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Effect of Calcium-phosphate Desensitizers on Staining Susceptibility of Acid-eroded Enamel

KY Kyaw • M Otsuki • MS Segarra • N Hiraishi • J Tagami

Clinical Relevance

Calcium-phosphate desensitizers have a remineralization effect on acid-eroded enamel that shows resistance to extrinsic staining.

SUMMARY

Objective: To investigate the effect of calcium-phosphate–based desensitizers, Teethmate AP paste (TMAP) and Teethmate Desensitizer (TMD) (Kuraray Noritake Dental, Tokyo, Japan), on the prevention of staining on acid-eroded enamel.

Methods and Materials: Forty polished enamel samples (4×4×1 mm) from bovine incisors were randomly divided into five groups (n=8). After immersion in 50 mL of 0.5% citric acid (pH 2.5) for 15 minutes to form acid-eroded surfaces, the surfaces were subjected to different treatments with TMAP, TMD, and NaF (0.21% means 950 ppm) for five minutes. Another eroded group was not treated with desensitizer. For the control group, the samples were not eroded or treated. All the samples were stored in artificial saliva (AS) at pH 7.2 for 24 hours at 37°C. The TMAP, TMD, or NaF was reapplied at eight and 16 hours during the 24 hours of storage time. The surface roughness (Sa) was evaluated following ISO 25178 for surface texture using confocal laser scanning microscopy (VK-X 150 series, Keyence, Osaka, Japan) before acid erosion, after acid erosion, and after 24 hours of incubation in AS. Afterward, the color difference was measured with a dental colorimeter (Shade Eye NCC, Shofu, Kyoto,

Khin Yupar Kyaw, BDS, Department of Cariology and Operative Dentistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Masayuki Otsuki, DDS, PhD, Department of Cariology and Operative Dentistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Michelle Sunico Segarra, DMD, Section of Operative Dentistry, College of Dentistry, University of the Philippines Manila, Manila, Philippines

*Noriko Hiraishi, DDS, PhD, Department of Cariology and Operative Dentistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Junji Tagami, DDS, PhD, Department of Cariology and Operative Dentistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

*Corresponding author: 1-5-45 Yushima, Bunkyo-ku, Tokyo 1138549, Japan; e-mail: hiraope@tmd.ac.jp

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Japan) before and after staining with tea solution.

Results: One-way repeated measures analysis of variance showed that acid erosion significantly increased Sa ($p<0.001$). TMAP- and TMD-treated groups exhibited lower Sa values than the NaF group and the no-desensitizer treatment group. The greatest staining was observed in the NaF group and the no-desensitizer group, while the TMAP and TMD groups significantly decreased the formation of stains.

Conclusions: Acid-eroded enamel increased surface roughness and tended to absorb more stains. However, the application of TMAP and TMD moderated the roughness and thus prevented the formation of extrinsic stains.

INTRODUCTION

Dental erosion is a chronic loss of dental hard tissue due to chemical removal of minerals by acid dissolution. Erosion is fundamentally different from caries because the erosive process does not involve acid of bacterial origin.[1] Risk factor assessment for dental erosion includes frequent use of acidic dietary products, gastroesophageal reflux disease, prolonged use of chewable acidic medications (especially vitamin C and aspirin), low saliva flow rate, and inadequate saliva buffering capacity.[2,3] In recent years, the changing lifestyle, easy availability, and consumption of acidic drinks have become the main etiological factor in erosion, making this condition a major problem in oral health.[4] The erosive potential of drinks is influenced by numerous factors, such as the pH, calcium and phosphate contents, and exposure time. The low pH of beverages is one of the most important factors in the erosion of enamel.[5] Erosion starts with enamel surface softening in the early stage, and enamel tissue loss develops progressively with continued erosive challenges.[1] If the acid challenge persists, the prism cores and interprismatic areas are further dissolved, resulting in a loss of hydroxyapatite crystals, calcium, and phosphate ions.[6] Softened enamel tissue is susceptible to abrasive wear. Brushing after erosive challenges accelerates enamel tissue loss.[7]

Three-dimensional areal measurements have been reported to quantify the microstructural surface of enamel and changes that occur during erosion *in vitro*.[8-11] Previous studies reported that polished eroded enamel samples exhibited an increase in diffuse reflection and surface roughness, showing a strong relationship between erosion and surface roughness.[12-14] A significant correlation was observed between pH changes and surface roughness on the enamel surface after exposure to soft drinks and orange juice in a previous study by Fujii and others.[15] Acid-eroded enamel could be more susceptible to stain absorption, leading to surface staining and discoloration. which is a major esthetic problem.[16-18] Increased roughness on enamel surface is related to differences in color stability of the surface.[17,18]

Fluoride has been shown to provide protection of dental erosion and reduce its progression as well as reduce and prevent hypersensitivity.[19-21] A study by Fowler and others showed that dentifrices containing at least 1400-ppm fluoride can remineralize enamel eroded by citric acid.[20] A more recent study by Junko and others showed that enamel remineralization with fluoride increases surface hardness and decreases surface roughness, which can in turn prevent stain absorption.[22]

Calcium-phosphate–containing products are also considered a treatment for dental erosion and for the prevention of hypersensitivity.[23-25] Kashkosh and others identified that the surfaces became significantly rough after erosion and then became significantly smoother after application of calcium-phosphate–containing products.[26] The calcium and phosphate ions leach out from these calcium-phosphate–containing materials. The mineral phase on the enamel/dentin surface becomes "supersaturated" with calcium and phosphate for remineralization.[23-26] As a result, the calcium phosphate minerals fill the voids in the eroded enamel surface and increase the surface hardness and decrease the surface roughness of the eroded enamel.[27]

More recently developed calcium-phosphate desensitizers are based on tetracalcium phosphates (TTCP) and dicalcium phosphate anhydrous (DCPA).[28-30] Dissolution of TTCP and DCPA can lead to supersaturation of calcium and phosphate, spontaneously forming hydroxyapatite (HA).[28-30] Although there have been studies on the effect of calcium-phosphate–containing desensitizers on remineralization and enamel surface roughness, their effectiveness in preventing or decreasing stain formation on eroded enamel surfaces is still unknown. To date, there has been no study on TTCP- and DCPA-based desensitizers regarding their effect in preventing staining on eroded enamel surfaces.

Therefore, the aim of this study was to investigate the effects of TTCP- and DCPA-based desensitizers on the reduction of staining on acid-eroded enamel.

Table 1: *Components and Their Ingredients*

Products and Manufacturers	Composition
Teethmate AP (TMAP) (Kuraray Noritake Dental, Tokyo, Japan)	Dicalcium phosphate anhydrate (DCPA) Tetracalcium phosphate (TTCP) 950-ppm sodium fluoride (NaF) Glycerin Polyethylene glycol
Teethmate Desensitizer (TMD) (Kuraray Noritake Dental, Tokyo, Japan)	Powder: DCPA TTCP Liquid: water, preservative
0.21% sodium fluoride (NaF) solution (Wako Pure Chemical, Osaka, Japan)	950-ppm NaF

The null hypothesis was that there is no difference in stain resistance on acid-eroded enamel after treatment with TTCP- and DCPA-based desensitizers vs 950-ppm fluoride solution.

METHODS AND MATERIALS

Materials and Specimen Preparation

The products, manufacturers, and components of materials used in this study are listed in Table 1. The labial surfaces of bovine incisors were ground to leave flat enamel surfaces of approximately 1 mm in thickness and were polished using silicon carbide papers from 600 to 2000 grit and 3-μm diamond pastes under running water. Two specimens (4×4×1 mm) were obtained from each flattened surface. The polished enamel surfaces were cleaned ultrasonically in deionized water for five minutes to remove any trace of the polishing materials. The specimens were distributed into five groups (n=8) as shown in Table 2. For the acid erosion challenge, the specimens were immersed in 50 mL of 0.5% citric acid solution at pH 2.5 for 15 minutes.

Surface Roughness Assessment and Morphological Changes

All the specimens were subjected to surface roughness (Sa) analysis using a confocal laser scanning microscope (CLSM) (VK-X 150 series, Keyence Corporation, Osaka, Japan) to assess a baseline Sa before acid exposure. The Sa (μm), or the extension of Ra (arithmetical mean height of a line) to a surface, is defined as $Sa = 1/A \iint_A |Z(x, y)| dxdy$, whereas A = the defined area, Z = the absolute value of the height of the points, and x, y = the measurement unit of the XY stage. The Sa measurement used in this study is the ISO 25178 surface texture parameter. It expresses, as an absolute value, the difference in height of each point compared to the arithmetical mean of the surface. Three measurement units (each 273×204 μm), equally spaced 1 mm apart, were selected from each sample. The mean Sa of the three Sa values was used to represent the surface roughness of the samples.

Surface Treatments

The detailed treatment procedures are shown in Table 2. For group 1, the surfaces were treated with 1-mm thickness of Teethmate AP paste (TMAP) (Kuraray Noritake Dental, Tokyo, Japan) by rubbing with an applicator for five minutes. The TMAP was rinsed with deionized water. For group 2, Teethmate Desensitizer (TMD) (Kuraray Noritake Dental, Tokyo, Japan) powder and liquid were mixed for 15 seconds to form a slurry and applied on the enamel surface with continued rubbing motion for 30 seconds using a microbrush, left for five minutes, and then was rinsed off with deionized water for two seconds. For group 3, the specimens were immersed in 2.1 g/L (0.21%) sodium fluoride solution for five minutes. All the specimens were stored at 37°C for 24 hours in artificial saliva (AS) containing 150 mM KCl, 0.9 mM NaH_2PO_4, and 1.5 mM $CaCl_2$ (pH 7.2) using an incubation shaker (Personal-11, Taitec, Saitama, Japan) at 80 rpm. Group 4 consisted of acid-eroded samples with no desensitizer treatment. Group 5 was not subjected to acid erosion or desensitizer treatment and served as a control.

The TMAP, TMD, and NaF were reapplied in the same manner on the enamel surfaces at eight and 16 hours during the 24-hours AS incubation period. Specimens from groups 4 and 5 were stored in AS without any treatment. The AS was changed at eight and 16 hours to maintain solution freshness.

After 24 hours, all specimens were removed from the AS, gently air-dried, and measured for Sa. The differences in Sa between baseline (prior to acid erosion), after acid erosion, and after 24 hours of incubation with/without treatment were calculated for all specimens and used for statistical analysis. Topography images of all the surfaces were taken using the CLSM.

Table 2: *Treatment Groups*

Group	Acid Erosion for 15 Min	First Treatment for Five Min	AS Incubation for Eight Hours	Second Treatment for Five Min	AS Incubation for Eight Hours	Third Treatment for Five Min	AS Incubation for Eight Hours	Staining for Seven Days
1	+	TMAP	+	TMAP	+	TMAP	+	+
2	+	TMD	+	TMD	+	TMD	+	+
3	+	0.21% NaF	+	0.21% NaF	+	0.21% NaF	+	+
4	+	–	+	–	+	–	+	+
5	–	–	+	–	+	–	+	+

Abbreviations: AS, artificial saliva; TMAP, Teethmate AP; TMD, Teethmate Desensitizer; NaF, sodium fluoride.

Color Stability Assessment

The baseline color was assessed using the CIE Lab system with a dental colorimeter (Shade Eye NCC, Shofu, Kyoto, Japan). The CIE L*a*b* values were recorded before staining as baseline data. Photographs of the tooth surfaces were taken with a digital camera before and after staining.

The staining solution was created by immersing a tea solution that was prepared with two tea bags (Lipton Yellow Label Teabag, Unilever Japan, Tokyo, Japan) in 100 mL of boiling water for five minutes. The specimens were stored in an incubator for seven days at 37°C. The solution was changed on the fourth day. After seven days of immersion, the stained specimens were dried gently, and the color was measured again. Photographs were also taken. The color difference (ΔE) from the values obtained at the baseline and after staining was calculated according to the following equation:

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{1/2}$$

Statistical Analysis

The Sa data for all groups were subjected to one-way repeated measures analysis of variance (ANOVA) to compare differences in Sa. One-way ANOVA was performed to compare ΔE followed by the Tukey multiple comparison test at the 0.05 level of significance for *post hoc* analysis.

RESULTS

Surface Roughness and Color Stability Analysis

Baseline Sa was not significantly different among all groups. One-way repeated measures ANOVA showed that the acid erosion significantly increased Sa ($p<0.001$). The Sa decreased for all acid-eroded groups after treatment with desensitizing agents and immersion in AS. The decrease in Sa was significant for TMAP (group 1) ($p<0.001$) and TMD (group 2) ($p=0.002$), while the decrease in Sa was not significant for NaF (group 3) ($p>0.05$) and the no-desensitizer treatment (group 4) ($p>0.05$). The Sa measurement of the control group (group 5) was not significantly different from the baseline measurement ($p>0.05$) (Figure 1a).

For ΔE (Figure 1b), TMAP treatment showed the least staining, which was not significantly different from group 5 ($p>0.05$). TMD treatment showed a slightly higher degree of staining than group 5 ($p<0.05$). NaF significantly showed a higher degree of discoloration than that of group 5 ($p<0.001$). The discoloration of the NaF group was not significantly different from that of group 4 (acid eroded but no treatment) ($p>0.05$).

DISCUSSION

The conditions of the current study were made to simulate acid erosion and staining in the oral cavity. We used bovine enamel because it has been verified in a number of *in vitro* studies to be an excellent alternative to human enamel in the evaluation of the effect of anticariogenic agents on enhancing enamel remineralization and inhibiting enamel demineralization.[31] Citric acid, commonly found in lemon juice and orange juice, was used as erosive potential in this study to simulate acidic challenge because of its being an important constituent of various acidic beverages.[32] The staining procedure utilized a tea beverage to induce discoloration on tooth because tea has no potential for calcification/decalcification.[33]

To evaluate discoloration, the present study used photography and a dental colorimeter that can generate parametric data that can be easily applied for statistical analysis. This method produces more objective results than the visual method.[34] L* represents the psychometric lightness from black to white, and the a* axis is red on the positive side and green on the negative side. The b* axis is blue on the positive side and yellow on the negative side.[35]

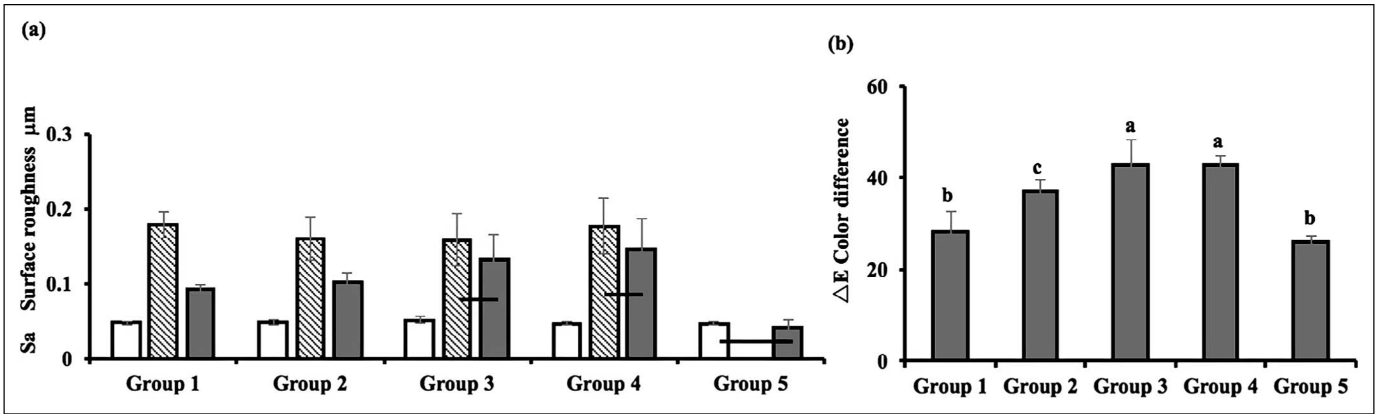

Figure 1. *(a): Mean (SD) enamel surface Sa at baseline and after erosion in citric acid (p<0.001) and after treatment with TMAP and TMD (p<0.001). Bars show no significant difference. White colors show baseline for all groups, line patterns show acid erosion groups, and gray colors indicate treatment for TMAP (group 1), TMD (group 2), NaF (group 3), with no desensitizer (group 4), and control group (group 5). (b): Mean △E, color measurement of discoloration in tea staining for seven days. Same characters show no significant difference.*

As expected, exposure to citric acid (pH 2.5) for 15 minutes significantly increased surface roughness and color difference due to minerals being leached out.[16-18,36,37] Enamel eroded by citric acid exhibited a pattern of demineralization with interprismatic mineral loss (Figure 2b), producing a roughened surface. The structural defects and porosities caused by mineral loss readily facilitate the diffusion of staining agents or chromogenic pigments.[16] A rough surface is generally considered more susceptible to staining, as the surface area for mechanical retention of discoloration pigments is increased, causing esthetic problems.[17,18,38,39]

The present study assessed the effect of two TTCP- and DCPA-based desensitizers (TMAP and TMD) to prevent staining on acid-eroded enamel surface and compared it with that of NaF, which is one of the most common agents used for remineralization of acid-eroded enamel.

The decrease in Sa of the acid-eroded groups after the application of TMAP, TMD and NaF is due to mineral precipitation as evidenced by the optical images taken with CLSM (Figure 2c,d,e). The enamel surfaces shown in Figure 2c,d were smoother than those on the acid-eroded enamel surface shown in Figure 2b,e,f. However, only the TMAP and TMD groups showed a significant decrease in surface roughness, with TMAP showing the lowest Sa value. The same result was observed for color change (ΔE), which indicated that the TMAP and TMD groups showed a significantly low ΔE. TMAP showed the lowest ΔE value among the tested groups. The results indicated that the lower the surface roughness value (smoother surfaces), the lesser the discoloration (more resistant to staining).[38]

These results can be explained by the components of the two calcium-phosphate–based desensitizers (Table 1). Both desensitizers contain TTCP and DCPA, which have different mechanisms of action

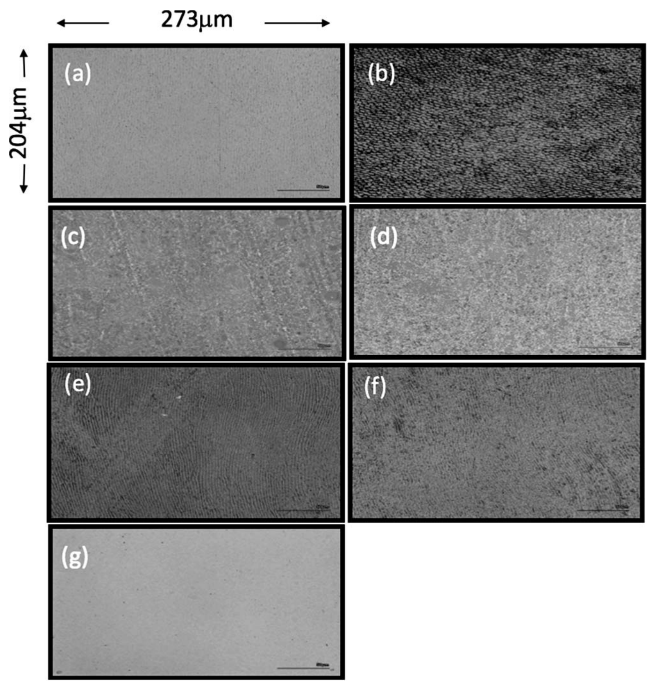

Figure 2. *Representative CLSM optical images of the bovine enamel surface texture before, after erosive challenges, and after treatment. (a): Baseline (before erosion) showed no morphological change. (b): Acid erosion for 15 minutes showed the typical honeycomb acid dissolution patterns on the enamel surface due to the loss of minerals. (c): Treatment with TMAP. (d): Treatment with TMD showed significant decrease in surface roughness. Treated surface was noticeably smoother than acid eroded enamel. (e): Treatment with 0.21% NaF treatment showed rough appearance of the enamel surface. (f): Acid-eroded enamel with no desensitizer treatment was not significantly different with 0.21% NaF treatment. (g): Control group stored in AS did not exhibit any morphological change.*

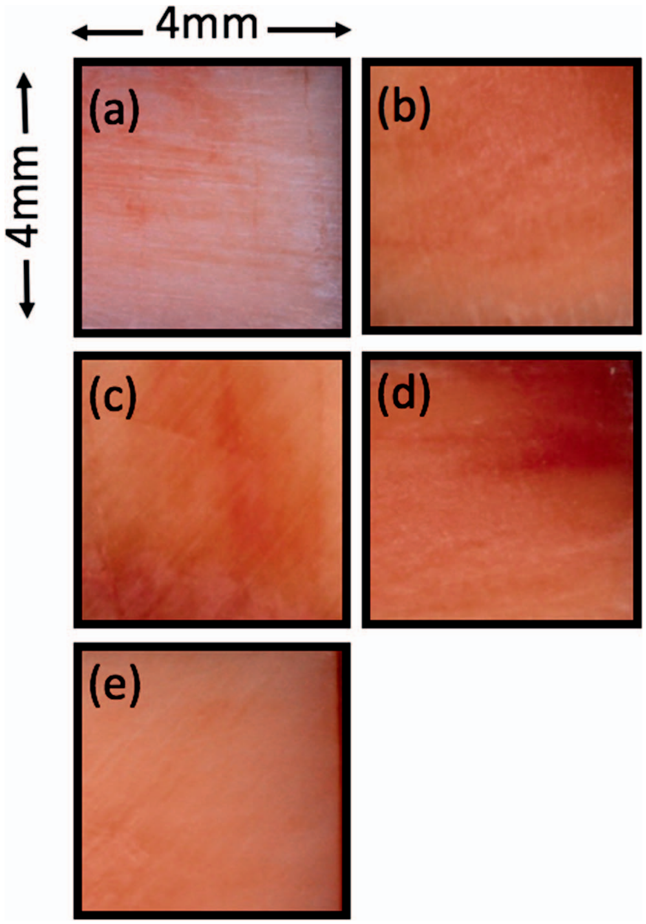

Figure 3. *Representative digital camera photos of the degree of staining on the acid-eroded enamel surfaces as to treatment groups. (a): Treatment with TMAP showed less staining. Staining was similar to control group because of smooth surface. (b): Treatment with TMD showed a slightly higher staining than control group. (c): Treatment with 0.21% NaF showed greater staining than control group. (d): No-desensitizer treatment indicated similar staining with 0.21% NaF treatment. (e): Control group.*

from other conventional desensitizers. The dissolution of TTCP and DCPA would lead to a supersaturation of calcium and phosphate ions with respect to hydroxyapatite. This increase in calcium and phosphate ion concentration leads to the formation of hydroxyapatite-like crystals,[28-30] mineralization, and consequently a decrease in surface roughness. The lower Sa values or smoother surfaces after treatment for TMAP compared to TMD may be due to the additive effect on enamel remineralization of the 950-ppm fluoride present in TMAP.

Figures 2c and 3a (TMAP) and Figures 2d and 3b (TMD) show the remineralized surfaces with the formation of a superficial homogeneous layer similar to that of intact enamel (Figure 2e,g), thereby minimizing discoloration.

A notable finding is that NaF (Figure 2e) did not significantly decrease Sa, nor did it prevent staining, as it showed the highest ΔE (Figure 3c), similar to that of group 4 (no desensitizer) (Figures 2f and 3d). This corresponds to the results of a previous study showing that fluoride alone is insufficient to cause significant remineralization.[40] In that study, it was reported that fluoride can induce remineralization only in the presence of adequate amounts of calcium and phosphate ions in the saliva; therefore, the enamel must be exposed to saliva long enough to enable the saturation of ions for fluoroapatite or fluorohydroxyapatite formation on the enamel surface.[40] In the present study, 24 hours of immersion in artificial saliva might be insufficient to attain enough concentration of calcium and phosphate ions to form fluoroapatite. The microporosities formed after acid erosion and fluoride application were not homogeneously remineralized, allowing stains to develop on the enamel surface within 24 hours.

Group 4, which did not receive any desensitizer treatment, exhibited almost the same Sa and staining as the NaF group, which might be indicative of the possible formation of remineralization from the calcium and phosphate ions present in the artificial saliva. However, remineralization was too little to prevent severe discoloration. According to Buzalaf and others,[41] one of the most important factors in the repair of softened enamel is saliva, as it contains calcium and phosphates to induce natural remineralization. However, natural remineralization through saliva alone is a very slow process, and thus calcium phosphate technologies and fluorides are employed to hasten the remineralization process.[42,43]

Group 5 (control), which was not subjected to acid erosion and did not receive any treatment, showed similar Sa compared with baseline and then indicated the lowest staining, which was not significantly different with group 1 because of the smooth surface.

The results of the present study show that desensitizers containing TTCP and DCPA (TMAP and TMD) are more effective in decreasing surface roughness and discoloration than sodium fluoride. These agents have clinical benefits, as they can prevent discoloration due to effective remineralization of eroded enamel.

CONCLUSIONS

Acid erosion increases surface roughness, which subsequently increases staining. TTCP- and DCPA-based desensitizers may produce HA formation and precipitation on eroded surfaces and therefore are effective in inhibiting enamel erosion and increasing resistance to tea stain absorption into acid-eroded surfaces.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the Tokyo Medical and Dental University.

Conflict of Interest

The authors of this article 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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