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ORIGINAL RESEARCH

Effects of Toothbrushing with Fluoride Abrasive and Whitening Dentifrices on Both Unbleached and Bleached Human Enamel Surface in Terms of Roughness and Hardness: An *in vitro* Study

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ABSTRACT

Aim: The aim of this *in vitro* study was to evaluate the surface roughness and hardness of both unbleached and bleached (opalescence; 10% carbamide peroxide) human enamel brushed with water (without dentifrice), fluoride abrasive dentifrice (Colgate Total) and whitening dentifrice (Natural White).

Materials and methods: Human enamel samples were obtained from third molars and randomly divided into five groups (n = 8): G1—Control (brushed with water without dentifrice), G2—Colgate Total (fluoride abrasive dentifrice), G3—Natural White (whitening dentifrice), G4—Opalescence (10% carbamide peroxide) and then brushed with Colgate Total, G5—Opalescence (10% carbamide peroxide) and then brushed with Natural White. Bleaching regimen was applied according to manufacturers' instructions. The brushing process was performed with a modified Nyffenegger's brushing machine. Surface roughness was analyzed with a profilometer. Microhardness testing was performed with a Brinell hardness tester.

Results: Results were statistically analyzed by Kruskal-Wallis, one-way ANOVA analysis and Mann-Whitney U, Wilcoxon matched-pairs signed-ranks tests. There were significant differences in surface roughness values for all groups, which showed an increase in roughness ($p < 0.05$). When the bleaching treatment combined with brushing with whitening dentifrice was performed (G5), there was a significant decrease in hardness values ($p < 0.05$). The other groups (G1, G2, G3, G4) showed no significant hardness differences ($p > 0.05$).

Conclusion: It was concluded that toothbrushing procedures increased the enamel surface roughness, and that bleaching regimen performed with cleaning treatment, through brushing with whitening dentifrice decreased hardness values.

Clinical significance: When applied together, bleaching and cleaning treatments may alter the enamel surface roughness and hardness values.

Keywords: Toothbrushing, Dentifrices, Roughness, Hardness, Bleaching.

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INTRODUCTION

Bleaching procedures have gained popularity with patients and dentists as conservative techniques to lighten teeth in order to improve the harmony of the smile. In recent years, tooth whitening is one of the most rapidly growing oral care sectors fuelled by the consumer's demand for both healthy and cosmetically attractive smiles. Indeed, for the majority of people, the appearance of the teeth is very important, and any discoloration or stain that may form on them will affect their esthetic qualities.[1] The public interest in esthetic dentistry has led to the development of new technologies to whiten teeth. Patients, who submit themselves to a tooth-bleaching treatment, are generally patients that brush their teeth three or four times a day to achieve health and beauty.[2]

The carbamide peroxide at home bleaching technique was formally introduced to dental profession by Haywood and Heymann in 1989 and has been universally accepted since then.[3] But since the introduction of the night guard vital bleaching technique using 10% carbamide peroxide, studies have been performed in order to evaluate the adverse effects during and after whitening procedures.[4] Evidence has demonstrated that enamel presents structural changes when exposed to 10% carbamide peroxide, compromising its composition and morphology.[4] Fluoride therapy has been

shown to avoid these side effects, and one of the most useful methods of fluoride application is the use of dentifrices. It has been showed that fluoride dentifrice could reduce the toothbrushing abrasion of the soft and hard dental tissues.[5,6]

Tooth whitening dentifrices play an important role in the cleaning process by removing extrinsic stains, and patients commonly use them during the bleaching treatment. Some complicating factors may be explained by acknowledging that cleaning effectiveness may not be solely related to abrasion, and there is concern that some abrasives may contribute to excessive tooth wear.[7-10] However, to maintain oral health, teeth need to be daily brushed and there is lack of evidence on the effects of bleaching agents combined with toothbrushing on the enamel surface as well as on its influence on enamel roughness.

Toothbrushing is now the most common means of oral prophylaxis and in the light of its potential benefits to oral health; the adverse effects or damage caused by tooth brushing can be regarded as insignificant. However, it would be an exaggeration to conclude that toothbrushing is totally harmless.[10] Toothbrushing abrasion depends not only on the dental substrate but also on the abrasiveness of the dentifrice used. Indeed, tooth wear may be influenced by the presence of fluoride ions in the environment during the abrasive challenges.[5]

The purpose of the study was to investigate the *in vitro* effects of both unbleached and bleached (10% carbamide peroxide, Opalescence/Ultradent Co., South Jordan, UT, USA) human enamel brushed with fluoride abrasive dentifrice (Colgate Total/Colgate Palmolive, Istanbul, Turkey) and whitening dentifrice (Natural White/Natural White Ltd Staines, Middlesex, England) on the surface roughness and hardness.

MATERIALS AND METHODS

A total of 40 recently extracted human third molar teeth with noncarious intact enamel surfaces were used in the study. The teeth were embedded in acrylic blocks (3 cm long, 1.3 cm wide and 0.6 cm thick). The cusp tips of the teeth were ground with silicon carbide slurries on rotating metal disks so that a small flat area of enamel would be exposed. The test surface of each tooth was then serially polished with 320-, 400-, 600- and 1200-grit aluminum oxide abrasive papers in a water-cooled mechanical grinder. All teeth were ultrasonically cleaned in a mild detergent for removal of polishing agents, labeled and stored in distilled water.

Specimens were randomly divided into five groups (n = 8), four of which with different bleaching procedures and a control group. Table 1 shows the composition, manufacturer and bleaching regimen of the whitening products used in this study. G1—brushed with water without dentifrice (control group), G2—brushed with fluoride abrasive dentifrice (Colgate Total), G3—brushed with whitening dentifrice (Natural White), G4—bleaching regimen (Opalescence, 10% carbamide peroxide) was applied to enamel for 1 hour a day for a month and then brushed with fluoride dentifrice (Colgate Total), G5—bleaching regimen (Opalescence, 10% carbamide peroxide) was applied to enamel for 1 hour a day for a month and then brushed with whitening dentifrice (Natural White). During the bleaching period, specimens were placed in 100% relative humidity 37ºC. Following the bleaching regimen, specimens were rinsed and stored in deionized water for 24 hours at 37ºC before being tested.

Brushing abrasion was performed with an automatic toothbrushing machine with a motor that produced a motion on 10 soft nylon bristle toothbrushing heads (Colgate), in a thermostatically controlled environment 37 ± 0.5ºC. The samples had been embedded in acrylic resin blocks 3 cm long, 1.3 cm wide and 0.6 cm thick, with the stained surface exposed, and attached to a toothbrushing machine. The brushing process was performed with a modified Nyffenegger's brushing machine.[11] This machine contains

Table 1: Chemical composition of the bleaching agent, whitening and fluoride dentifrice

Bleaching agent		
Opalescence (10% carbamide peroxide)	Ultradent Co, South Jordan, UT, USA	Glycerine, buffered polycarboxylic acid, peppermint oil
Whitening dentifrice		
Natural White	Natural White Ltd, Staines, Middlesex, England	Aqua, hydrated silica, glycerin, potassium nitrate, pentasodium triphosphate, PVP, sodium lauryl sulfate, titanium dioxide, cellulose gum, aroma, sodium saccharin, sodium benzoate, sodium fluoride, sorbitol, sodium methyl cocoyl taurate, CI 16035
Fluoride dentifrice		
Colgate total	Colgate Palmolive, Istanbul, Turkey	Aqua, hydrated silica, sorbitol, sodium lauryl sulphate, aroma, *Carrageenan*, sodium fluoride, cellulose gum, sodium saccharin, glycerin, triclosan, PMV/MA copolymer, sodium hydroxide, titanium dioxide

eight arms that apply horizontal brush strokes and allowed brushing of all eight specimens simultaneously. The load to each specimen was adjusted to 600 gm, including the weight of the brush. The range of the brushing movement was 37 mm. The specimens were fixed in place with screws to the bases of the protective reservoirs. The machine contained seven reservoirs filled with toothpaste slurry made from a mixture of 75 gm of toothpaste (Colgate Total), into which the resin blocks with attached samples were placed. Samples were exposed to 20.000 brush strokes. Hard nylon multitufted toothbrushes (Colgate) were used. Brushes and toothpaste mixture were replaced after every 200 brush strokes.

A profilometer (Mitutoyo Surf Test 402, Mitutoyo Corp, Tokyo, Japan) was used to measure the surface roughness (Ra) before and after toothbrushing treatments. Two measurements were made on each specimen. A mean was obtained from the two readings and recorded.

Surface hardness of the specimens was accessed using a Brinell hardness tester (Frank Prufent-Messen, Germany). The instrument was calibrated according to manufacturer's instruction. A 10 mm diameter steel bar was pressed into the specimen by a 3,000 kg load for 15 to 30 seconds. The hardness was determined by measuring the amount of deformation which occurred due to the applied load. Brinell hardness number (BHN) was calculated according to following formula:[12,13]

$$BHN = \frac{F}{\frac{\Pi}{2} D.\left(D - \sqrt{D^2 - D_i^2}\right)}$$

F: Imposed load in kg

D: The diameter of the spherical indenter in mm

D_i: Diameter of the resulting indenter impression in mm

Three measurements were performed on each specimen and a mean was obtained. The data were subjected to statistical analysis. Kruskal-Wallis, one-way ANOVA analysis and Mann-Whitney U, Wilcoxon matched-pairs signed-ranks tests were used to determine any significant change in surface roughness and hardness.

RESULTS

The results of the surface roughness and hardness measurements of the specimens before and after brushing with the selected fluoride and whitening dentifrices and bleaching regimen have been given in Tables 2 and 3. At the baseline values, all groups showed statistically similar Ra values ($p > 0.05$) and BHN values ($p > 0.05$) (Kruskal-Wallis test). However, a statistically significant increase was observed in the surface roughness ($p < 0.05$) of the five groups—the first one of which was treated with only water, the second and third of which were unbleached and the last two bleached—after brushing treatment with water without dentifrice G1 (control group, $p = 0.028$), fluoride abrasive dentifrice (G2, $p = 0.046$), whitening dentifrice (G3, $p = 0.012$), and bleached enamel surfaces treated with abrasive dentifrices brushing treatment (G4, $p = 0.012$; G5, $p = 0.012$) (Wilcoxon matched-pairs signed-ranks test). Besides, there was a significant difference between the other four groups and the control group except G2 ($p = 0.350$). The groups (G3, G4, G5) exposed to surface treatment presented a statistically significant increase in the surface roughness as compared to the control group (G1) ($p < 0.05$) (Mann-Whitney U test). As for hardness values, there was a decrease in the enamel surface hardness in G5 bleached with 10% carbamide peroxide gel (Opalescence) and then brushed with whitening dentifrice ($p = 0.025$). Yet, there were no statistically significant differences among the other treatment groups in enamel hardness ($p > 0.05$) (Wilcoxon matched-pairs signed-ranks test).

DISCUSSION

It has been known for a long time that toothbrushing has some undesirable effects on the hard tooth tissues.[5,7,14] But *in vitro* studies have demonstrated that a toothbrush alone has no clinically significant effects on hard tissues. When toothbrushes are used with toothpaste, measurable enamel loss occurs and this is primarily related to the abrasiveness of the dentifrice. The simple act of cleaning away dental deposits from teeth requires that the toothbrush-dentifrice combination possesses some level of abrasivity. The abrasivity of a modern dentifrice on enamel is such that after about 50,000 brush strokes an average layer about 0.5 μm enamel is removed. This suggests that toothbrushing with a dentifrice *per se* constitutes little risk to the integrity of the enamel.[11,12,14]

Among the factors that affect *in vitro* enamel by toothbrushing are the type of testing device, load, number of strokes, type of toothbrush and type of dentifrice.[11-13] In the present study, has been used a mean value of 600 gm for the evaluation of the abrasive effect of dentifrices. The movement of the brushing machine used in the study is similar to that of a horizontal or scrub-brushing, machine that reproduces the horizontal brushing procedure. The scrub method was described as a more effective brushing procedure for the removal of dental plaque,[15] so a brushing machine with horizontal movement was selected in this study.

Dentifrices are sometimes recommended for specific purposes, like cleaning or abrasion, in order to improve the bleaching process by removing superficial stains and polishing teeth. It is generally accepted that, *in vitro* tooth-brushing with abrasives can cause loss of dental hard tissue,

Table 2: Mean values and standard deviation of surface roughness (Ra) in μm before and after brushing

	G1			*G2*			*G3*			*G4*			*G5*		
	Control group brushed with water without dentifrice			*Colgate Total brushed with fluoride abrasive dentifrice*			*Natural White brushed with whitening dentifrice*			*Opalescence and Colgate Total bleaching regimen brushed with fluoride abrasive dentifrice*			*Opalescence and Natural White bleaching regimen brushed with whitening dentifrice*		
	Before brushing	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*
$\bar{X}$ ± SD	0.137 ± 0.18	0.245 ± 0.20		0.216 ± 0.05	0.479 ± 0.27		0.190 ± 0.40	0.599 ± 0.23		0.16 ± 0.30	0.466 ± 0.13		0.229 ± 0.02	0.456 ± 0.16	
Median	0.140	0.175	0.028	0.220	0.505	0.046	0.200	0.640	0.012	0.150	0.460	0.012	0.235	0.402	0.012
Min-max	0.11-0.16	0.15-0.74		0.15-0.30	0.17-0.84		0.12-0.26	0.26-0.87		0.13-0.20	0.34-0.60		0.20-0.25	0.31-0.70	

Table 3: Mean values of surface hardness (BHN) before and after brushing

	G1			*G2*			*G3*			*G4*			*G5*		
	Control group brushed with water without dentifrice			*Colgate Total brushed with fluoride abrasive dentifrice*			*Natural White brushed with whitening dentifrice*			*Opalescence and Colgate Total bleaching regimen brushed with fluoride abrasive dentifrice*			*Opalescence and Natural White bleaching regimen brushed with whitening dentifrice*		
	Before brushing	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*	*Before brushing*	*After brushing*	*p*
$\bar{X}$ ± SD	56.088 ± 3.58	52.813 ± 5.38		57.850 ± 3.39	56.012 ± 3.84		56.438 ± 3.15	55.513 ± 3.24		56.500 ± 5.36	55.000 ± 1.99		61.400 ± 3.04	56.800 ± 1.08	
Median	56.200	54.500	0.483	58.300	56.800	0.327	56.300	55.750	0.779	56.100	54.500	0.674	62.150	56.350	0.025
Min-max	52.20-60.50	43.50-58.30		54.80-63.80	50.90-60.40		52.70-62.60	50.90-60.50		49.50-64.10	52.70-58.30		56.50-64.80	55.20-58.30	

while little damage occurs with toothbrushing alone. It is encouraging to note that in Europe, over the last decade, there has been a general trend toward reduced toothpaste abrasivity without loss of cleaning efficacy. This may be mostly due to the increased use of high-performance abrasives, such as hydrated silica. The most common abrasives these days are hydrated silica, calcium carbonate, dicalcium phosphate dehydrate.[16] Addy et al[14] described a simple and reproducible method for comparing the abrasivity of toothpaste *in vitro*. Their measurements were expressed as mean loss in micrometers. However, their abrasiveness may be responsible for superficial tooth wear and other complicated factors, and abrasion may be more severe, when associated to bleaching treatment regimens.[17] Use of carbamide peroxide (10%) for prolonged periods (2-5 weeks) during the day or night is a bleaching technique recommended by dentists. According to clinical experiences and research, daily exposure to 10% carbamide peroxide is an apparently safe and effective procedure for whitening tooth hard tissues.[18-20] However, the exposure of tooth hard tissues to bleaching agents can result in microstructural changes in the enamel surface.[21-24] While alterations on enamel surface produced by bleaching oxidizing process roughened the surface, the loss of mineral content and organic matrix decreased enamel microhardness. The oxidative process involved in and low pH of tooth bleaching products have been considered as the main source of adverse effects on mineralized tissues during bleaching treatments. Low concentrations of carbamide peroxide promote varying degrees of surface porosity and structural change, depending on the bleaching agent. The use of abrasive-containing dentifrice might result in enamel roughness.[24-26] In this study, after all treatments, enamel surface presented increase in surface roughness, and the G5, G4, G3 roughness values were significantly different to G1. G3, G4 and G5, whose specimens differ statistically from G1, demonstrate that the brushing had an effect on the enamel surface roughness. Our results indicate that the fluoride abrasive dentifrice present in G2 was not able to prevent the increase in the surface roughness but the similar results were obtained brushed with water. The enamel surface roughness was increased after the surface cleaning treatment with whitening dentifrice in G3. Also G4 and G5 showed that the brushing had effect on bleached enamel surface roughness. Attin et al[6] have shown that the application of a fluoride solution cannot prevent, but may reduce, the loss of mineral from enamel during at home bleaching treatment with 10% carbamide peroxide. According to Neves et al[27] the appearances of enamel surfaces brushed with either a fluoridated or nonfluoridated dentifrice were similar. The same study showed that the control group, brushed with toothbrush and water, showed a smooth surface caused by the toothbrushing treatment. In spite of these results, the use of rational and daily fluoride therapy, especially mouth rinsing with fluoride and neutral solutions, associated with fluoride dentifrices without abrasives is an important method to prevent possible erosion caused on the enamel due to the use of superficial cleaning treatments after bleaching agents.[28]

Most studies have evaluated the microhardness of bleached enamel. Those studies have reported that the hardness of bleached enamel is similar to that of untreated enamel.[29-31] However, few studies showed that a 10% carbamide peroxide gel decreased enamel hardness.[4,32] This study reported no surface hardness alterations after bleaching procedures (G2, G3, G4) except that bleaching regimen combined with a brushing treatment with whitening dentifrice (G5). Because carbamide peroxide, an oxidizing agent, may cause superficial alterations and reduction in the calcium-phosphorus ratio.

CONCLUSION

After toothbrushing procedures, bleaching regimen and whitening dentifrice promoted increase in surface roughness on enamel. When the bleaching regimen combined with cleaning treatment was performed through brushing with whitening dentifrice, a significant decrease in hardness values was observed.

CLINICAL SIGNIFICANCE

As bleaching and cleaning treatments not only increased enamel surface roughness values but also decreased enamel surface hardness values in our study, dentists should take this into account in clinical procedures.

When applied together, bleaching and cleaning treatments may alter the enamel surface roughness and hardness values.

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