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Remineralizing potential of a fluoride varnish modified by bioactive nanoparticles

Potencial remineralizante de um verniz fluoretado modificado por nanopartículas bioativas

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ABSTRACT

Objective: Evaluate a fluoride varnish modified by nanostructures with the bioactive qualities of silica (SiO2) and niobium pentoxide (Nb2O5), testing its remineralizing potential by surface hardness (SH) and energy-dispersive X-ray spectroscopy (EDX). **Material and Methods:** Bovine enamel specimens (6×4×2mm) were prepared and submitted to a demineralizing/remineralizing process to produce a subsurface caries-like lesion, evaluated by transversal microradiography image (TMR) and subsequently distributed randomly into three groups: fluoride varnish (VZ); fluoride varnish + silica gelatin (VZ-SiO2) and fluoride varnish + niobium nanoparticles (VZ-Nb2O5). The specimens were subjected to a pH-cycling demineralizing/remineralizing process for 7 days at 37ºC. The %SH loss and %SH recovery (after the pH-cycling regimen) were calculated (n=10/group). The Ca/P weight ratio before and after the pH-cycling regimen was evaluated through SEM/EDX. A two-way ANOVA followed by Tukey's test ($p<0.05$) was performed for hardness and EDX. **Results:** TMR image showed the formation of an artificial subsurface lesion, and a significant SH increase was observed in the VZ-Nb2O5 group ($p<0.05$). Regarding the %SHL and %SHR, the VZ-Nb2O5 and VZ-SiO2 were significantly different compared to the VZ group ($p<0.001$), but VZ-Nb2O5 presented higher values. The Ca/P ratio showed that blocks treated with VZ-SiO2 and VZ-Nb2O5 showed greater ion deposition, particularly in the presence of Nb. **Conclusion:** The bioactivity of niobium facilitated greater interaction between the enamel and the varnish, leading to a slow release of nanoparticles and a longer-lasting remineralizing effect.

Keywords
Fluorides; Nanostructures; Niobium; Operative dentistry; Tooth remineralization.

RESUMO

Objetivo: Avaliar um verniz fluoretado modificado por nanoestruturas com a bioatividade da sílica (SiO2) e pentóxido de nióbio (Nb2O5), testando seu potencial remineralizador pela dureza de superfície (SH) e espectroscopia de energia dispersiva de raios-X (EDX). **Material e Métodos:** Espécimes de esmalte bovino (6×4×2mm) foram preparados e submetidos à desmineralização/remineralização para produzir uma lesão subsuperficial semelhante a cárie, avaliada por imagem de microrradiografia transversal (TMR) sendo distribuída em três grupos: verniz fluoretado (VZ); verniz fluoretado+gelatina de sílica (VZ-SiO2) e verniz fluoretado+nanopartículas de nióbio (VZ-Nb2O5). As amostras foram submetidas à desmineralização/remineralização por ciclagem de pH durante 7 dias a 37°C. A porcentagem de perda e recuperação de SH foram calculadas (n=10/grupo). A relação em peso Ca/P antes e depois da ciclagem foi avaliada através de MEV/EDX. ANOVA a dois critérios seguida do teste de Tukey ($p<0,05$) foi realizada para dureza e EDX. **Resultados:** A TMR mostrou a formação de uma lesão subsuperficial e um aumento significativo de SH foi observado no grupo VZ-Nb2O5 ($p<0,05$). Em relação ao %SHL e %SHR, o VZ-Nb2O5 e o VZ-SiO2 foram significativamente diferentes em relação ao grupo VZ ($p<0,001$),

mas o VZ-Nb2O5 apresentou valores maiores. A relação Ca/P mostrou que os blocos tratados com VZ-SiO2 e VZ-Nb2O5 apresentaram maior deposição de íons, principalmente na presença de Nb. **Conclusão:** A bioatividade do nióbio facilitou maior interação entre o esmalte e o verniz, levando a uma liberação lenta de nanopartículas e a um efeito remineralizante mais duradouro.
PALAVRAS-CHAVE
Fluoretos; Nanoestruturas; Nióbio; Dentística operatória; Remineralização dentária.

INTRODUCTION

In recent years, there has been a shift in dental approaches towards preventive methods, risk control, and early diagnosis of tooth decay, moving away from extensive restorative procedures [1]. One of the key focuses is on remineralizing subsurface carious lesions before they progress to cavitation, thus avoiding the need for invasive restorative interventions [2-4]. The prevention, diagnosis, and early treatment of active white spot lesions through remineralizing products are crucial in preventing the need for more invasive treatments [5]. These agents can recover the mineral loss, and they are regarded as a very helpful alternative and a preventive step to control the demineralization process instead of invasive techniques [6]. White spot lesions (WSL) are identified with the loss of minerals, despite the hard dental surfaces. Most of the situations are associated with poor oral hygiene, hypofunction of salivary glands, and early WSL having a reversible hard surface [7].

Fluoride varnish is a noninvasive treatment option in the remineralization process. It is safe, effective, and can enhance the results of topical fluoride therapies by increasing enamel exposure to fluoride. Sodium fluoride (NaF) varnish is one derivative that is topically applied. The concentration of fluoride and the duration of contact between fluoride and the tooth structure determine the extent to which the tooth structure absorbs fluoride. Multiple application sessions are typically required annually [8], requiring patient cooperation [9]. Fluoride varnish aids in remineralization by releasing calcium, phosphate, and fluoride ions, thereby increasing the saturation level in the liquid medium surrounding dental hard tissues. This promotes the remineralization process [10]. Fluoride has also been shown to significantly reduce bacterial adhesion to tooth surfaces [11] and increase enamel surface hardness [12].

Different remineralizing agents are constantly being evaluated to achieve a more consistent and stable effect on enamel remineralization. The use of silica (SiO_2) in the remineralization of enamel caries lesions has shown promising results and more effective in enamel remineralization compared to other topical agents such as fluoride and Casein Phosphopeptide - Amorphous Calcium Phosphate (CPP-ACP) [13]. It has been demonstrated that incorporating tailored hybrid nanofibers doped with SiO_2 and SiO_2-CaP into enamel resin infiltrants can inhibit demineralization and increase enamel hardness [14].

Nanoparticles containing SiO_2 derived from the sol-gel process have shown increased apatite deposition [15], demonstrating bioactivity potential. When in contact with saliva, sodium ions from silica particles react with hydrogen cations from saliva, leading to the release of calcium and phosphate ions. The increase in salivary pH helps to precipitate the extra calcium and phosphate ions, resulting in the formation of calcium fluoride and eventually hydroxyapatite. This enhances the remineralization of tooth structures [13,16]. However, one challenge with bioactive materials is their low mechanical strength [17]. With advancements in nanotechnology, bioactive materials with improved mechanical properties have been investigated for dentistry and biomedical applications [14,18-20].

One example is niobium pentoxide (Nb_2O_5), a metallic oxide that exhibits bioactivity, develops hydroxyapatite crystals when in contact with human saliva and offers excellent mechanical properties [18]. It was demonstrated that the incorporation of nanofibers doped by Nb_2O_5 in one self-adhesive resin cement promotes significant improvements in the mechanical properties of the material [21]. In addition, Nb_2O_5 has optical properties similar to tooth structure, being interesting for dental materials applications [22].

Therefore, modifying a fluoride varnish with bioactive glass particles could enhance the deposition of calcium and phosphate ions, thus improving its cariostatic effect. Additionally, the

incorporation of Nb_2O_5 particles could increase the varnish's resistance to cariogenic challenges and prolong its effectiveness, reducing the need for frequent reapplications. The objective of this study was to evaluate the remineralizing potential of a varnish modified with bioactive nanomaterials (Nb_2O_5 and SiO_2) through surface hardness and energy-dispersive X-ray spectroscopy (EDX) analyses. The null hypothesis tested was that the experimental materials would not possess remineralizing potential and bioactivity.

MATERIAL AND METHODS

Sample preparation

Sixty-five extracted non-carious bovine mandibular incisors were obtained under a protocol registered and approved by the Animal Use Ethics Committee (#004/2020). The teeth were selected using 10 × magnifying glass support, excluding those with cracks, caries and/or fractures. The roots of each tooth were sectioned 1 mm below the enamel-cementum junction using an IsoMet low-speed saw (Buehler; Lake Bluff, IL, USA) equipped with a diamond disc (Extec; Enfield, CT, USA). Subsequently, enamel slabs were prepared ($6\times4\times2mm^3$) at a speed of 300 rpm under continuous water irrigation. The specimens were then polished flat using abrasive papers (#600-grit to #1200-grit) followed by a felt disk with a diamond suspension (Buehler; Lake Bluff, IL, USA), in a polishing machine (Politriz APL-4 AROTEC, Cotia, SP, Brazil).

The baseline SH of the dental slabs was determined by three indentations (n = 10) in specimens, using a Knoop diamond indenter with intervals of 100 μm from each other. Assessments were made with a 50-g load for 10 s (MicroMet 6040; South Bay Technology, Lake Bluff, IL, USA). Before submitting the specimens to any further process for the production of a simulated caries-like lesion, blocks were divided into three areas of $2\times4\times2mm^3$ (control, only demineralized and treated) by acid-resistant varnish. The demineralizing solution was developed with the composition: 1.3 mM $Ca(NO_3)2.4H_20$; 0.78 mM $Na_2HPO_4.2H_20$; 0.05 M glacial acetic acid; 0.0315 ppm F; pH 5.0 at 37°C [23]. The selected specimens remained for 16 hours in this solution (30 ml per specimen). The SH of the dental slabs was again determined after caries-like induction by three indentations in specimens and submitted to Transverse microradiography.

Transverse microradiography (TMR)

The specimens were longitudinally sectioned at the center of the carious surface using an IsoMet low-speed saw (Buehler; Lake Bluff, IL, USA) and a double-sided diamond disc – XL 12205, cooled with purified water. This step enabled the acquisition of a fragment with a thickness of approximately 500 μm to prevent dentin fracture. To achieve a thickness suitable for analysis (120–130 μm), the fragments were manually polished using abrasive papers #600-grit to #1200-grit moistened with purified water. The final thickness was confirmed using a digital micrometer (Mitutoyo, Tokyo, Japan).

After cleaning, enamel specimens (n=3) of each group were submitted to microradiograph exposure (20 kV and 20 mA, Softex, Tokyo, Japan). The developed plates were analyzed using a transmitted light microscope fitted with a 20 × objective. Two images per sample were obtained using data acquisition (version 2012) and interpreted using calculation (version 2006) software (Inspektor Research System; Amsterdam, Netherlands). The mineral content was calculated assuming 87 vol% of mineral content for sound enamel and that the lesion depth ends when enamel contains around 82.5% of mineral volume [24,25].

Treatment of bovine enamel blocks with fluoride varnish doped with nanoparticles

The fluoride varnish used in this study was produced by FGM Produtos Odontológicos LTDA (Joinville, Santa Catarina, Brazil), composed of artificial resin as a base and ethanol as a solvent (6% NaF + 6% CaF_2). All specimens were cleaned first with Robinson brush/pumice stone and treated with a thin layer of material by a micro brush, following the three groups: experimental varnish (VZ); experimental varnish + 1 wt% silica gelatin (VZ-SiO_2) and experimental varnish + 1 wt% niobium nanoparticles (VZ-Nb_2O_5) (Table I).

After treatment, specimens were kept for 24 hours in relative humidity and then, varnishes were removed with a scalpel blade, being cleaned with an acetone-water (1:1) solution in a microbrush [24].

Table I - Chemical composition of fluoride varnishes

Group/ Manufacturer	Composition	Filler
VZ-control/ FGM	Artificial resin, ethanol, 6% NaF and 6% CaF_2	-
VZ-SiO_2/ FGM	Artificial resin, ethanol, 6% NaF and 6% CaF_2	1 wt.% SiO_2
VZ-Nb_2O_5/ FGM	Artificial resin, ethanol, 6% NaF and 6% CaF_2	1 wt.% Nb_2O_5

pH-cycling regimen

After varnish removal, the blocks were subjected to a pH-cycling model. For 8 days, the blocks were kept for 22 hours in a remineralizing solution and 2 hours in a demineralizing solution at 37°C. The proportions of the de-remineralizing solutions used were 6.25 ml/mm² and 3.12 ml/mm² of enamel, respectively. The solutions were replaced on the fourth day of cycling with new solutions, and after the end of the period of 8 days of cycling, the surface hardness of the post-treatment enamel was evaluated [23].

Knoop hardness assessment

At the end of each time condition (after treatments and after pH-cycling regimen), SH was also determined (n = 10) in triplicate. Three indentations at a standard distance from the treatment area were made (100 μm) and then, the mean values from the three indentations and the percentage of surface hardness change were calculated after treatments. The percentage of surface hardness loss (%SHL) and recovery (% SHR) was calculated after the pH-cycling regimen, according to the formula:

$$\%SHL = \frac{SH\ baseline - SH\ after\ pH\ cycling}{SH\ baseline} X\ 100 \quad (1)$$

$$\%SHR = \frac{SH\ after\ pH\ cycling - SH\ after\ artificial\ caries\ lesion}{SH\ baseline - SH\ after\ artificial\ caries\ lesion} X\ 100 \quad (2)$$

EDX analysis

For the EDX analysis, a total of 6 groups were evaluated in different conditions: G1 (control area of VZ), G2 (VZ after pH-cycling), G3 (control area of VZ-SiO_2), G4 (VZ-SiO_2 after pH-cycling), G5 (control area of VZ-Nb_2O_5) and G6 (VZ-Nb_2O_5 after pH-cycling). Before the EDX analysis, the specimens were kept in artificial saliva for 7 days to obtain ion shift between the saliva and enamel specimens. The composition of the artificial saliva was: 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2 \cdot 2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4 and traces of ascorbic acid (pH 6.8) (all chemicals were purchased from Merck; Darmstadt, Germany) [26]. The specimens were sputter-coated with a thin layer of gold and examined using scanning electron microscopy (SEM) (Aspex Express; Fei Europe, Eindhoven, Netherlands) at an accelerating voltage of 15–20 kV in relative vacuum. Elemental analysis by EDX, which was fully integrated into the Aspex Express SEM, was conducted over the entire area to determine the relative amounts of Ca and P by atomic percentage, carried out in standardless mode. Ca/P ratio was calculated for all groups.

Statistical analysis

The data were statistically analyzed with the Statistica software, version 10.0. Normal distribution and equality of variances were checked for all the variables using the Shapiro-Wilk test. For enamel specimens, Two-way ANOVA was performed to evaluate initial SH, SH after the demineralization process and after pH-cycling regimen, %SHL, %SHR and Ca/P ratio, followed by a multiple comparison test performed with the Tukey HSD test ($p < 0.05$).

RESULTS

Microhardness of the fluoride varnishes

Initial surface hardness was not significantly different between groups ($p = 0.179$). Changes in surface hardness values after each step are shown in Table II.

The two-way ANOVA showed that there were statistical differences between the varnishes, and the moments ($p < 0.05$), revealing a significant interaction between the two study factors (varnishes x moment). The incorporation of niobium and silica nanoparticles into the fluoride varnish resulted in significant differences in surface hardness. Surface hardness after treatment was higher for both experimental groups (VZ-SiO_2 and VZ-Nb_2O_5), showing significant differences when compared to the control group (VZ). The group with the incorporation of Nb_2O_5 presented the highest hardness values, differing from the others

(p=0.001), consequently recovering the hardness in values (%) very close to the initial hardness with sound enamel as shown in Table II.

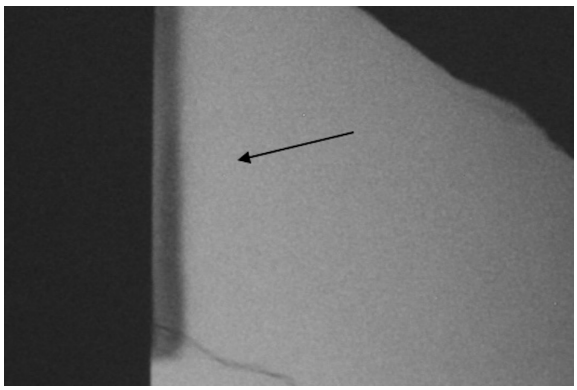

Figure 1 - TMR image evidencing an artificial caries-like subsurface lesion. Black arrow shows the subsurface lesion.

Caries-like lesion evidence and EDX evaluation of the bovine enamel blocks

Figure 1 shows a transversal microradiography image (TMR), evidencing the production of artificial caries-like subsurface lesions without surface erosion in the enamel blocks after being submitted to the demineralizing solution.

For EDX, the areas of the sound enamel blocks are represented in Figures 2a, b and c. Figures 2d, e and f represent the demineralized area of the enamel blocks. After treatment with varnish and pH-cycling (Figures 2g, h and i), the blocks treated with VZ-SiO_2 and VZ-Nb_2O_5, showed increased phosphate (P) and calcium (Ca) ion deposition, being superior in the presence of Nb_2O_5.

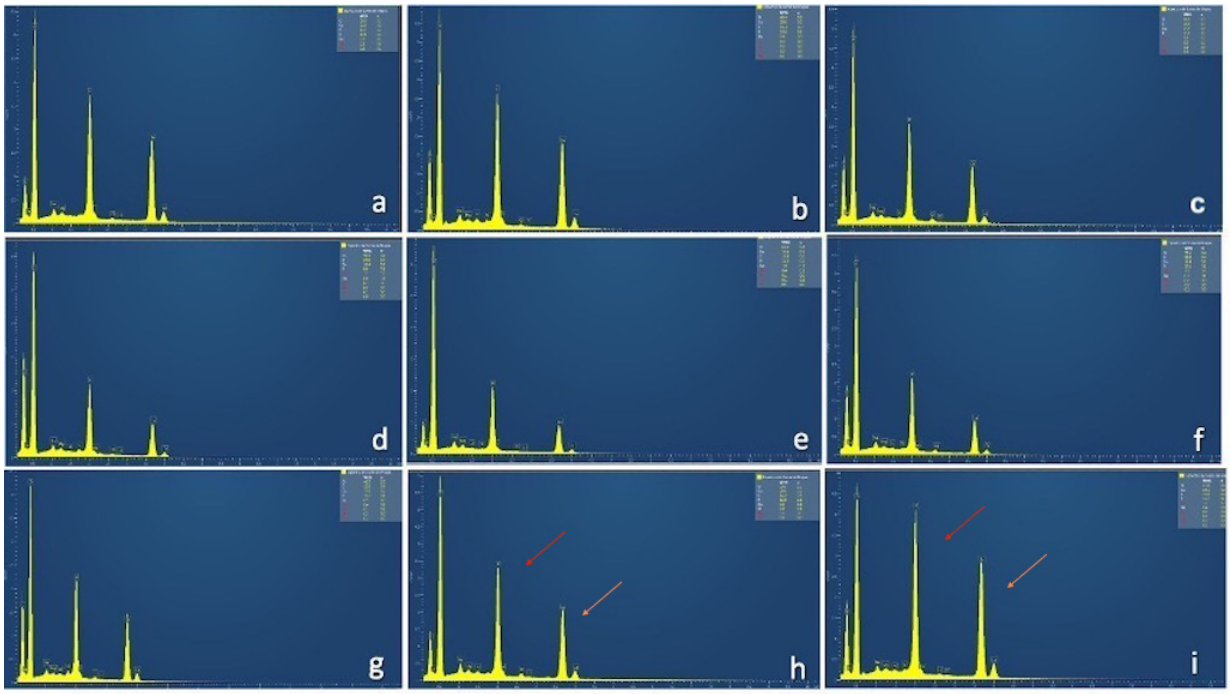

Figure 2 - EDX analysis of the different groups: Sound blocks (a-VZ; b-VZ-SiO_2; c-VZ-Nb_2O_5); Demineralized blocks (d-VZ; e-VZ-SiO_2; f-VZ-Nb_2O_5) and blocks treated with fluoride varnish and after pH-cycling (g-VZ; h-VZ-SiO_2; i-VZ-Nb_2O_5). Red arrows indicate phosphate (P) ion deposition, and orange arrows indicate calcium (Ca) ion deposition.

Table II - Mean ± SD of SH after pH-cycling regimen, %SH loss and %SH recovery after pH-cycling regimen

Groups	Initial SH (Kg/mm^2)	SH after demineralization process (Kg/mm^2)	SH after pH-cycling regimen (Kg/mm^2)	Percentage of SH loss (%SH loss)-after pH-cycling regimen	Percentage of SH recovery (%SH recovery)-after pH-cycling regimen
VZ	342.4±16.3^a	204.2±3.7^a	230.1±5.8^a	40.2±2.8^a	19.9±6.0^a
VZ-SiO_2	333.7±11.1^a	203.8±1.9^a	303.0±7.1^b	38.8±2.3^a	77.6±9.5^b
VZ-Nb_2O_5	331.8±11.7^a	204.5±0.9^a	325.2±9.7^c	38.1±1.9^a	95.0±5.3^c

Values in the same column with different superscript lower – case letters significantly differ from each other ($p < 0.05$).

DISCUSSION

Several remineralizing strategies have made significant progress in recent years, and conventional methods for caries prevention, such as topical fluoride application, have been shown to decrease demineralization by depositing fluoride in the enamel crystal lattice, reducing its solubility [27]. This understanding of the biomineralization process of dental hard tissue has led to the development of biomimetic remineralization strategies that mimic the crystallization pathway using amorphous precursors of hydroxyapatite (HAP) [28].

Fluoride treatment remains the standard therapy for remineralization of white spot lesions and has the highest level of scientific evidence [29,30]. NaF varnishes are also valid approaches for remineralization [9] and even in enamel samples submitted to erosion and abrasion protocols [31]. However, since fluoride does not penetrate deeper than the subsurface demineralized zone [32], many researchers have started developing new therapies, especially for individuals at high risk for caries lesions who require professional application of products with a higher concentration of fluoride. Thus, most therapies aim to enhance the effects of existing fluoride therapies rather than replacing them [33,34]. The present study aimed to evaluate the percentage composition of enamel components after demineralization and application of fluoride varnish modified by bioactive nanoparticles. The bioactivity of Nb_2O_5 allowed greater interaction between the enamel and the material. Therefore, the null hypothesis of this study was rejected.

One concern with bioactive materials is their low mechanical strength [17]. Nanotechnology is an emerging trend in dentistry, and new bioactive materials with good mechanical properties, such as Nb_2O_5 and SiO_2, have been investigated and used in the biomedical and dental fields [14,21]. Literature suggests that a minimal fraction of bioactive fillers should be used in composites to promote remineralization through ion release, and they should be combined with reinforcing fillers, such as whiskers [35,36]. In this study, a concentration of only 1 wt% of nanoparticles was chosen for better particle distribution within the material. Surface microhardness and EDX analysis were performed to assess the effects of chemical/physical agents on hard tissues of teeth [37] and to determine the Ca/P ratio, analyzing the bioactivity potential of the experimental varnishes.

The experimental varnishes (VZ-SiO_2 and VZ-Nb_2O_5) were able to remineralize the enamel surface, inducing a significant increase in hardness recovery. These results are promising and suggest that the incorporation of bioactive nanoparticles into the fluoride varnish adds an anticaries action, allowing ion exchange with the solutions and protecting the surface for a longer period.

In our study and similar studies [14], surface microhardness decreased after demineralization but increased after all fluoride varnish applications. One interesting finding was that the initial surface microhardness was almost reached in the VZ-Nb_2O_5 group, which is important for preventing enamel demineralization.

Most remineralizing strategies aim to prolong the supersaturation periods by creating stable systems that can supply bioavailable calcium, phosphate, and fluoride directly to the lesion or the surrounding biofilm [33]. During remineralization, ion substitution takes place in forming apatite, including the substitution of calcium ions with magnesium and sodium, substitution of hydroxyl sites with fluoride and chloride, and substitution of phosphate and hydroxyl sites with carbonate. Therefore, minerals other than calcium and phosphate can have a significant influence on the remineralization process, and considerable variation in apatite properties can occur. For example, carbonate substitution increases the solubility of apatite, while fluoride substitution decreases its solubility [38]. One significant challenge for remineralizing therapies is to provide the right concentration of minerals at the right time, as elevated mineral concentration at the wrong time could lead to unwanted surface precipitation, limiting the efficacy of the therapy [33]. The VZ-Nb_2O_5 group in our study showed a slow release of nanoparticles, which may favor subsurface mineral gain and longevity of the remineralizing treatment.

One limitation of the present study was that mineral loss was only evaluated on the surface, using surface hardness measurements. Therefore, it would be interesting to assess cross-sectional hardness to observe the progression and determine the depth to which the material is capable of remineralizing.

CONCLUSION

The experimental varnishes modified by nanoparticles were able to significantly increase the remineralizing potential, being more effective in improving surface remineralization, with the one modified by Nb_2O_5 the group that presented promising results.

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Author's Contributions

ATO: Writing – Review & Editing, Writing – Original Draft, Resources, Methodology, Investigation and Conceptualization. MMACV: Writing – Review & Editing, Writing – Original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. TRLN: Writing – Review & Editing, Resources, Methodology, Investigation, Conceptualization. DLE: Writing – Review & Editing, Writing – Original Draft, Methodology, Investigation, Funding Acquisition. LSA: Writing – Review & Editing, Visualization, Supervision. JFSB: Writing – Review & Editing, Writing – Original Draft, Visualization, Validation, Supervision, Software, Resources, Project Administration, Methodology, Investigation, Funding Acquisition, Formal Analysis, Data Curation, Conceptualization.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Regulatory Statement

This study protocol was reviewed and approved by the Animal Use Ethics Committee from Bauru School of Dentistry, University of São Paulo (#004/2020).

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