Article published online: 2021-11-01

ORIGINAL ARTICLE

Chromatic and surface alterations in enamel subjected to brushing with desensitizing whitening toothpaste

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ABSTRACT

Aim: This study evaluated the chromatic and surface changes on enamel after toothbrushing with whitening and desensitizing toothpaste. **Materials and Methods:** Sixty enamel blocks were prepared, pigmented, and stratified according to initial Knoop microhardness and divided into six groups. The average roughness (Ra) was determined from two readings. After 24 h in artificial saliva, 10,000 cycles of simulated brushing were applied. The Ra was measured after 5000 and 10,000 cycles, and tooth wear was determined. The mean roughness was evaluated, and tooth color was recorded before and after treatment. **Results:** Brushing with dentifrices increased the roughness of enamel in all groups. It was lower for Colgate Sensitive Pro-Relief + Bleaching, Colgate maximum protection anti-caries, and the control group. Greater roughness was observed in dentifrices containing silica. Greater wear was found with Sensodyne bleaching extra fresh and in the control group. The best bleaching effect was found with Colgate Sensitive Pro-Relief + Bleaching. Colgate Sensitive Whitening, Oral-B Pro-Health Whitening, and Sensodyne Whitening Extra Fresh showed major changes on surface roughness. **Conclusion:** The physical characteristics of the minerals of the toothpaste appear to be the major determinant of dental abrasion, not their quantity or whitening capacity, or rather their ability to remove enamel surface stains.

Key words
Dentifrices, dentin desensitizing agents, dentin sensitivity, tooth bleaching, tooth wear

INTRODUCTION

With the increasing longevity of the population and better access to dental care, caries prevention has been effective. However, in parallel with the decreased prevalence of caries, an increase in noncarious lesions with nonbacterial causes has been observed. This loss of dental tissue, independently of its cause (erosion, abrasion, and abfraction), is commonly associated with dentin hypersensitivity. Several products are currently widely available for the treatment of hypersensitivity, i.e., desensitizing toothpaste. Most of these products act by occluding dentinal tubules by the deposition of crystals on the substrate's surface.[1-3]

Access this article online	
Quick Response Code:	Website: www.ejgd.org
	DOI: 10.4103/2278-9626.189256

Moreover, nowadays, there is a greater awareness of esthetic dentistry and the search for a beautiful smile, which is often translated as having whiter teeth. Because of this demand, the market has released toothpaste with whitening action in associated with desensitizing agents. Whitening toothpaste mainly acts due to higher abrasiveness, with the aim of removing extrinsic staining. This seems to occur in opposition to the occlusive action of desensitizing agents.[4,5]

The objective of this study was to evaluate the chromatic and superficial changes (roughness and wear) of bovine enamel after simulated toothbrushing with desensitizing

How to cite this article: de Melo Monteiro GQ, Macedo de Oliveira IL, Fonseca de Brito OF, Guedes BP, Lula de Amorim MS, Araujo Maia AM. Chromatic and surface alterations in enamel subjected to brushing with desensitizing whitening toothpaste. Eur J Gen Dent 2016;5:115-21.

toothpaste with whitening action, and the relationship between these results and the abrasive composition of the products. The hypothesis was that desensitizing toothpaste with whitening action does not promote chromatic changes; however, they do lead to an increase in the roughness and wear of the enamel surface, which are directly related to the abrasive composition of the toothpaste.

MATERIALS AND METHODS

Experimental design and specimen preparation

This study was subjected to the Ethics Committee on Animal Research at the Universidade Federal de Pernambuco and approved under the protocol nº 23076.045898/2012-17.

Bovine incisors recently extracted were selected and donated from the public slaughterhouse of the city of São Lourenço da Mata, Pernambuco, Brazil. The teeth were stored in 0.5% chloramine T solution for 7 days for disinfection. Sixty teeth with sound crowns and free of stains were selected. The roots were sectioned using a diamond saw at low speed. Enamel blocks (4 mm × 4 mm) were then prepared and stored in distilled water. All blocks were individually embedded in acrylic resin. The exposed enamel surface was ground with water-lubricated silicon-carbide paper (SiC) of decreasing grit (#600 and 1200). The specimens were then polished using a metallographic cloth (SUPRA - Arotec, São Paulo-SP, Brazil) with 1-μm diamond suspension (Buehler, Chicago, IL, USA). All specimens were then sonicated in distilled water.

The specimens were immersed for 2 weeks in a 25% of coffee solution prepared using 250 g of powdered coffee (Nescafé, Nestle, São Paulo-SP, Brazil) in 750 mL of water at 100°C. After cooling to room temperature, the specimens were immersed in the solution and stored at 37°C. The solution was changed after 7 days. After this period, the specimens were washed with water and stored in artificial saliva for 24 h at 37°C.[6]

The artificial saliva comprised Ca 1.5 mM, PO_4 0.9 mM, KCl 150 mM, and hydroxypropyl methylcellulose buffer 20 mM (pH 7).[7,8]

Experimental groups

For standardization, the specimens were subjected to microhardness analysis (HMV MicroHardness Tester, Shimadzu, Japan). This allowed an even distribution among all experimental groups. A Knoop (KHN) pyramidal diamond indenter was used with a 25-g static load for 10 s. Five indentations were performed on each specimen, 100 μm apart.[9] The resulting hardness was obtained by calculating the average of the five indentations. The specimens were then stratified into six groups (n = 10) according to the test product. A control group was also prepared with no dentifrice applied to evaluate the brushing effect over the enamel.

Enamel wear analysis

Enamel wear was determined by measuring changes in the length of the longer diagonal. As the Knoop diamond indenter is more long than deep, very small changes in depth due to enamel wear (Δd) lead to measurable changes in the diagonal length. Thus, the length of the longer diagonal was measured before and after mechanical testing (tooth brushing). Changes in the length of the diagonal (Δl) were determined, and the change in the depth of the indenter was calculated using the equation:

$$\Delta d = 0.032772\ \Delta l$$

The wear (Δd) was calculated for each specimen, with an overall average calculated for each group.

Enamel surface roughness analysis

Roughness was evaluated before and after the simulated brushing procedure in contact mode (Surfpak - SJ Version 1300, Mitutoyo, Japan).The Ra (average roughness) parameter was considered for statistical analysis. Two areas were evaluated in each specimen covering a total of 1.7 mm.

Color analysis

Color measurement was carried out using a clinical spectrophotometer (Vita Easy-Shade, Vita Zahnfabrik, Bad Säckingen, Germany). The colorimetric value was obtained with spectrophotometer readings in the color space L*, a*, b*; where L* represents lightness, a* represent the hue, and b* represents saturation. In addition, color change (ΔE) was calculated using the differences between the values obtained at the initial and final L*, a*, b* readings (ΔL, Δa, and Δb). The following equation was used to determine ΔE:

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

The results were interpreted as follows: $\Delta E \leq 1$, undetected by the human eye; $1 < \Delta E < 3.3$, detected by the human eye and clinically acceptable; and $\Delta E > 3.3$, detected by the human eye and clinically unacceptable.

Three readings were obtained in the central area of the specimen after pigmentation and after simulated toothbrushing, and the mean value was considered for analysis.

Simulated tooth brushing

Toothbrushing was simulated using specific equipment (MSET/*ElQuip*, São Carlos, SP, Brazil). The dentifrices used in the experiment were diluted in distilled water at a ratio of 1:3 by weight [Table 1]. This ratio was used to allow the solution to be injected into the

Table 1: Products, manufacturers, and their components

Product	Composition
Colgate Cavity Protection	Sodium monofluorophosphate (1450 ppm fluoride), calcium carbonate, water, sorbitol, sodium lauryl sulfate, sodium monofluorophosphate, flavor, cellulose gum, tetrasodium pyrophosphate, sodium silicate, sodium saccharin, methylparaben, propylparaben
Colgate Sensitive Whitening	Sodium monofluorophosphate 1.1% (1450 ppm fluoride), potassium citrate, water, sorbitol, glycerin, hydrated silica, potassium citrate, PEG-12, tetrasodium pyrophosphate, copolymer PVM/MA, sodium lauryl sulfate, sodium monofluorophosphate, flavor, cellulose gum, sodium hydroxide, saccharin sodium, xanthan gum, titanium dioxide (CI 77891), blue pigment 15 (CI 74160)
Colgate Sensitive Pro-Relief Whitening	Arginine 8%, sodium monofluorophosphate 1.10% (1450 ppm fluoride), calcium carbonate, water, sorbitol, arginine bicarbonate, sodium lauryl sulfate, flavor, cellulose gum, sodium bicarbonate, potassium acesulfame, sodium silicate, xanthan gum, sucralose, titanium dioxide (CI 77891), sodium monofluorophosphate, titanium dioxide (CI 77891), blue pigment 15 (CI 74160)
Sensodyne Extra Whitening	Sodium fluoride (1384 ppm fluoride), potassium nitrate 5%, sorbitol, water, silica, glycerol, triphosphate pentasodium, polyethylene glycol, flavor, titanium dioxide (CI 77891), sodium methyl cocoyl taurate, cocamidopropyl betaine, xanthan gum, sodium hydroxide, sodium saccharin, sodium fluoride
Crest/Oral-B Pro-Health Whitening	Tin fluoride (1100 ppm fluoride), sodium fluoride (350 ppm fluoride), glycerin, hydrated silica, sodium hexametaphosphate, propylene glycol, PEG-6, zinc lactate, flavor, sodium lauryl sulfate, sodium gluconate, carrageenan, silica, trisodium phosphate, mica, saccharin sodium, carnauba wax, xanthan gum, titanium dioxide, blue pigment15

Colgate – Colgate-Palmolive Company, New York, USA, Sensodyne – GlaxoSmithKline, London, UK, Crest/Oral B – Procter and Gamble Manufacturing, Ohio, USA. PEG – Propylene glycol, PVM/MA - copolymer of methyl vinyl and maleic anhydride, CI - colour index

toothbrushing machine without obstructing the syringe tip.[10] In addition, this dilution seems to reproduce the intraoral situation. The toothpaste suspensions were manually applied, 1 mL every 200 cycles. For the control group, only distilled water was applied.[8,11,12]

All specimens were subjected to 10,000 cycles at a speed of 4.5 cycles/s and 200 g axial load. At 5000 cycles, the specimens were analyzed for all methods. After brushing, the specimens were thoroughly washed under running water, followed by sonication in distilled water for 10 min to completely remove residual toothpaste. Between analyses, the specimens were stored in distilled water.

Characteristics of the abrasive particles: size and morphology.

All toothpaste was diluted to remove all soluble components leaving only the insoluble or less soluble abrasive particles. Thus, the dilution was carried out using 2 g of toothpaste in 100 mL of distilled water. This solution was agitated for 15 min using an ultrasound cube. From this solution, 13 mL was transferred to Falcon tubes and centrifuged for 3 min at 2000 rpm at 19°C (relative centrifugal force 680; Universal 320R Hettich Zentrifugen/LHNH Uniscience, Tuttlingen, Germany). The supernatant solution was discarded, and more of the initial solution was added until all 100 mL was used. On average, this procedure was performed ten times. Two centrifugations were then performed for 5 min each until the liquid inside the Falcon tube remained clear. After eliminating the supernatant liquid, another 13 mL of distilled water was added to the tube, and vortex agitated. Then, 200 μL of each solution was pipetted into another Falcon tube containing 10 mL of distilled water. After homogenization, 1 mL of the new solution was then placed in an Eppendorf tube.

Subsequently, a polyvinyl pyrrolidone (molecular weight 5500) solution (PVP 5500) was prepared to prevent agglomeration of the particles. This solution was prepared using 10 mL of distilled water and 0.0055 g of PVP 5500. One milliliter of PVP 5500 solution was added to the Eppendorf tube containing the toothpaste dilution.

A drop of this new solution was poured onto a microscopy glass slab and stored at 60°C for 1 h. The glass slabs were mounted on stubs, metalized, and analyzed by scanning electron microscopy at 1000–20,000×.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences version 13 (SPSS, Chicago, IL, USA). Descriptive statistics were obtained, and the Kolmogorov–Smirnov test was applied to evaluate the normality of the data. One-way analysis of variance (ANOVA) and the Kruskal–Wallis test were used to evaluate the data. The Tukey and Mann-Whitney tests were applied whenever differences were observed between groups. The Wilcoxon signed-rank test was used to compare the results at different times. For all tests, a $P = 0.05$ was considered statistically significant.

RESULTS

The results for wear (Δl) after 10,000 cycles are shown in Table 2. One-way ANOVA showed significant differences between groups ($P = 0.026$).

The average surface roughness values are represented in Table 3. The Kruskal–Wallis test showed differences between groups after 5000 and 10,000 cycles of tooth brushing ($P < 0.001$). Note the similarity among the

Ra values at the initial stage (P = 0.926). The Wilcoxon signed-rank test showed significant differences among the valuation times (initial vs. 5000 cycles, 5000 vs. 10,000 cycles, initial vs. 10,000 cycles). Exceptions were observed for the control group (initial vs. 5000 cycles, P = 0.799; 5000 vs. 10,000 cycles, P = 0.093) and for Colgate Sensitive Whitening toothpaste (5000 vs. 10,000 cycles, P = 0.114).

Table 2: Mean enamel wear (µm) and standard deviation after 10,000 cycles of simulated brushing (n=10)

Toothpastes	Wear (Δ*l*) (µm)
Sensodyne Extra Whitening	0.269 (0.257)^{B}
Colgate Sensitive Whitening	0.207 (0.107)^{A,B}
Colgate Sensitive Pro-Relief Whitening	0.105 (0.048)^{A}
Crest/Oral-B Pro-Health Whitening	0.164 (0.089)^{A,B}
Colgate Cavity Protection	0.096 (0.051)^{A}
Control (distilled water)	0.174 (0.207)^{A,B}

Different superscript letters indicate differences between groups; ^{A,B}Sensodyne Extra Whitening x Colgate Sensitive Pro Relief Whitening (P = 0.45*); xColgate Cavity Protection (P = 0.30*)

Table 3: Mean surface roughness (µm) and standard deviation before and after 5000 and 10,000 cycles of simulated brushing (n=10)

Toothpastes	Ra (cycles)		
	0	5000	10,000
Sensodyne Extra Whitening	0.06 (0.01)^{a}	0.12 (0.10)^{A,b}	0.13 (0.11)^{B,c}
Colgate Sensitive Whitening	0.06 (0.02)^{a}	0.14 (0.05)^{A,b}	0.24 (0.18)^{A,b,c}
Colgate Sensitive Pro-Relief whitening	0.06 (0.01)^{a}	0.06 (0.01)^{B,b}	0.07 (0.01)^{B,c}
Crest/Oral B Pro-Health whitening	0.05 (0.01)^{a}	0.10 (0.04)^{A,b}	0.20 (0.13)^{A,c}
ColgateCavityProtection	0.07 (0.04)^{a}	0.06 (0.01)^{B,b}	0.09 (0.04)^{B},^{c}
Control (distilled water)	0.05 (0.01)^{a}	0.06 (0.01)^{B,a,b}	0.07 (0.02)^{B,b,c}

Different superscript letters indicate differences between groups (uppercase in columns and lowercase in lines). Ra – Roughness average; (a x b) P < 0.001*; (a x c) P = 0.007*; (b x c) P = 0.047*; (a x b) P = 0.009*; (a x c) P = 0.005*; (b x c) P = 0.114

The color parameters at all analysis times are shown in Figure 1. One-way ANOVA detected differences between groups only for ΔE between initial and 5000 cycles (P = 0.003). Tukey honest significant difference *post hoc* test showed statistically significant differences between Colgate Sensitive Pro-Relief Whitening, Colgate Cavity Protection, Crest/Oral-B Pro-Health Whitening (ΔE = 8.15, 7.91, 7.75, respectively) and Sensodyne Extra Whitening (ΔE = 4.04).

The fillers, composition, size, shape, and superficial aspects are described in Table 4. Representative images of all types of particles are shown in Figures 2a-e.

DISCUSSION

The hypothesis of this study regarding chromatic changes was accepted, that is, desensitizing toothpaste with whitening action does not promote chromatic changes. Differences between products were only observed when the baseline results were compared with the results after 5000 cycles. No statistically significant differences were observed between them and the control group.

Our results corroborate previous results that disagree about the whitening potential of this toothpaste. It seems that these products act by extrinsic stain removal due to their higher abrasiveness.[13-15] Further composition analysis did not identify any substance with a bleaching action. Dentifrices containing hydrogen peroxide can produce some increase in the L*parameter. However, they cannot reduce the pigmentation of the yellow color (b*) with clinical efficacy.[13,16]

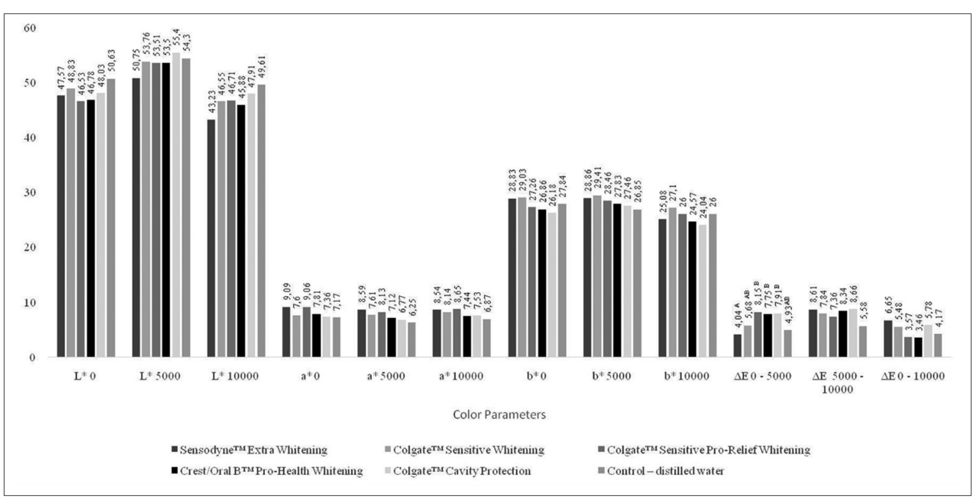

Figure 1: Mean values for the tooth color parameters (L*a*b*) and ΔE with their corresponding standard deviation at all evaluation times. Different superscript letters indicate differences between groups

Table 4: Description of the type, size, shape, and aspects of the fillers in the dentifrices

Dentifrices	Composition	Size (μm)	Shape and aspects
Sensodyne Extra Whitening [Figure 2a]	Silica	5.06-17.20	Irregular with rounded edges; porous particles
Colgate Sensitive Whitening [Figure 2b]	Silica	3.94-5.26	Irregular with rounded edges; porous particles
Crest/Oral-B Pro-Health Whitening [Figure 2c]	Silica	6.41-20.4	Irregular with rounded edges; dense particles
Colgate Sensitive Pro-Relief whitening [Figure 2d]	Calcium carbonate	Width, 0.263; length, 0.853-0.995	Irregular, needle crystals
Colgate Cavity Protection [Figure 2e]	Calcium carbonate	1.07-1.40	Irregular, needle crystals

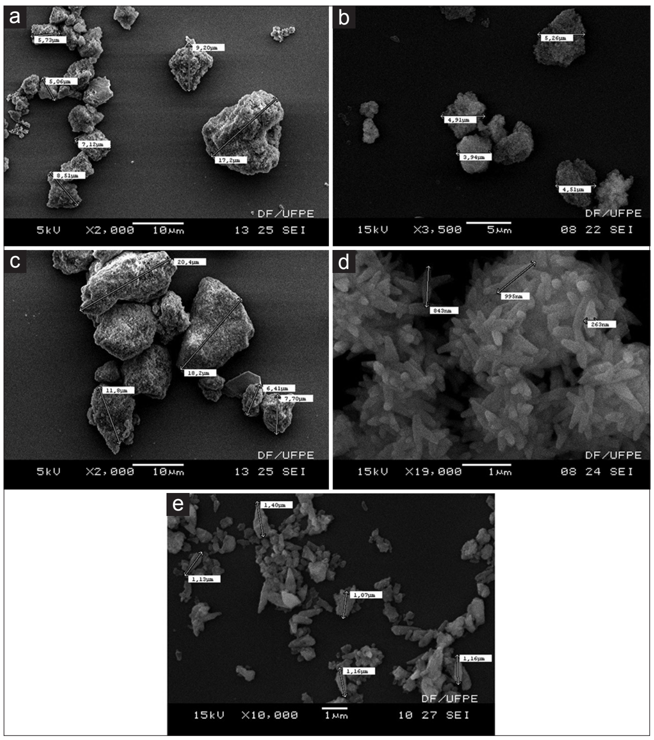

Figure 2: (a) Sensodyne extra whitening filler size and morphology; (b) Colgate Sensitive Whitening filler size and morphology; (c) Crest/Oral B Pro-Health Whitening filler size and morphology; (d) Colgate Sensitive Pro-Relief Whitening filler size and morphology; (e) Colgate Cavity Protection filler size and morphology

Individual analysis of each color parameter (L*, a*, and b*) showed no statistical differences between groups for each evaluation period. Color changes were mainly attributed to the reduction in b* (yellow-blue) and an increase in L* (brightness).[17] Reduction in b* has been reported to be the most important indicator because it occurs more rapidly and to a greater extent compared with a* (red-green).[16]

Evaluation of color changes produced by abrasive particles is accomplished by studying the color parameters. In addition, superficial alterations must be considered due to the abrasive capacity of the products. It is therefore very important to assess enamel roughness after their use. To do this, we tested the hypothesis that these products enhance surface roughness. Our results partially accepted this hypothesis as surface roughness seemed to be product related. By analyzing the data obtained for the mean roughness of the enamel surface at baseline, we can conclude that any differences occurring in the surface roughness of the groups after 5000 and 10,000 cycles of brushing are the result of the treatment applied (brushing + toothpaste). Considering the time of treatment, a significant increase in roughness was observed on the enamel surface after brushing regardless of the evaluation period. An exception was observed in the control group because of the absence of toothpaste. On the other hand, in a study evaluating the effect of three toothpaste on the enamel surface using a qualitative method of scanning electron microscopy, it was reported that the surface changes were inconsistent.[18]

Our results rejected the hypothesis of increased wear. Yet again, differences were observed between the products, however, without any differences between them and the control group.

We attempted to find a relationship between abrasive particle morphology and size and the results obtained for roughness and wear, however, no association was found. For example, Colgate Sensitive Pro-Relief Whitening produced one of the highest roughness values. This material has calcium carbonate as its abrasive particle. Despite having an irregular needle-like shape, the particles were very small (width, 0.263 μm; length, 0.853–0.995 μm). Conversely, Crest/Oral-B Pro-Health Whitening produced the smallest roughness values, and its particles were irregular with rounded edges but much bigger (6.41–20.4 μm).

The presence of other constituents that may act as abrasive particles must also be taken into account, for example, sodium bicarbonate. This component is considered to have low abrasiveness due to low intrinsic hardness and high solubility, providing low wear. The low abrasiveness of calcium carbonate in association with sodium bicarbonate has also been demonstrated.

Toothpaste that contains a high concentration of sodium bicarbonate seem to whiten teeth more than regular toothpaste and those containing silica or calcium phosphate.[19] Our results confirm the low roughness of this association as observed by the results for Colgate Sensitive Pro-Relief + Whitening. An improved whitening effect (ΔE) between the first and second evaluation periods (0–5000 cycles) was also observed for this group. However, there were no significant differences from the control group. In the literature, the whitening potential of sodium bicarbonate is still controversial, with many results demonstrating no whitening efficacy related to sodium bicarbonate.

The concentration, type, shape, size, and hardness of the abrasive particles are essential factors for appropriate product selection. However, the lack of information contained on the packaging of these products is very common. In general, manufacturers only indicate the types of abrasives.[7,20] For example, two toothpaste based on silica with similar particle size gave different results regarding roughness: Crest/Oral-B Pro-Health Whitening 0.20 μm (particle size 6.41–20.4 μm) and Sensodyne Extra Whitening 0.13 μm (particle size 5.06–17.20 μm). This highlights the influence of the different variables that affect superficial roughness.[21]

It was interesting to observe the color change obtained by Colgate Cavity Protection. With the stain removal potential of calcium carbonate in comparison with another silica compounds, it removed significantly more stains.[22] The higher capacity for stain removal was not associated with higher enamel wear.[12] In this study, this product also produced very low surface roughness.

It is important to emphasize that some studies attribute a higher bleaching action of toothpaste containing silica. However, in some studies, blue covarine is associated with silica, which may explain the better bleaching effect.[12,22]

CONCLUSION

It can be concluded that desensitizing toothpaste with bleaching action does not promote color changes. Toothpaste containing calcium carbonate promotes less abrasion and wear with improved stain removal capacity compared with silica compounds. The composition, concentration, size, and shape of the abrasive particles play an important role in dental abrasion. However, the lack of this information makes the product choice difficult for the dentist and the patient. However, whitening capacity, or rather superficial stain removal, is not necessarily associated with an increase in surface roughness; the reciprocal is also true.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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