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ORIGINAL ARTICLE

Enamel remineralization effect of a dentifrice containing calcium sodium phosphosilicate: an optical coherence tomography observation

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ABSTRACT

Objective: The purpose of this study was to examine the effects of a dentifrice containing 5% calcium sodium phosphosilicate (CSP) on the remineralization of the enamel using optical coherence tomography (OCT).

Materials and methods: Bovine incisors were sliced and shaped in a rectangular form. One group of five specimens was treated with undersaturated 0.1 M lactic acid buffer solution (pH 4.75) for 10 min and then placed in artificial saliva (pH 7.0) (De group). Other specimens were stored in solutions of toothpaste containing CSP for 10 min, followed by 10-min immersion in the lactic acid buffer solution twice a day before storage in artificial saliva (CSP group). An additional group was stored in only artificial saliva (control group). OCT imaging on the selected location of the enamel surface was performed. The peak intensity and width at $1/e^2$ were recorded in each of the six areas on the sample and averaged, and the sample size of each group was six. The integrated value in units ($dB \times \mu m$) was calculated in the area of peak intensity. The data for each group was subjected to one-way repeated-measures ANOVA and Tukey HSD tests ($\alpha = 0.05$).

Results: The changes in integrated values of each group were different. A slight but significant increase in the integrated value was observed in the control group, whereas a slight but significant decrease in the value was observed the De group. Integrated values increased in the CSP group.

Conclusions: Remineralization occurred upon immersion in the toothpaste containing CSP.

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Introduction

Continuous demineralization and remineralization of the tooth structure is common in the oral environment. However, when this balance is disrupted, demineralization may progress and lead to changes in the tooth structure [1]. During the initial stages of carious lesions, non-uniform mineral loss and increasing porosity may be observed in the subsurface areas of the enamel. A dentifrice with calcium sodium phosphosilicate (CSP) is one of the oral hygiene products that have the ability to help prevent demineralization [2]. CSP is a bioactive glass that reacts and deposits hydroxycarbonate apatite, a mineral chemically similar to the enamel and dentin, when exposed to body fluids such as saliva [3]. A previous study examined the release of Si from bioactive glass particles and reported that it was absorbed onto the hard tissue, thereby providing sites for heterogeneous CaP nucleation and subsequent creation of an apatite layer [4].

The layer formed by CSP has been demonstrated to exhibit a reduction in permeability when challenged with citric acid [5]. A review paper suggested that the use of a dentifrice containing 5% CSP was effective in reducing hypersensitivity by promoting dentinal tubule occlusion [6]. It has been hypothesized that CSP forms a mechanically strong hydroxyapatite-like layer that can resist degradation from repeated acid challenges on the dentine surface [7]. A previous study reported that the desensitizing dentifrices tested produced a similar rate of erosive dentin wear to the conventional dentifrice; however, only the dentifrice containing CSP was able to promote tubule occlusion [8].

By detection and diagnosis of early stage carious lesions, dentists can prevent progression of the decay and avoid invasive removal of sound tooth structure. However, an accurate diagnosis of the initial stages of carious lesions, including an assessment of the affected depth, can be particularly challenging due to hypermineralization of the outer enamel surface that masks the underlying lesion. In recent years, several diagnostic techniques have been developed to detect the early stages of enamel demineralization [9]. Optical coherence tomography (OCT) has considerable potential as a non-invasive imaging technique for carious lesions as it obviates the need to remove the surface and eliminates radiation exposure [10]. Time domain OCT is a form of OCT that uses low-coherence interferometry to determine the echo time delay and magnitude of back-scattered light reflected from a transparent or semi-transparent structure. This technique combines light from a low-coherence light source (broadband superluminescent diode) with a

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Michelson interferometer to produce cross-sectional images of tissue structures, which are generated through an interaction between a partially coherent beam of optical radiation and the tissue components [11]. This allows it to be more compact and cost effective than spectral domain OCT is [12].

The principal purpose of this study was to demonstrate the efficacy of dentifrices containing CSP in promoting enamel remineralization using OCT. The null hypothesis tested was that there were no differences between the specimens treated with the dentifrice and the other groups.

Materials and methods

A diluted (1:3 dilution) dentifrice suspension was prepared by suspending 12 g of the dentifrice (Sensodyne Repair & Protect; Glaxo Smith Kline, Surrey, UK) in 36-mL deionized water. The suspension was thoroughly stirred with a stirring rod and mechanically agitated using a vortex mixer for 1 min.

Bovine incisors from 2- to 3-year-old cattle were used as substitutes for human teeth. After separating the root with a low-speed diamond saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL), the pulps were removed. Thereafter, the lingual surfaces of the bovine incisors were removed with a diamond saw, and a super-fine diamond point (ISO #021, Shofu Inc., Kyoto, Japan) was used to carefully shape each slab into a rectangular form that was 4 mm ×4 mm ×2 mm in size. Each surface of the specimen was ground using successive grit sizes (range #600 to #2000) of wet silicon carbide paper. The thickness and size of the specimens were measured using a dial gauge micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan). After the preparation, a total of six specimens in each group were treated as follows:

1. DE group: The specimens were treated with undersaturated 0.1 M lactic acid buffer solution (pH 4.75, 0.75 mM $CaCl_2{\cdot}2H_2O$; 0.45 mM KH_2PO_4) for 10 min and then placed in artificial saliva (pH 7.0, 14.4 mM NaCl; 16.1 mM KCl; 0.3 mM $MgCl_2{\cdot}6H_2O$; 2.0 mM KH_2PO_4; 1.0 mM $CaCl_2{\cdot}2H_2O$; 0.10 g/100 mL sodium carboxymethyl cellulose). These procedures were performed twice a day throughout the 4-week test period. All specimens were stored in artificial saliva at 37 °C between treatments.
2. CSP group: The specimens were first placed in a demineralizing undersaturated 0.1 M lactic acid buffer solution for 10 min, followed by a suspension of dentifrice containing CSP for 3 min, and then artificial saliva. These procedures were performed twice a day throughout the 4-week test period. All specimens were stored in artificial saliva at 37 °C between treatments.
3. Control group: All control specimens were stored in only artificial saliva for the same period of time.

Figure 1 shows a schematic representation of the OCT system used in the current study. The focused light beam was projected onto the selected locations and scanned across the area of interest in two dimensions using a probe attached to a mounting device. Superluminescent diodes with a central wavelength of 1310 nm, a spectral bandwidth of 40 nm, and an optical output power of 7.5 mW (DL-CS3184B, DensLight Semiconductors, Singapore, Singapore) were used as the light source. The emission light was coupled to a single-mode fibre-optic Michelson interferometer and delivered to

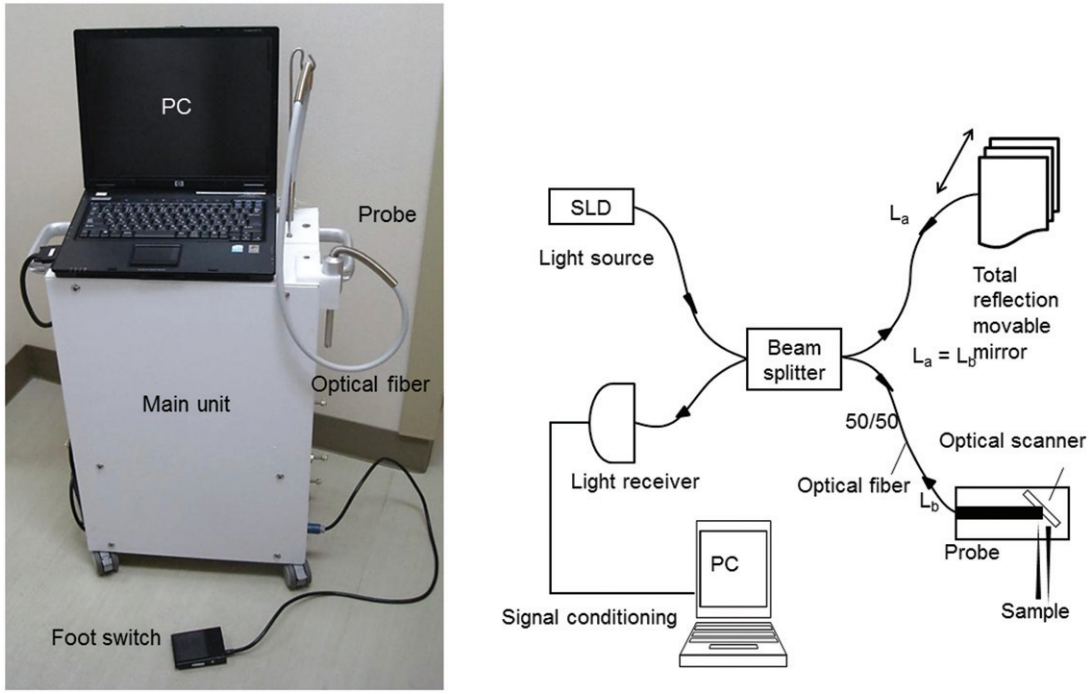

Figure 1. Three dimensional OCT system used in this study. The emission light was coupled to a single-mode fibre-optic Michelson interferometer and delivered to both the reference mirror and the sample. The reference mirror was mounted on a linearly translating galvanometer that was driven by a triangular voltage waveform with a fringe modulation frequency of 1 kHz. The light was reflected off the mirror and back onto the retroreflector, and then re-imaged on the reference arm fibre.

both the reference mirror and the sample. The reference mirror was mounted on a linearly translating galvanometer driven by a triangular voltage waveform with a fringe modulation frequency of 1 kHz. The light was reflected off the mirror and back onto the retroreflector, and then re-imaged on the reference arm fibre. These signals were amplified and demodulated by an amplifier. Subsequently, the voltage from the lock-in amplifier was converted to a digital signal using a data acquisition board, and then processed on a personal computer using analysis software (Origin 9, OriginLab Corp., Northampton, MA).

The scanning probe connected to the OCT was set at a fixed distance (2.0 mm) from the enamel surface, and the scanning beam was set at a right angle to the surface of the tooth. Enamel surface changes were analyzed based on the peak intensity values of the OCT images. Our analyses also calculated the width between the points where the intensity decreased to a value of $(1/e^2)$ and corresponded to the peak intensity of the line profile. The $(1/e^2)$ width is equal to the distance between the two points where the intensity falls to $(1/e^2) = 0.135$ times the maximum value. The peak intensity and width at $(1/e^2)$ were recorded at six areas in every sample and then averaged. A total of six samples were examined for each group. Calculations of the integrated value in units (dB × μm) were based on the area of the peak intensity determined by the OCT.

Specimens from each group were treated using the same methods and observed using three-dimensional laser-scanning microscopy (LSM; VK-8700; Keyence Corp., Osaka, Japan). The excitation light had a maximum wavelength of 658 nm. Both the intensity of the excitation light and amplification of the photomultiplier remained constant during the investigation. The image size recorded was $81.5 \times 71.5\ \mu m^2$, and the resolution was 1024×768 pixels. Images were obtained for four different sites on each specimen.

The data were analyzed using a repeated measures analysis of variance (ANOVA), followed by a Tukey–Kramer post-hoc multiple comparison ($\alpha = 0.05$). All statistical analyses were carried out using a software system (SigmaStat 4.0, Systat Software Inc., San Jose, CA).

Results

The OCT images (B-scans) of the specimens are shown in Figure 2. The abscissa of the tomograms corresponds to the scan depth, while the ordinate corresponds to the vertical measurement position on the enamel surface. A weak and narrow signal was visible and the back-scattered light was well above the noise level in the DE group on day 1, resulting in a grainy appearance of the OCT images. After 28 days, although an area of strong scattering on the enamel surface was visible, the back-scattered grainy appearance was very weak. For the CSP group, the signal from the enamel surface was visible and became broader after 28 days of treatment, with a slight back-scattered intensity observed.

Changes in signal intensities (dB), widths at the $(1/e^2)$ (μm), and integrated values are shown in Tables 1, 2, and Figure 3, respectively. There were no significant changes in the signal intensities for the control (−42.6 to −43.5 dB), De (−33.8 to −43.4 dB) and CSP (−42.5 to −57.2 dB) groups during the test period. Significant changes in widths at the $(1/e^2)$ were observed for the De group (82.2–100.6 μm) and CSP group (82.5–138.4 μm), which occurred during days 0–7. Different changes in the integrated values were observed in each of the groups. The control group exhibited a slight but significant increase in the integrated value (from 3565 to 3973), while no significant changes were observed in the De group (from 3400 to 3567). The integrated values were doubled in the CSP group 7 days after the start of the experiment, after which there was a slight increase in the value (from 3506 to 7805).

Representative LSM images of the enamel specimens (Figure 4) illustrate the morphologic changes caused by the different treatments. Although evitable differences were not found in the control group, pronounced morphologic changes including build-up of a reaction layer on the enamel surface occurred in the CSP group for 28-day specimens. In contrast, specimens in the De group showed morphologic changes such as roughening of the enamel surface and appearance of an enamel etching pattern for 28-day specimens.

Discussion

Comparative data on the properties of human and bovine hard dental tissue are scarce; however, bovine enamel is widely used as a substitute for human enamel [13]. Human teeth are thought to be most relevant for conducting *in vitro* studies [14]. However, bovine teeth were employed in the present study, as they are easy to obtain in large quantities and in good condition, and have fewer composition variables. Bovine teeth have large flat surfaces, and have not undergone prior caries challenges that might affect test results [15]. Moreover, structural changes and the mineral distribution of carious lesions are reported to be similar in human and bovine teeth [16].

Permanent enamel contains about 2 wt% (around 6 vol%) water in both free and bound forms [17,18], has a fine porous structure, and inter-rod and intra-rod spacing. Because of its small molecular size and its compatibility with the mineral and protein components, water can penetrate even the smallest pores in the enamel [19]. This pore structure affects the optical properties of enamel. A decrease in the translucency of the enamel is associated with the replacement of water around the enamel prisms by air blowing [20]. Such changes in the optical properties of enamel may have affected the OCT images obtained [21].

A previous study reported finding four phenomena that occurred during interactions between a tooth and light flux. These include specular transmission of the light flux through the tooth, specular reflection at the surface, diffuse light reflection at the surface and absorption and scattering of the flux within the tooth [22]. Thus, accurate determination of optical constants from OCT image data is of principal importance in dental applications as these constants describe the interaction of the optical field with the sample and its

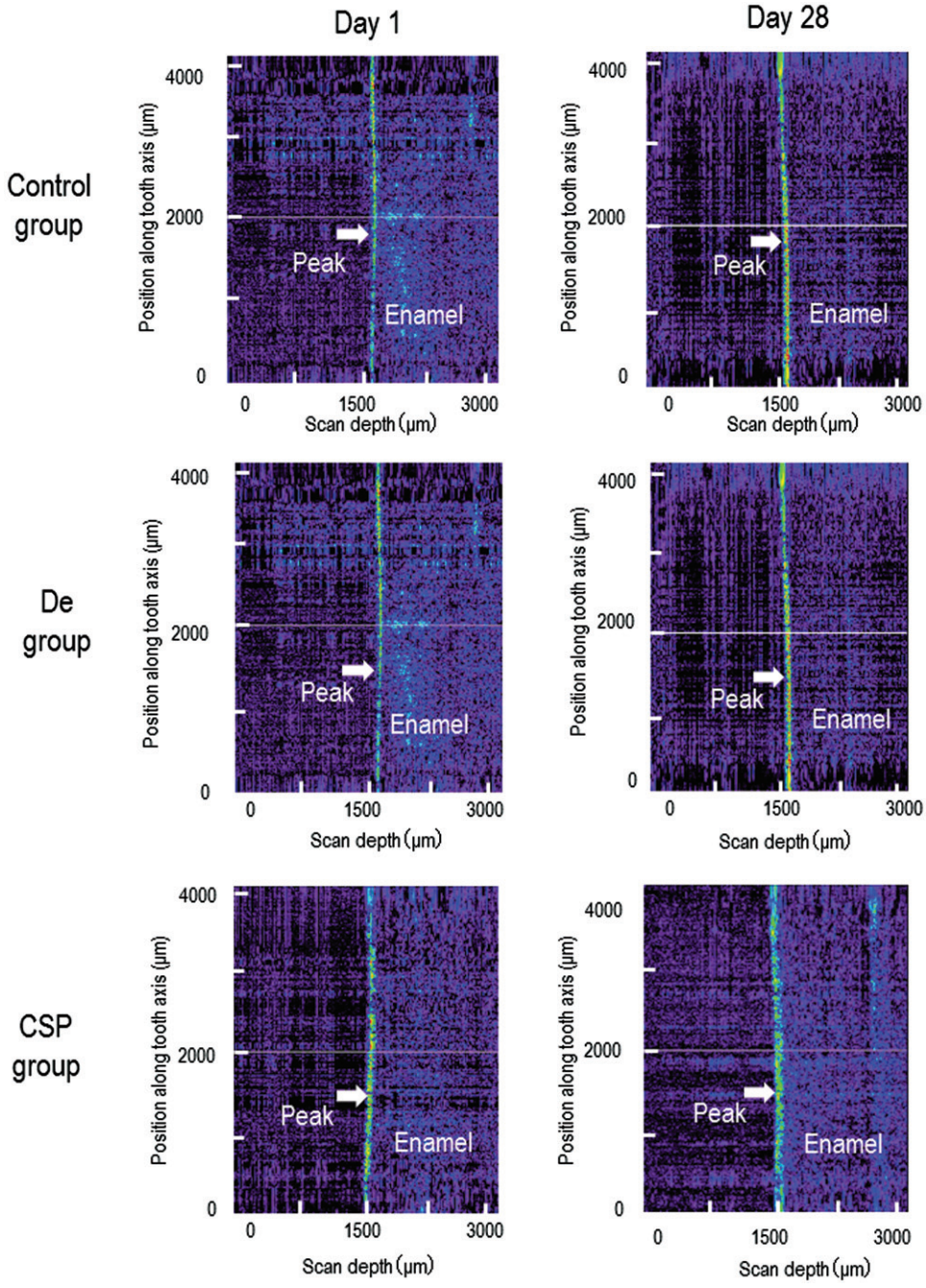

Figure 2. Representative B-scan images of enamel surfaces. The abscissa of the tomograms corresponds to the scan depth, while the ordinate corresponds to the vertical measurement position on the tooth surface. A weak and narrow signal without any back-scattered intensity was observed in the control group, with no other changes in the OCT images observed during the actual test period.

fundamental constitution [23]. It has been suggested that the reflectivity of the lesion area can be directly determined and used as a measure of the lesion severity [24]. It has also been shown that shallow lesions exhibit a loss of penetration depth in OCT images, and this is correlated with the mineral loss that can be detected with microradiography [25].

An intense reflection at the enamel surface was observed in the control group, and the rest of the signal that propagated into the tooth substrate decayed in conjunction with the scan depth. These tendencies remained unchanged throughout the test period. A relatively similar increase in intense reflection at the enamel surface was observed in the De group on day 1, and the signal exhibited back scattering in line with the depth beyond the enamel surface. On day 28, the De group did not exhibit any significant differences in the signal due to the intense depolarization caused by the light scattering on the acid-attacked, roughened enamel surface. This is thought to be responsible for masking the subsurface scattering and creating speckles in the image. Because incident light was strongly scattered by the sound and demineralized enamel surfaces, light propagation may have been affected by convoluted structures on the enamel surface [26]. Therefore, definition of the light penetration depth can be difficult, especially on complicated sound and demineralized enamel surfaces.

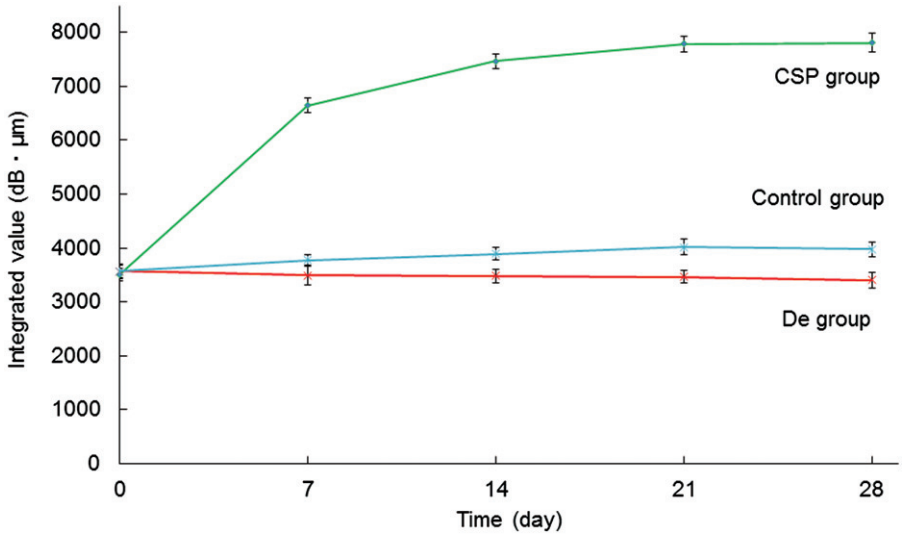

Figure 3. Integrated value of bovine enamel under different treatment conditions with different storage periods. Though there were no significant changes in the integrated values for the control and the De groups during the test period, significant increases in the values were observed for the CSP group during the first test period, and this occurred during days 0–7.

Table 1. Influence of different treatment procedures on signal intensity.

	Treatment time (days)				
Group	0	7	14	21	28
Control	−42.6 (11.4)^{aA}	−42.7 (10.6)^{aA}	−43.1 (10.1)^{aA}	−43.5 (10.9)^{aA}	−43.1 (10.2)^{aA}
De	−43.4 (11.8)^{aA}	−36.6 (13.6)^{aA}	−34.7 (11.8)^{aA}	−34.5 (10.9)^{aA}	−33.8 (10.6)^{aA}
CSP	−42.5 (10.2)^{aA}	−54.8 (11.9)^{aA}	−57.2 (9.9)^{aA}	−56.7 (10.6)^{aA}	−56.4 (11.5)^{aA}

Unit: dB; (): SD; $n = 6$. Within groups: means sharing the same lower-case letter are not significantly different ($p > 0.05$). Between groups at the same treatment times: means sharing the same upper-case letter are not significantly different ($p > 0.05$).

Table 2. Influence of different treatment procedures on width at $1/e^2$.

	Treatment time (days)				
Group	0	7	14	21	28
Control	83.7 (10.3)^{aA}	88.3 (10.4)^{aA}	90.3 (10.8)^{aA}	92.4 (13.1)^{aA}	92.2 (13.2)^{aA}
De	82.2 (11.3)^{aA}	95.5 (13.2)^{bA}	100.3 (10.4)^{bA}	100.5(10.7)^{bA}	100.6 (13.4)^{bA}
CSP	82.5 (11.1)^{aA}	121.2 (11.3)^{bB}	130.6 (13.4)^{cB}	137.4 (13.7)^{cB}	138.4 (15.0)^{cB}

Unit: μm; (): SD; $n = 6$. Within groups: means sharing the same lower-case letter are not significantly different ($p > 0.05$). Between groups at the same treatment times: means sharing the same upper-case letter are not significantly different ($p > 0.05$).

When attempting to measure lesion depth and severity, definition of the cut-off point is important because of the high dynamic range of reflectivity. Moreover, determination of the signal fall-off is very complicated because the light is exponentially attenuated during its propagation into the tooth substrate. Because it was previously proposed that the depth at which there is a ($1/e^2$) decrease in intensity can be used to determine the cut-off intensity values [27], we used this method in the current study, as reported previously [28]. Additionally, in order to define the changes in quantity of the tooth substrate, we used the area of peak intensity to calculate the integrated value, signal intensity, and band width at the ($1/e^2$). This resulted in lower signal intensity with wider ($1/e^2$) width seen in the CSP group on day 28. These results indicate that the OCT signals generated by light travelled a longer pathway than the light in the samples of the De group. Due to the presence of ions from the dentifrice containing CSP particles, the porosities of the enamel surfaces may accumulate minerals that can ultimately lead to changes in the optical properties [29].

Drastic changes in integrated values after 28 days of storage were observed in the CSP group (Figure 3). The LSM observation revealed an existence of precipitation material on the enamel surface, and these results were in agreement with previous studies reporting scanning microscopic observations [30] that demonstrated the existence of an ion-enriched layer firmly attached to the enamel surface. When bioactive glass is incorporated into toothpaste formulations, the ions released from the amorphous calcium phosphate layer are believed to contribute to the remineralization process of the tooth surface [31]. Among the ions released from the CSP particles, Si was thought to play an important role in the mineralization of the tooth substrate through the promotion of hydroxyapatite formation by triggering hydroxyapatite nucleation [32]. In aqueous environments, a rapid exchange of sodium ions (Na^+) with hydrogen cations (H^+ or H_3O^+)

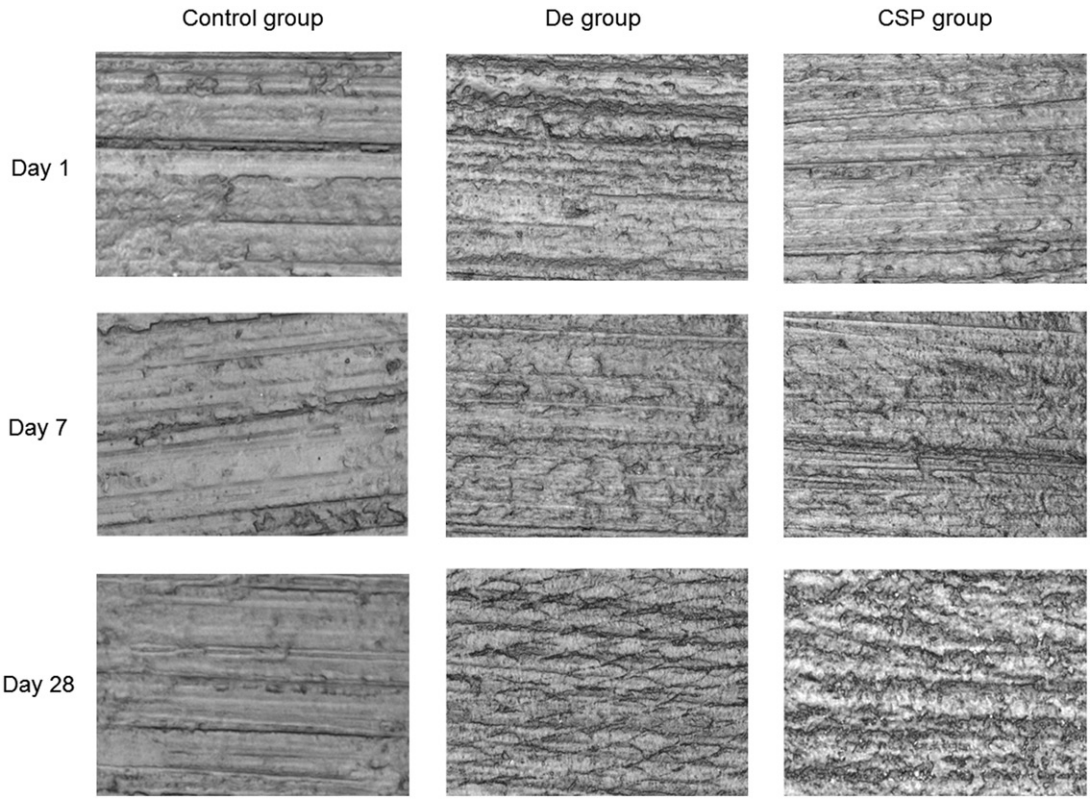

Figure 4. Representative laser scanning microscopy images of the enamel surfaces. Pronounced morphologic changes in the tooth surfaces occurred in the De group where the enamel surface was roughened by acid attack. In contrast, specimens in the CSP showed morphologic changes after day 28.

from the solution occurs. There is a loss of soluble silica in the form of $Si(OH_4)$ into the solution, resulting from a breakage of Si–O–Si bonds and formation of Si–OH (silanols) at the glass–solution interface. Condensation and repolymerization of a SiO_2^--rich surface is depleted in alkalis and alkaline-earth cations. Migration of Ca^{2+} and PO_3^{4-} groups to the CSPS particles through the SiO_2^--rich surface results in the formation of a $CaO–P_2O_5$-rich film on the particle surface, which then crystallizes into hydroxycarbonate apatite [33]. It has been proposed that the chemical reactions that promote apatite formation may also be useful in the enhancement of remineralization and/or prevention of demineralization of early carious lesions [34].

One limitation of the current study would be the manner in which the slurries were prepared. This study used distilled water instead of artificial saliva, and because artificial saliva contains Ca^{2+} in its composition, additional sources of this ion would be beneficial for better action of the desensitizing agents in clinical conditions. However, similar to previous investigations [35], this method was used to prevent the agents from reacting during mixing and before reaching the dental hard tissue. In this sense, care should be taken when considering the results of this study in relation to the clinical scenario.

Based on the present results and under the current experimental condition, it was concluded that dentifrices containing CSP appear to promote remineralization and inhibit demineralization of the enamel. Because the remineralization of enamel structure in oral environment may be quite different compared to the condition of this study, further researches are needed to prove clinical effectiveness of the dentifrice containing CSP under *in vivo* conditions.

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Disclosure statement

The authors certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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