Micromorphology and surface roughness of sound and demineralized enamel and dentin bleached with a 10% carbamide peroxide bleaching agent

ROBERTA TARKANY BASTING, DDS, MS, SCD, PHD **ANTONIO LUIZ RODRIGUES JR.**, DDS, MS, SCD
& **MÔNICA CAMPOS SERRA**, DDS, MS, SCD

ABSTRACT: Purpose: To evaluate the micromorphology and surface roughness of sound and demineralized enamel and dentin treated with a 10% carbamide peroxide bleaching agent *in situ* for 3 weeks. **Methods:** A 10% carbamide peroxide bleaching agent, Opalescence (OPA) was evaluated and a placebo agent (PLA) not containing carbamide peroxide, was used as a control group. Forty dental slabs [10 sound enamel slabs (SE), 10 demineralized enamel slabs (DE), 10 sound dentin slabs (SD) and 10 demineralized dentin slabs (DD)] were randomly fixed on the facial surface of the first maxillary molars and second maxillary premolars of 10 volunteers. Demineralized enamel and dentin fragments were obtained by a dynamic model using demineralizing and remineralizing solutions. The volunteers were divided into two groups that received the bleaching or the placebo agent in different sequences and periods in a double blind 2 x 2 cross-over study with a wash-out period of 2 weeks. Roughness was performed on the slab surfaces followed by scanning electron microscope (SEM) evaluations. The images were evaluated by three calibrated examiners in duplicate in two different periods at x1500 (SE and DE) and x2000 original magnification (SD and DD). **Results:** The Wilcoxon test showed significant differences in roughness for SE and DE treated with OPA or PLA, with rougher surfaces treated with OPA. No differences in roughness were observed for SD or DD treated with OPA or PLA. As the Kappa value for the inter-examiner agreement of SEM evaluations showed "moderate" reproducibility ($0.41 < k < 0.60$) in Period 1 and "substantial" ($0.61 < k < 0.8$) in Period 2, the latter was considered for the statistical analysis. The McNemar test showed no significant differences between SE, DE, SD or DD treated with OPA or PLA. (*Am J Dent* 2007;20:97-102).

CLINICAL SIGNIFICANCE: In a roughness evaluation, enamel surfaces seemed to be more affected by bleaching agents than dentin. However, these changes were not reflected as micromorphology alterations.

✉: Prof. Dr. Roberta Tarkany Basting, Faculdade de Odontologia e Centro de Pesquisas Odontológicas São Leopoldo Mandic, Departamento de Odontologia Restauradora – Dentística, Rua José Rocha Junqueira, 13, Swift, Campinas, SP, CEP: 13045-755 Brazil. E-✉: rbasting@yahoo.com

Introduction

The bleaching technique for home-use has become one of the most accepted esthetic procedures for stained teeth due to its safety and cost-effectiveness. It provides the least risk for the greatest benefits when using 10% carbamide peroxide agents in a custom-fitted tray after a proper examination and diagnosis by a dentist.[1] Treatment time is generally 1 to 6 weeks, compared with a single in-office treatment, but has the advantage of causing less or no sensitivity.[1,2]

Due to the close contact of bleaching agents with the tooth structure, microscopic alterations of enamel and dentin surfaces have been reported by *in vitro* models.[3-10] However, indirect clinical evaluations by SEM, using replicas[11] or extracted teeth after bleaching treatment[12] show alterations that seem to cause no clinical harm to the tooth structure.

Changes in micromorphology were associated with an increase in surface roughness.[13] As surface roughness and electrostatic and/or hydrophobic interactions are factors related to bacterial adherence to the tooth surface,[14] an increase in enamel surface roughness *in vitro* after using carbamide peroxide at 10% was shown,[13] but no differences were shown[15] *in vivo* in the *S. mutans* counts during and after bleaching. Considering that it may not be entirely possible to extrapolate the results of *in vitro* studies to the clinical setting, *in situ* models have been useful to evaluate the effect of bleaching agents on the microhardness of dental structures.[16,17] However, there are no reports about micromorphology or roughness evaluations *in situ.*

This study evaluated the micromorphology and surface roughness of sound and demineralized enamel and dentin exposed *in situ* to a 10% carbamide peroxide bleaching agent or a placebo for 3 weeks.

Materials and Methods

Experimental design - Ten volunteers were randomly divided into two equal groups of five to take part in this double-blind experiment, performed in two periods of 3 weeks, with a wash-out period of 2 weeks, as described by Basting *et al*.[17] Each group received the bleaching or the placebo agent for 3 weeks in different sequences, in two distinct periods (bleaching agent – placebo agent; placebo agent – bleaching agent) in a cross-over 2 x 2 study design.[18] The factors under study were treatment agents: experimental (Opalescence[a]) and a control consisting of a non-carbamide peroxide containing placebo agent (pharmacy-mixed formula[b]). The experimental units consisted of 20 sound enamel slabs; 20 demineralized enamel slabs; 20 sound dentin slabs; and 20 demineralized dentin slabs. One fragment of each dental tissue was randomly distributed in complete blocks among 10 volunteers. Each volunteer was considered as a block. All of the volunteers were subjected to the treatment with the bleaching agent for 3 weeks and with the placebo agent for another period of 3 weeks. The quantitative response variable was surface roughness (micrometers) and the qualitative response variable was micromorphology (SEM scores).

Selection of volunteers and experimental periods - This study was approved by the FOP/UNICAMP Ethical Committee, in

agreement with the National Health Council in Brazil.[19] The volunteers were 10 adults (seven women and three men) from 20 to 22 years of age, who were candidates for at-home bleaching treatment.

Maxillary and mandibular dental arch impressions were taken with alginate (Jeltrate[c]) and stone cast molds were made. The maxillary casts were horseshoe shaped and without a palate to avoid interference with the efficiency of the vacuum pull on the hot thermoplastic sheet. In the molds, facial reservoirs with three coats of nail varnish were prepared on all teeth. On the maxillary first molars and maxillary second premolars (or, when the latter were missing, the maxillary first premolars), reservoirs were prepared with resin composite (Charisma[d]) corresponding to the dental slabs that would be fixed in the volunteers.

Two scalloped trays were manufactured for each volunteer, using a 0.4 mm thick flexible ethyl vinyl acetate (EVA) polymer[e] in a vacuum forming machine (P7[e]).

A 10% carbamide peroxide bleaching agent, Opalescence (OPA) was evaluated. The pH level of the bleaching agent was measured with a pH meter (SA 720[f]). The control group consisted of a placebo agent (PLA) prepared with carbopol 940 and glycerin. The color, taste, flavor, consistency and packaging of the placebo agent were similar to that of the bleaching agent, but the placebo had a neutral pH and no active component (carbamide peroxide). Table 1 presents the basic composition, and pH level of each treatment agent.

Preparation of enamel and dentin slabs - Thirteen non-erupted third molars were used. Immediately after extraction, the teeth were kept in 10% formaldehyde at pH 7.0. They were sectioned with double-faced diamond discs[g] with a low speed handpiece.[h] Only the cervical region was used to obtain 80 dental slabs (40 enamel slabs and 40 dentin slabs) measuring 4 mm x 4 mm x 2 mm.

The dental slabs were embedded, polished and steam sterilized, as described by Basting *et al.*[17] Artificial caries-like lesions were obtained by using a dynamic model of demineralization and remineralization cycles, similar to the model proposed by Featherstone *et al.*[20] Twenty enamel slabs were subjected to seven cycles of de-remineralization,[21] while the dentin slabs were submitted to three cycles of de-remineralization.[22] The 20 dental slabs of enamel and 20 of dentin, which made up the sound group of each dental tissue, were not subjected to the de-remineralization cycles but kept immersed in distilled and deionized water.

Experimental periods - Two weeks before the experiment began, toothbrushes (Oral B 35[i]), fluoride toothpastes, and instructions (Colgate MFP[j]) were given to the volunteers to standardize the toothbrushing method and the fluoride levels in the mouth. This period was called the "run-in" period and it lasted for 2 weeks.

The 10 volunteers were randomly divided into two equal groups of five. Group 1 received the bleaching treatment while Group 2 received the placebo treatment. In a second period, Group 1 received the placebo treatment while Group 2 received the bleaching treatment.

In the experimental Phase I, four dental slabs (one of sound enamel, one of demineralized enamel, one of sound dentin, and one of demineralized dentin) were randomly fixed to the vestibular surfaces of the maxillary first molars and maxillary second premolars (or, when the latter were missing, the maxillary first premolars) of each volunteer. The slabs were fixed using an adhesive system (Scotchbond MultiPurpose[k]) and a resin composite (Charisma). The bleaching or placebo treatment was applied in the maxillary dental arch of each volunteer during 3 weeks.

Table 1. Composition, pH, and entation form of each treatment agent.

Treatment	Composition	pH	Packaging
Opalescence	10% carbamide peroxide; bopol; glycerin; flavoring*	6.7	Dispensable syringe
Placebo	5% glycerin; 1.2% carbopol 940	7.0	Dispensable syringe with package identical to Opalescence

* The manufacturer does not indicate the percentage of each component.

Group 1 (five volunteers) were instructed to apply the bleaching agent while Group 2 (five volunteers) were instructed to apply the placebo agent (Group 2) in the tray and to wear it during the night for about 8 hours. They were instructed to clean the tray after removing it from the mouth and keep it in a container provided. They did not know if the product was the bleaching or the placebo agent.

After 3 weeks of the treatment with the bleaching or placebo agent (experimental Phase I), the slabs were removed with appropriate pliers. The composite resin that adhered to the volunteer's tooth was removed with resin polishing carbide burs[g] and aluminum oxide discs (Sof-Lex[k]). The volunteers were subjected to a wash-out period of 2 weeks to eliminate the residual effects of the treatment previously applied. The volunteers were given new toothbrushes and the fluoride toothpaste (the same toothpaste was used during run-in, experimental and wash-out periods); and the toothbrushing technique was reinforced. New trays were used to eliminate the possibility that residues left by the previously applied agent would interfere with the effects of the other agent to be used.

In the experimental Phase II, another four dental slabs, sound and demineralized enamel and sound and demineralized dentin, were fixed in the same way as described for experimental Phase I. This time the volunteers used the treatment agent (placebo or bleaching agent) that they had not received in the experimental Phase I, for another period of 3 weeks. The slabs were removed again with appropriate pliers.

During experimental Phases I and II, a syringe of bleaching or placebo agent was given to the volunteers weekly. They were also evaluated with regard to sensitivity and dental color changes related to the bleaching treatment.

Roughness measurements and scanning electron microscope (SEM) evaluations - The roughness measurements were randomly performed at the surface of the dental slabs with a profilometer (Surf Corder SE 1700[l]). To record roughness measurements, the needle moved at a constant speed of 0.05 mm/second. The cut-off value was set at 0.08 μm and the surface roughness was characterized by the arithmetical mean of the absolute values of the profile departures within the evaluation length (Ra), in micrometers (μm). Three tracings were made on each specimen at different locations. The mean roughness, taken after three measurements on each slab, was considered for statistical analysis.

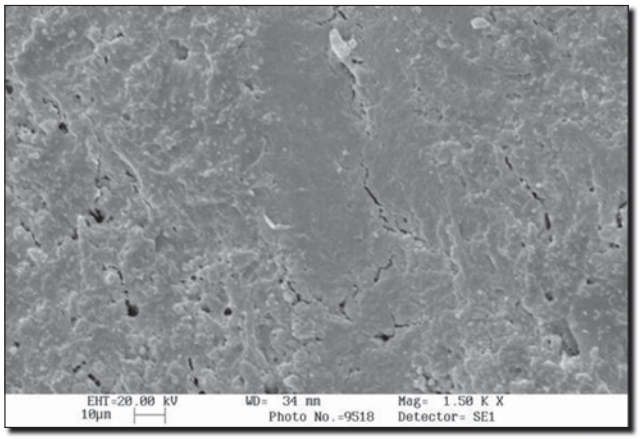

Fig. 1. Scanning electron microscopic image of sound enamel treated with OPA.

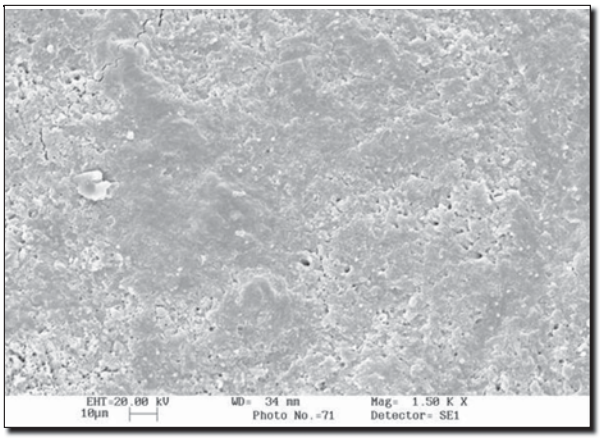

Fig. 2. Scanning electron microscopic image of demineralized enamel treated with PLA.

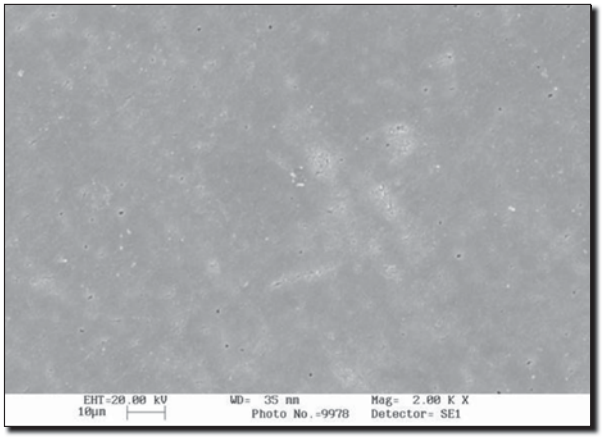

Fig. 3. Scanning electron microscopic image of sound dentin treated with OPA.

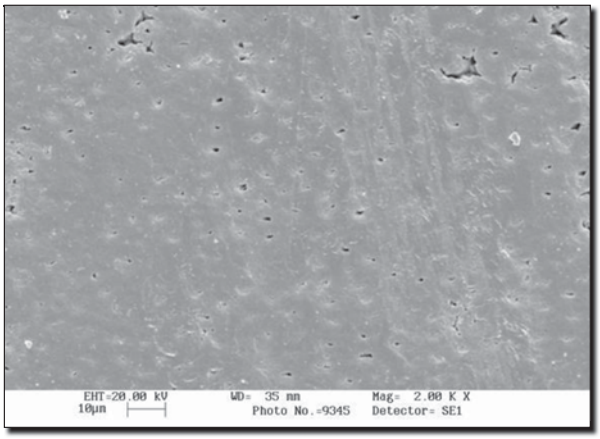

Fig. 4. Scanning electron microscopic image of demineralized dentin treated with PLA.

Table 2. Scores attributed to enamel and dentin during image observations by the examiners according to the evaluation criteria.

Substrate	Score	Criteria
Enamel	N	No porosities and/or erosions suggesting no changes in enamel surface
	Y	Presence of mild to severe porosities and/or erosions suggesting changes in enamel surface
Dentin	0	No image-like smear layer removal. No porosities and/or erosions. The dentin tubules are obliterated and could not be observed.
	1	Partial removal of image-like smear layer, with some dentin tubules opened. Mild changes in dentin surface.
	2	Total removal of image-like smear layer, with severe changes in dentin surface. Dentin tubules are opened.

For the SEM evaluations, enamel slabs were submitted to a gold sputtering process. Dentin slabs were dehydrated by a critical point drying process. Images were taken at original magnification of x1500 for enamel slabs (Figs. 1, 2) and x2000 for dentin (Figs. 3, 4). The images were recorded, coded and evaluated by three calibrated examiners (A, B and C) in duplicate, at two different times. The examiners had experience with SEM images, and received information about defects related to the SEM technique, which were not to be considered during evaluation. The images were projected on the computer monitor for 10 seconds and each examiner had 5 seconds to fill out the scoring form (Table 2).

Statistical analysis - For roughness measurements, the Wilcoxon non-parametric test was used to compare period and treatment agents. Enamel and dentin roughness were evaluated separately. The roughness of each sound and demineralized tissue treated with bleaching or placebo agents was compared. The significance level was 5%.

The statistical analysis for SEM evaluations considered the scores attributed by the three examiners independently. The Kappa estimator was used to assess intra and inter-examiner agreement. The most reliable examiner was selected as a result of the highest Kappa value and his score results were considered for evaluating the dental tissues (enamel or dentin, sound or demineralized), using the McNemar test.

Results

No statistical differences ($P< 0.05$) between first and second periods were found, irrespective of the dental tissue (enamel or dentin, sound or demineralized). Figures 5 and 6 show mean values of the surface roughness provided by bleaching or placebo applied on sound and demineralized enamel and dentin, at first and second periods.

There were statistically significant differences in roughness for sound enamel treated with OPA (0.135 µm) or PLA (0.082 µm) ($P< 0.05$). For demineralized enamel, statistical differences between OPA (0.425 µm) and PLA (0.330 µm) were also significant ($P< 0.05$). Sound dentin treated with OPA (0.099 µm) or PLA (0.069 µm) showed no differences in roughness

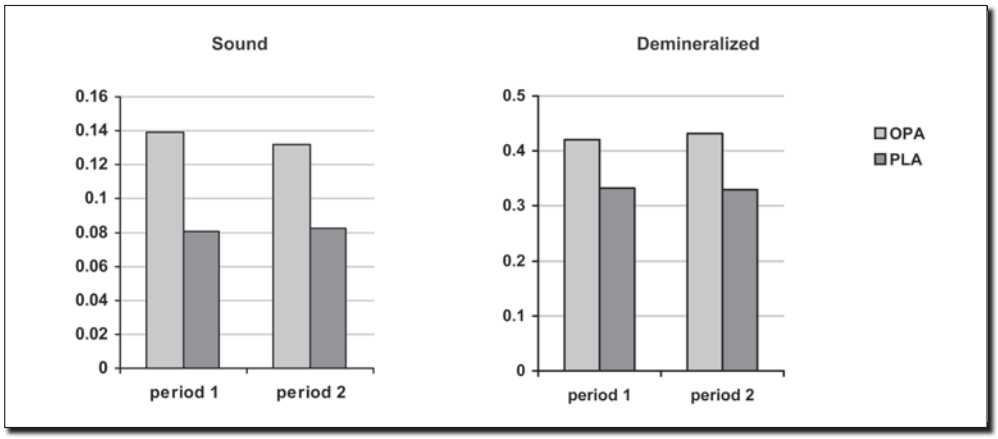

Fig. 5. Bar chart of mean values for surface roughness of sound and demineralized enamel treated with a bleaching or placebo agent in different periods.

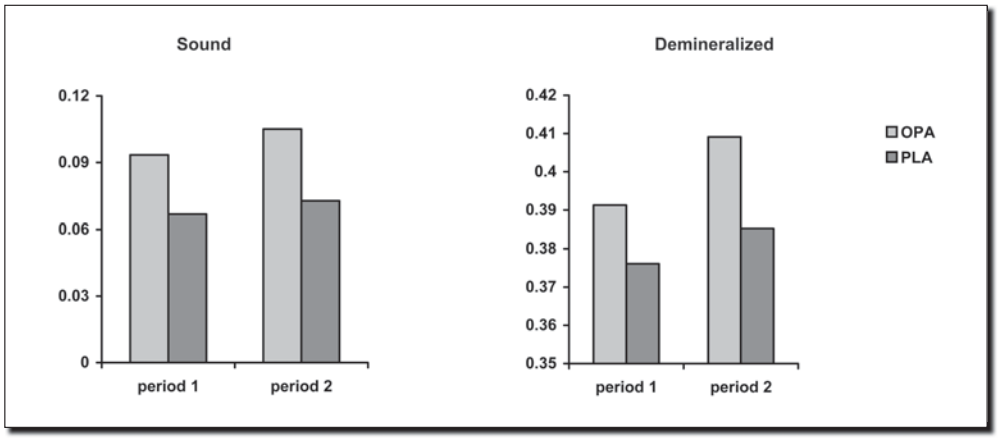

Fig. 6. Bar chart of mean values for surface roughness of sound and demineralized dentin treated with a bleaching or placebo agent in different periods.

Table 3. Mean roughness (Ra) in μm, standard deviation (SD) and median (M) for enamel and dentin according to the quality of dental slabs, treatment agents and periods.

Quality of the dental slabs	Treatment agents	Enamel						Dentin					
		Period 1			Period 2			Period 1			Period 2		
		Ra	SD	M	Ra	SD	M	Ra	SD	M	Ra	SD	M
Sound	OPA	0.139	0.009	0.141	0.083	0.007	0.082	0.091	0.008	0.094	0.072	0.009	0.068
	PLA	0.081	0.005	0.079	0.131	0.005	0.132	0.066	0.004	0.065	0.102	0.004	0.102
Deminera-lized	OPA	0.420	0.012	0.422	0.329	0.007	0.329	0.391	0.017	0.389	0.385	0.023	0.387
	PLA	0.331	0.009	0.335	0.431	0.009	0.437	0.376	0.027	0.369	0.409	0.013	0.409

(P> 0.05). No statistical differences in roughness were observed for demineralized dentin treated with OPA (0.400 μm) or PLA (0.380 μm) (P< 0.05).

With regard to the SEM evaluations, the intra-examiner concordance values (A, B and C) were 0.6977, 0.7500 and 0.7368 for enamel, and 0.6205, 0.6452 and 0.5863 for dentin, considered respectively "moderate" to "substantial", according to Landis & Koch.[23] Table 4 shows Kappa values for inter-examiner concordance for enamel and dentin at both evaluations. For the statistical analysis, the results of evaluator B in the second evaluation were selected, since the Kappa value that represented the agreement of the proposed scores as a response variable, was considered to be "substantial", according to Landis & Koch.[23] The McNemar test did not show statistically significant differences for sound (P= 0.375) or demineralized (P= 0.375) enamel treated with OPA or PLA. For dentin, no statistical differences were found between sound (P= 0.625) or demineralized dentin (P= 1.0000) exposed to OPA or PLA.

Table 4. Kappa values for inter-examiner concordance.

Period	Examiners	Enamel			Dentin		
		A	B	C	A	B	C
1	A	--	0.5908	0.5181	--	0.3951	0.3195
	B		--	0.5181		--	0.6940
	C			--			--
2	A	--	0.7500	0.5545	--	0.6800	0.6341
	B		--	0.7000		--	0.6603
	C			--			--

Discussion

Scanning electron microscopic analysis and roughness measurements have been used to evaluate the effects of bleaching agents on dental structures.[3-11,13] However, these evalu-

ations were taken under *in vitro* conditions, in which exposure doses and times are artificially exaggerated leading to results that have limited clinical relevance. Even in studies where great care is taken to simulate intraoral conditions accurately, replication of the complex *in vivo* chemistry and kinetics is difficult. Therefore, this study used an *in situ* model, using a 10% carbamide peroxide agent, the concentration most used in the home-use bleaching technique, to provide substantial information about the effects on dental structures.

In vitro studies have demonstrated that a 10% carbamide peroxide bleaching agent causes micromorphological changes in enamel and dentin, such as the presence of pores, erosions and increased roughness.[3-11,13] Clinically, the increase in pore diameters allows the bleaching product to penetrate easily through enamel and dentin and to break down the pigmented macromolecules into smaller and whiter molecules, but it also causes transient sensitivity during use.[24]

Morphological changes observed in indirect clinical evaluations[11,12] have been attributed to carbamide peroxide degradation by-products, mainly urea and oxygen. Urea has the ability to denature proteins in the organic content of dental structures, with the potential to penetrate through enamel and affect the prismatic and inter-prismatic structures,[25,26] contributing to the permeability increase and microstructural changes. Hegedus *et al*[27] showed that free oxygen also increases surface porosity, with greater effects on dentin. Oxygen-free radicals are non-specific and react with the organic content of dental tissues, through which their passage is easier than through the mineralized structure where the stained molecules are located.

In this *in situ* investigation, the differences between placebo and bleaching agents were observed only for sound or demineralized enamel roughness. Considering the lower inorganic content of dentin, it seems possible that intraoral challenges, like erosion and abrasion, masked the effect of the bleaching agent.

The design and experimental strategies used in this study did not find differences in the micromorphology of sound or demineralized enamel and dentin surfaces exposed to a 10% carbamide peroxide or a placebo agent, evaluated by SEM. Despite this, it could be assumed that both products cause some changes in dental tissues. This may be due to carbopol (one of the products used to prepare the placebo agent), which causes changes in enamel and dentin microhardness and changes in enamel mineral content.[28,29]

Bacterial adherence to the dental structure is dependent on the properties of the surface that will adsorb it. Surface roughness, electrostatic and hydrophobic interactions, as well as sucrose-dependent mechanisms may facilitate plaque retention.[14] A 10% carbamide peroxide agent seems to decrease the adherence of *S. mutans, S. sobrinus* and *A. viscosus in vitro*.[14] *In vivo*, there are reports on the decrease of *Lactobacillus*[30] or no changes in *mutans Streptococcus* counts.[15] Although profilometric traces may be useful for evaluating the surface irregularities of enamel and dentin, it is questionable whether texture favors bacterial adherence, and if it does, how this occurs.[31]

Although the surface roughness of sound and demineralized enamel were compromised after the use of a 10% carbamide peroxide bleaching agent, these changes may be reversible. If they were not perceptible by SEM, it seems very likely that surface alterations would not be perceptible clinically. It is possible that the presence of saliva, frequent use of fluoride toothpaste and plaque control would interact with the surface of sound or demineralized dental tissue during and after exposure to bleaching agents.

a. Ultradent, Provo, UT, USA.
b. Proderma, Piracicaba, Brazil.
c. Dentsply, Milford, DE, USA.
d. Hereaus Kulzer, Wehrheim, Germany.
e. Bio-Art Equip. Odontologicos Ltda., São Carlos, Brazil.
f. Procyon, São Paolo, Brazil.
g. K.G. Sorensen, Barueri, Brazil.
h. Kavo do Brazil, Joinville, Brazil.
i. Gillette do Brazil, Manaus, Brazil.
j. Kolynos do Brazil, Osasco, Brazil.
k. 3M/ESPE, St. Paul, MN, USA.
l. Kozaka Corp., Tokyo, Japan.

Acknowledgments: To Prof. Dr. Elliot Kitajima for the use of the scanning electron microscope and other equipment at the Luiz de Queiroz Agricultural College, University of São Paulo, Piracicaba, Brazil, and to Patrícia Moreira de Freitas, José Augusto Rodrigues and Ana Cecília Aranha for the SEM image evaluations. This study was partially granted by the Foundation for Research Support of the state of São Paulo (FAPESP) - grants: 98/14425-1, 01/08774-8 and 03/02150-8 and by the National Council for Scientific and Technological Development (CNPq) - grant 500828/2003-2.

Dr. Basting is Professor, Department of Restorative Dentistry, School of Dentistry and Research Center São Leopoldo Mandic, Campinas, SP, Brazil. Dr. Rodrigues is Professor, Department of Social Medicine, School of Medicine of Ribeirão Preto (FAEPA-HCRP), University of São Paulo (USP), Ribeirão Preto, SP, Brazil. Dr. Serra is Professor, Department of Restorative Dentistry, School of Dentistry of Ribeirão Preto (FORP), University of São Paulo (USP), Ribeirão Preto, SP, Brazil.

References

1. Haywood VB. New bleaching considerations compared with at-home bleaching. *J Esthet Restor Dent* 2003, 15: 184-187.
2. Matis BA, Moura HN, Cochran MA, Eckert GJ. Clinical evaluation of bleaching agents of different concentrations. *Quintessence Int* 2000; 31: 303-310.
3. Ben-Amar A, Liberman R, Gorfil C, Bernstein Y. Effect of mouthguard bleaching on enamel surface. *Am J Dent* 1995; 8: 29-32.
4. Bitter NC, Sanders JL. The effect of four bleaching agents on enamel surface: A scanning electron microscopic study. *Quintessence Int* 1993; 24: 817-824.
5. Ernst CP, Marroquin BB, Willershausen-Zönnchen B. Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel. *Quintessence Int* 1996; 27: 53-56.
6. Flaitz CM, Hicks MJ. Effects of carbamide peroxide whitening agents on enamel surfaces and caries-like lesion formation: An SEM and polarized light microscopic *in vitro* study. *J Dent Child* 1996; 63: 249-256.
7. Josey AL, Meyers IA, Romaniuk K, Symons AL. The effect of a vital bleaching technique on enamel surface morphology and the bonding of composite resin to enamel *J Oral Rehabil* 1996; 23: 244-250.
8. Shannon H, Spencer P, Gross K, Tira D. Characterization of enamel exposed to 10% carbamide peroxide bleaching agents. *Quintessence Int* 1993; 24: 39-44.
9. Smidt A, Weller D Roman I, Gedalia I. Effect of bleaching agents on microhardness and surface morphology of tooth enamel. *Am J Dent* 1998; 11: 83-85.
10. Zalkind M, Arwaz JR, Goldman A, Rotstein I. Surface morphology changes in human enamel, dentin and cementum following bleaching: A scanning electron microscopy study. *Endod Dent Traumatol* 1996; 12: 82-88.
11. Türkun M, Sevgican F, Pehlivan Y, Aktener BO. Effects of 10% carbamide peroxide on enamel surface morphology: A scanning electron microscopy study. *J Esthet Restor Dent* 2002; 14:238-244.
12. Bitter NC. A scanning electron microscope study of the long-term effect of bleaching agents on the enamel surface *in vivo*. *Gen Dent* 1998; 46: 84-88.
13. McGuckin RS, Babin JF, Meyer BJ. Alterations in human enamel surface morphology following vital bleaching. *J Prosthet Dent* 1992; 68:754-760.

14. Steinberg D, Mor C, Dogan H, Zacks B, Rotstein I. Effect of salivary biofilm on the adherence of oral bacteria to bleached and non-bleached restorative material *Dent Mater* 1999; 15: 14-20.
15. Alkmin YT, Sartorelli R, Flório FM, Basting RT. Comparative study of the effects of two bleaching agents on oral microbiota. *Oper Dent* 2005; 30: 417-423.
16. Araújo Jr EM, Baratieri LN, Vieira LCC, Ritter AV. *In situ* effect of 10% carbamide peroxide on microhardness of human enamel: Function of time. *J Esthet Restor Dent* 2003; 15: 166-174.
17. Basting RT, Serra MC, Rodrigues Jr AL. The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *in situ*. *Oper Dent* 2001; 26: 531-539.
18. Montgomery D. *Design and analysis of experiments*. New York: John Wiley & Sons, 1991; 688.
19. Brazil National Health Council. *Resolution no 196*. Brasília: 1996 (In Portuguese).
20. Featherstone JDB, O'Really MM, Shariati M, Brugler S. Enhancement of remineralization *in vitro* and *in vivo*. In: Leach SA. *Factors relating to demineralization and remineralization of the teeth.* Oxford: IRL, 1986; 23-34.
21. Serra MC, Cury JA. The *in vitro* effect of glass-ionomer cement restorations on enamel subjected to a demineralization and remineralization model. *Quintessence Int* 1992; 23: 143-147.
22. Hara AT, Magalhães CS, Rodrigues Jr AL, Serra MC. Cariostatic effect of adhesive restorations on root surfaces: *In vitro* study. *Pesq Odontol Bras* 2000; 14: 113-118 (In Portuguese).
23. Landis J, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159-174.
24. Leonard Jr RH, Bentley C, Eagle JC, Garland GE, Knight MC, Phillips C. Nightguard vital bleaching: A long-term study on its efficacy, shade retention, side effects, and patients' perceptions. *J Esthet Restor Dent* 2001; 13: 357-369.
25. Arends J, Jongebloed WL, Goldberg M, Schuthof J. Interaction of urea and human enamel. *Caries Res* 1984; 18: 17-24.
26. Goldberg M, Arends J, Jongebloed WL, Schuthof J, Septier D. Action of urea solution on human enamel surfaces. *Caries Res* 1983; 17: 106-112.
27. Hegedüs C, Bistey T, Nagy F, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent* 1999; 27: 509-515.
28. Basting RT, Rodrigues Jr AL, Serra MC. The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time. *J Am Dent Assoc* 2003; 134: 1335-1342.
29. Freitas PM, Basting RT, Rodrigues Jr AL, Serra MC. Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals. *Quintessence Int* 2002; 33: 370-375.
30. Bentley CD, Leonard RH, Crawford JJ. Effect of whitening agents containing carbamide peroxide on cariogenic bacteria. *J Esthet Restor Dent* 2000; 12: 33-37.
31. Turssi CP, Faraoni JJ, Rodrigues Jr AL, Serra MC. An *in situ* investigation into the abrasion of eroded dental hard tissues by a whitening dentifrice. *Caries Res* 2004; 38: 473-477.