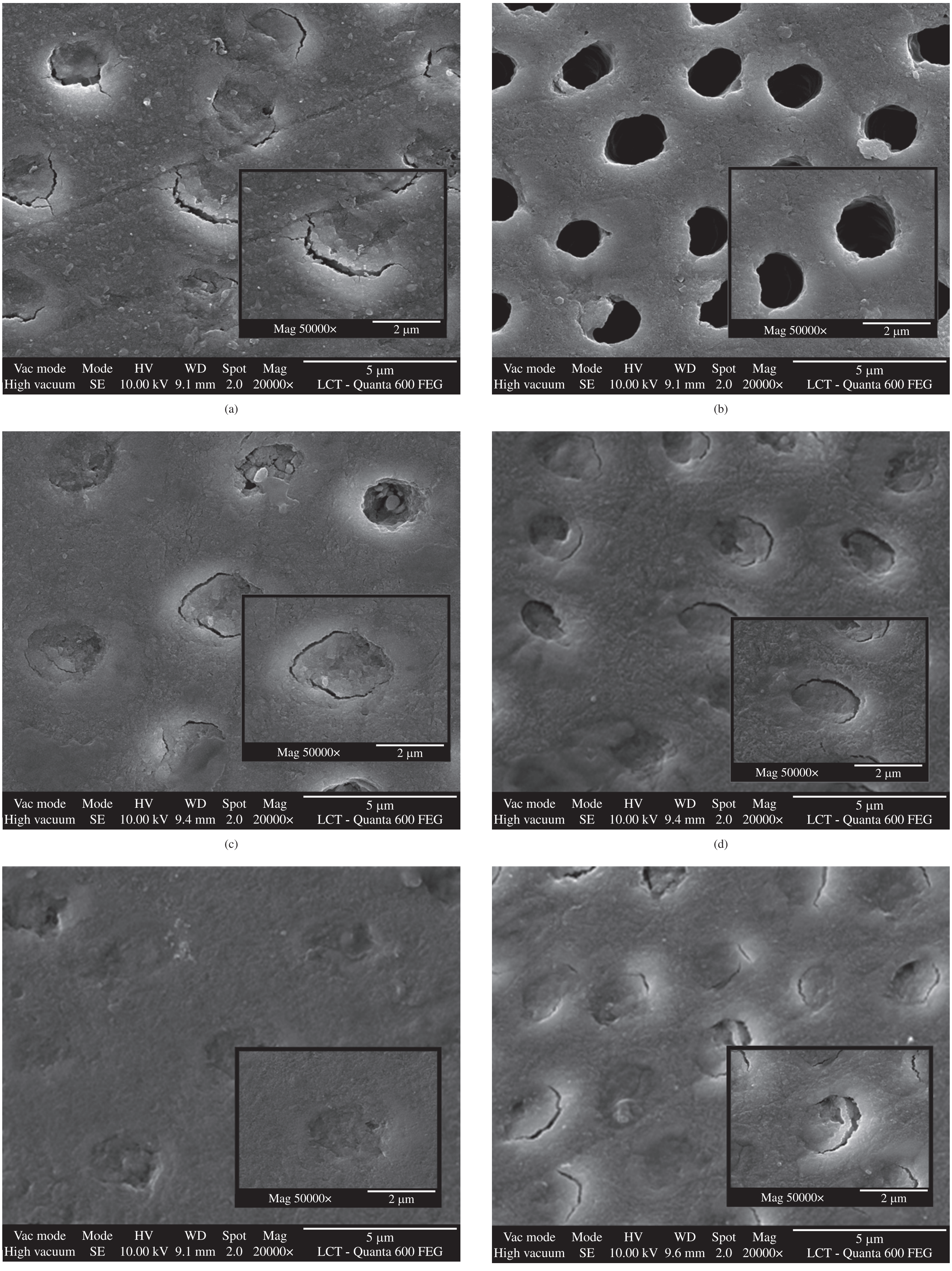

Figure 2. Representative FEG-SEM micrographs (original mag. = 20.000×) of dentin tubular surface: a) Control – untreated dentin; b) dentin surface after whitening treatment (WT); c) after WT + MI Paste; d) after WT + Relief ACP 30 minutes; e) after WT + Relief ACP 60 minutes; f) after WT + Biosilicate® (Insets = original mag. = 50.000×).

Table 2. Score means and statistical data of changes found in the different groups.

Group	Score mean
WT (whitening treatment)	3.7 A
WT + MI Paste Plus	0.3 B
WT + ACP 30 minutes	0.6 B
WT + ACP 60 minutes	0.0 B
WT + Biosilicate®	1.0 B
Control (without treatment)	0.0 B

Kruskal-Wallis test; means labeled with the same superscript letters were not statistically different ($p < 0.05$).

reported a reduction on the trans- and post- treatment sensitivity with the use a whitening gel enhanced with Ca and PO_4[29]. This mineral deposition may be related to the reduction of sensitivity, but additional clinical evidence is needed.

In the specimens that were whitened and treated with Relief ACP for 60 minutes daily, with an application time twice as long as recommended by the manufacturer, the dentinal tubules were completely obliterated (Figure 2e). The longer application time may have positively influenced the obliteration of the tubules leading to a higher level of remineralization. However, the statistical analysis of micrographs revealed no statistically significant difference between this and the other groups bleached and treated with bioactive materials. This may be related to the use of non-parametric analysis and limited sample size.

Because of the sensitivity[23,28], and the structural[37-39] and chemical[36,40,41] changes caused by whitening agents, the supply of Ca and PO_4 ions (through ACP technology) in conjunction with the whitening treatment may be a helpful approach to minimizing the loss of minerals, as well as the occurrence of porosity and erosion in the dental structure resulting from whitening treatments[14,28]. This combination is likely to reduce the trans- and post- treatment sensitivity by obliterating the dentinal tubules[11].

Other factors that show the efficiency of bioactive materials on tooth structure could be further investigated, for example, precipitation depth and chemical analysis of the precipitate.

5. Conclusion

The use of bioactive materials, such as ACP and Biosilicate® glass-ceramic, together with whitening treatments can promote remineralization and close the dentinal tubules. Further investigation is needed to evaluate the role of these changes in reducing whitening-related dental sensitivity.

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