©Operative Dentistry, 2010, **35-2,** 139-146

Clinical Research

In Situ Study of In-office Bleaching Procedures Using Light Sources on Human Enamel Microhardness

FO Araujo • LN Baratieri • É Araújo

Clinical Relevance

Regardless of the light sources used, the microhardness of human dental enamel did not present significant changes 14 days after in-office bleaching.

SUMMARY

Objective: To evaluate the influence of various light sources on the microhardness of human dental enamel following treatment with an in-office vital bleaching agent (35% hydrogen peroxide). Methods and Materials: One-hundred and sixty dental enamel slabs (~ 2.5 x 2.5 x 1.0 mm) were obtained from 32 recently extracted human third molars, polished and subjected to hardness testing (KHN, 50g-load, 5 seconds) after four time periods (baseline and after 1, 7 and 14 days). The specimens were placed in intraoral appliances and delivered to eight volunteers after being equally divided into five groups each according to the light source treatment to be performed extraorally (n=32): Group LA (35% hydrogen peroxide + argon laser unit); Group HA (35% hydrogen peroxide + halogen light-curing unit); Group LED (35% hydrogen peroxide + LED-laser unit); Group OX (35% hydrogen peroxide + no light source unit); or Group CO (control: saliva only). Microhardness values were analyzed by ANOVA and Tukey's post-hoc test (α=0.05). Results: Significant decreases in KHN were found in enamel for the HA group one day and seven days after treatment (5.81% and 2.35%, respectively) (p<0.0001). However, no significant differences were found between the baseline and final microhardness values for all groups submitted to bleaching. Conclusion: The different tested light sources did not significantly influence the micro-

*Fabiano de Oliveira Araujo, DDS, MS, PhD, graduate student, Operative Dentistry, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil; professor, Operative Dentistry, University Tuiuti of Paraná, Curitiba, Paraná, Brazil

Luiz Narciso Baratieri, DDS, MS, PhD, professor and chair, Operative Dentistry, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

Élito Araújo, DDS, MS, PhD, full-time professor, Dental Clinics, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

*Reprint request: Rua Vereador Antonio dos Reis Cavalheiro, 315, Cabral, Curitiba-PR, Brazil 80.035-210; e-mail: fabianoaraujo_@hotmail.com

DOI: 10.2341/08-033-C

hardness of human enamel following treatment with 35% hydrogen peroxide.

INTRODUCTION

The main goal of tooth bleaching is to whiten tooth tissues using oxidizing chemical agents that act both in enamel and dentin.[1] Because tooth shade depends on the composition of tooth tissues, severe chemical, mechanical and biological changes can damage the esthetic equilibrium of the smile.[2]

Bleaching tooth structure is possible due to the non-invasive nature of the bleaching systems that offer treatment options capable of fulfilling expectations of the most demanding patients. However, it is paramount that clinicians have an adequate knowledge of the methodologies and implications of their procedures in order to select and use the best products and technology available.[3-4]

Bleaching agents that use hydrogen peroxide and were specifically developed for enamel and dentin are considered to be the most effective, versatile and popular, since they are available in a range of formulations, concentrations and activation modes.[1]

There is no consensus regarding *in vitro* changes produced on enamel surfaces from bleaching treatments.[5-8] However, there are concerns about the direct contact of bleaching products on tooth surfaces because of the duration and activation mode when using light-sources for activation. A number of authors claim that the application of light-sensitive, high-concentration bleaching agents associated with a power-unit can be made in a single appointment to reduce the time required to achieve the expected results.[9-10] Basically, the bleaching material takes up energy from the light-source, which has the potential to heat or accelerate the breakdown of peroxide molecules, increasing the peroxide decomposition rate and improving the bleaching result.[11] *In situ* studies are required to evaluate the interaction between the association of light-sources, bleaching agents, saliva, soft tissues and tooth structure.[12-15]

This *in situ* study evaluated the influence of different light activation sources and 35% hydrogen peroxide on the microhardness of human enamel subjected to in-office dental bleaching.

METHODS AND MATERIALS

Eight medically healthy 19- to 45-year-old patients without periodontal conditions, who presented four sound, erupted third molars with an indication for extraction, were selected after patient approval of the Research Protocol and the Informed Consent Form that had been previously approved by the Committee of Ethics on Human Subjects Research at the university. All the extracted teeth were stored for up to two months in 0.2% thymol aqueous solution.

The patients were informed regarding the nature and aim of this study and that they would wear intraoral appliances containing enamel fragments obtained only from their own teeth. A written consent form was obtained from all participants prior to treatment.

The methods are schematically presented in Figure 1. After extraction, the teeth were cleaned with periodontal curettes. Visual inspection with 4x magnifying glasses (Bio-Art Equipamento Odontológico, São Carlos, SP, Brazil) was performed to exclude teeth with occlusal alterations or caries. The proximal and free enamel surfaces were ground using 1000 grit SiC paper disks (T467, Norton, São Paulo, SP, Brazil) mounted on a water-cooled wheel (DP-10/Panambra Struers, Panambra, Denmark) to obtain flattened surfaces. To standardize positioning of the teeth on a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA), the tooth roots were placed in PVC circular matrixes (2.5 x 2 cm) filled with self-curing acrylic resin. The teeth were sectioned longitudinally using a

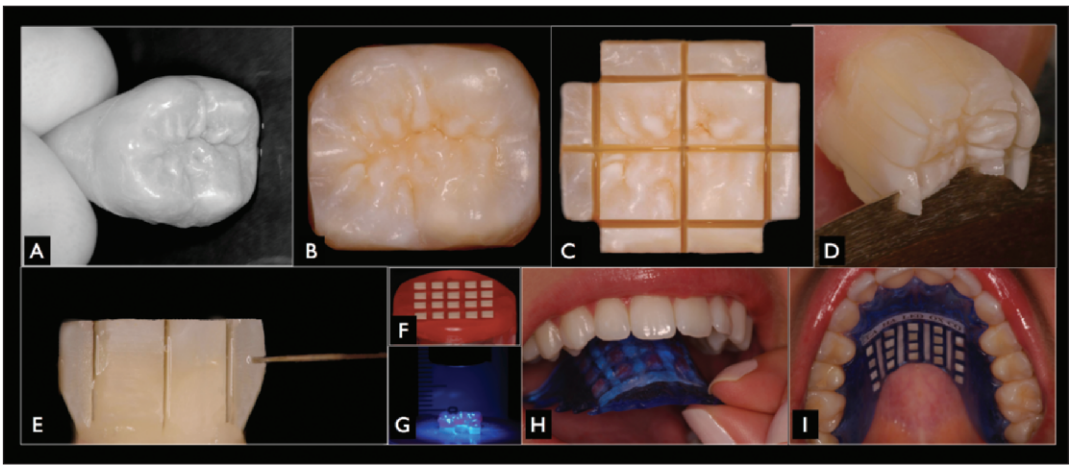

Figure 1. *Schematic representation of the steps performed. The proximal and free surfaces of enamel were ground using 1000 SiC paper disks (A) to obtain flat surfaces (B). Then, the specimens were serially sectioned to obtain 2.5 x 2.5 x 1.0 mm enamel slabs (C, D, E). The slabs were identified by numbers and leveled on a wax sheet (F), finished (1000 and 1200 SiC paper disks) and polished (1 µm, .3 µm and .05 µm alumina pastes). Baseline Knoop hardness measurements were taken (Shimadzu HMV/2000, Shimadzu Corporation, Kyoto, Japan) after application of the in-office bleaching agent and light-source (G) (except as noted in the research). The patients were instructed to wear the appliances continuously, except during meals (H, I), and microhardness measurements were taken after 1, 7 and 14 days (3 indentations per specimen, 50g -load, for 5 seconds).*

cutting machine (Isomet 1000, Buehler Ltd) in a parallel direction to the flattened proximal and free surfaces using a diamond blade (Diamond Wheel 012" x fine, South Bay Technology Inc, California, USA) under water cooling at 400 rpm, resulting in 1 mm enamel sections. To obtain the enamel slabs needed for the current study, a bucco-lingual cut was performed through the middle of the occlusal face. After turning the specimen 90°, another cut was made in the mesio-distal direction. Two final cuts were performed: the first as close as possible to the occlusal face; the second was 2.5 mm cervically from the first cut. Five enamel slabs from each of the 32 teeth were used in this study, resulting in 160 enamel slabs with dimensions of 2.5 x 2.5 x 1.0 mm. After ultrasonic cleaning (Ultrasonic Cleaner 1440D/Odontobrás, Ribeirão Preto-SP, Brazil) with distilled water for two minutes to remove excess debris, the slabs were identified by numbers and placed on a wax sheet with the flattened surfaces taller than the wax. Identification ensured that the patients were provided with slabs obtained only from their own teeth. To eliminate variations during finishing/polishing, the slabs were progressively finished with moist silicon carbide sandpaper with grits of 1000 (SH4, 3M ESPE, São Paulo, SP, Brazil) and 1200 (T469, Norton, São Paulo, SP, Brazil), mounted on a water-cooled wheel (DP-10/Panambra Struers, Panambra, Denmark) with 10 passes for each grit. The finishing was standardized by rotating the slabs 90° after each grit. A special device fixed on the base of the water-cooled wheel permitted simultaneous finishing of all slabs, ensuring that all specimens were prepared at the same time and under the same conditions. The slabs were cleaned in an ultrasonic cleaner for five minutes. The slabs were polished with 1 µm, .3 µm and .05 µm alumina pastes (Alumina Slurry/Water Base, Electron Microscopy Sciences, Fort Washington, PA, USA) and cleaned again in an ultrasonic cleaner for five minutes. Immediately after polishing, the slabs were stored in plastic boxes with deionized water at 37°C for 24 hours.

Stone casts were obtained from the volunteers and intraoral appliances were fabricated on the upper arch, with five groupings defined on the appliances. Each grouping had four slots where the enamel slabs were placed. Before the bleaching procedures, initial Knoop indentations (100g-load for 5 seconds) were performed. The baseline Knoop Hardness measurements were taken (Shimadzu HMV/2000, Shimadzu Corporation, Kyoto, Japan) with three indentations per specimen using a 50g-load for five seconds and a 100 µm distance between each indentation. This method used visualization of the surface indentations through the microscope of the testing machine. A mean value of microhardness was calculated for each slab.

The specimens were assigned to five groups according to the following bleaching protocols (n=32): Group LA, 35% hydrogen peroxide + argon laser unit (Accu Cure 3000, LaserMed, Salt Lake City, UT, USA); Group HA, 35% hydrogen peroxide + halogen light-curing unit (Optilux 501, Demetron Research Corp, Danbury, CT, USA); Group LED, 35% hydrogen peroxide + LED-laser unit (Whitening Lase II, DMC Equipamentos Ltda, São Carlos, SP, Brazil); Group OX, 35% hydrogen peroxide + no light source unit; Group CO (Control: saliva only *in situ*). The materials and equipment used in this research, along with the group parameters, are listed in Table 1.

The slabs were placed in acrylic cylinders covered by a wax sheet. After the application of 35% hydrogen peroxide in-office bleaching agent (Opalescence Xtra,

Table 1: *The Materials and Equipment Used in This Research, Along with the Group Distributions*

Material Equipment	**Group**	**(Name, Manufacturer)**	**Composition and Features**
Bleaching agent (35% hydrogen peroxide gel)	LA, HA, LED, OX[1]	Opalescence Xtra, Ultradent, South Jordan, UT, USA	35% hydrogen peroxide gel. Application time: 30 minutes
Light-source[2]	LA	Accu Cure 3000, LaserMed, Salt Lake City, UT, USA	Argon laser with concentrated light beam. Wavelength: 488 nm, light output: 200mW/cm^2. Application time: 90 seconds (2x)
	HA	Optilux 501, Demetron Research Corp, Danbury, CT, USA	QTH light-curing unit. Light output: 700mW/cm^2. Application time: 90 seconds (2x)
	LED	Whitening Lase II, DMC Equipamentos Ltda, São Carlos, SP, Brazil	Hybrid LED/Laser light-source. This presents two different wavelengths (one is produced by six high-intensity LEDs–470 nm; and another generated by three infrared laser diodes–800 nm and 100mW/cm^2 light output). Application time: 90 seconds (2x).
	CO[3]	-	-

[1] *OX Group: no light-source was applied.*
[2] *Application time: 90 seconds (2x).*
[3] *CO Group (Control): No light-source or bleaching agent was applied.*

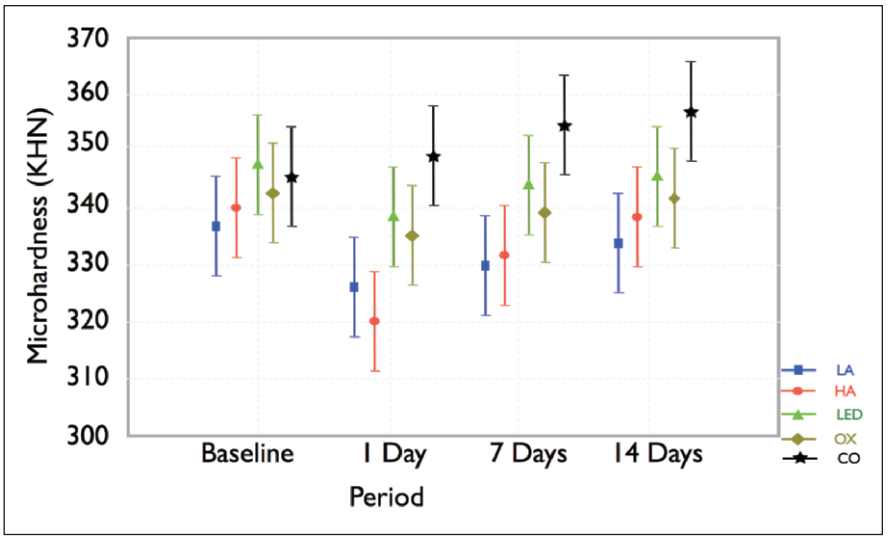

Figure 2. *Period X Group interaction: mean microhardness values (no significant differences were found between groups: p=.005131; confidence level: 95%).*

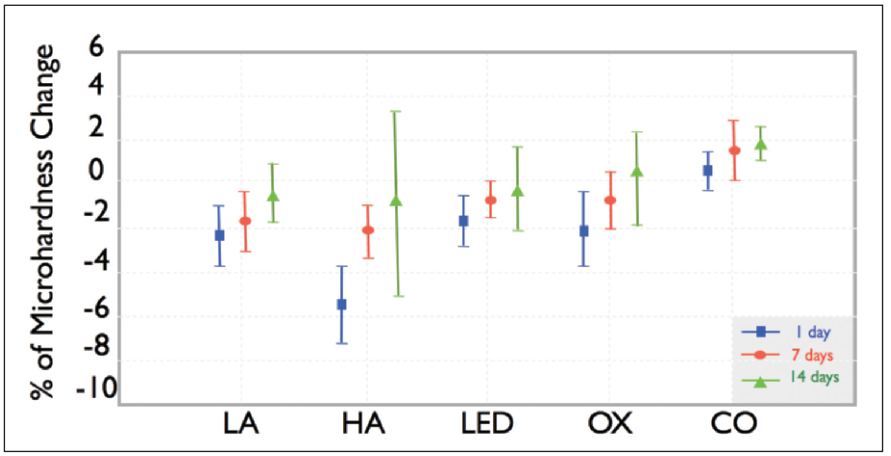

Figure 3. *Mean percent of microhardness change %SMC (95% confidence level), by group and evaluation periods.*

Ultradent, South Jordan, UT, USA) to the polished surfaces of the slabs in a 1 mm thick layer, one minute passed to allow for penetration of the bleaching agent into the dental structure. A special appliance was used to keep a 10 mm uniform distance between the light tip and the enamel surface. The specific light source for the appropriate group was then applied for 90 seconds, with five minutes without light activation, and the light source was reapplied for an additional 90 seconds, for a total of nine minutes from the start of the procedure. The enamel surfaces were rinsed with oil-free air/water spray for one minute, gently dried and the bleaching technique was repeated twice. With this procedure, the enamel slabs were submitted to 30 minutes of bleaching. The application time of the bleaching gel was similar in all groups, including the group that did not receive light-activation. The specimens of the CO group were not bleached or light-activated. Immediately after the bleaching session, the specimens were attached to an intraoral appliance using sticky wax. The polished surfaces were kept free and level with the surface of the appliance to prevent patient discomfort.[14]

The patients were instructed to wear their intraoral appliance continuously, except during meals. The enamel slabs were removed, tested and immediately reattached after 1, 7 and 14 days to perform microhardness measurements (three indentations per specimen, 50g - load, for five seconds). The results were subjected to the one-way ANOVA and Tukey's multiple comparison tests (α=0.05).

RESULTS

The results of this study are shown in Figures 2 and 3 and Tables 2 and 3.

A complementary analysis of the percent of change in microhardness was performed to evaluate whether the light-source significantly influenced microhardness. The values were converted to Percent Change of Surface Microhardness, following the formula: %SMC = ([MI – MF]/MI]) x 100, where MI = baseline microhardness and MF = final microhardness. Positive values indicated that the microhardness increased; negative values indicated that the final microhardness was lower than the baseline value.

DISCUSSION

Hardness can be defined as resistance against permanent deformation of the surface of a material or tissue subjected to penetration, involving complex stresses. Considering that the hard tooth tissues are subjected to constant pressure during function, hardness is a relevant property, since wear resistance is proportional to tissue hardness.[16] Although the microhardness test does not provide specific information on changes that occur within a substance or material, it is commonly used to detect changes in tooth tissues after experiments involving demineralization and remineralization; it is considered a suitable tool for investigating the softening of enamel surfaces.[17-19]

Notwithstanding, the in-office bleaching technique is considered to be a fast procedure to improve smile esthetics.[13] There are several questions about the potential effects resulting from the interaction between hydrogen peroxide-based products and tooth surfaces,

due to the complex physicochemical reaction involved in the bleaching process.[20]

It is reasonable to expect that baseline microhardness values range from tooth to tooth. This is possibly explained due to the mechanical properties that change with aging and the medical conditions of the subjects, as well as other conditions, such as exposure to fluoride and cariogenic challenges that occur in the oral cavity.[21] Additionally, the consistent, standardized preparation of specimens is laborious and time-consuming due to their small dimensions. Unfortunately, it is not possible to test the enamel surface without changing its morphology and microstructure, because microhardness testing requires a smooth, plane surface. The sum of these factors may be considered responsible for the variation in the obtained values.[22] Other possible reasons for the variation in the baseline values are mineralization, the orientation and density of enamel prisms, the moisture of the specimens, changes in mineral structure and the variable configuration of enamel crystals,[23] as well as different methodologies.[24]

Products based on the same active principle as that used in the current research (35% hydrogen peroxide) can present different formulations, pH and additives (thickener, fluoride, desensitizing agents). For this reason, it is not appropriate to compare the results obtained between studies that use products with the same concentration but from different manufacturers.[25] Therefore, only one bleaching gel was used in the current study.

To reduce the time of in-office bleaching to a single appointment, there is a trend towards using highly concentrated (35% to 50%) hydrogen peroxide with a high power light-source, such as a QTH lamp, argon laser or diode lasers

Table 2: *Mean Knoop Microhardness Values (KHN) and Results of Tukey's Multiple Comparison Test, According to the Group and Periods of Evaluation*

Group	Period	N	Mean ± SD	Tukey
LA	Baseline	32	336.6354 ± 15.45124	A
	1 day		326.7188 ± 14.33574	B
	7 days		330.2917 ± 13.20387	B
	14 days		334.8750 ± 14.46260	A
HA	Baseline	32	340.2188 ± 17.77107	A
	1 day		320.1771 ± 16.58474	B
	7 days		332.0104 ± 15.47237	B
	14 days		337.1354 ± 19.54644	A
LED	Baseline	32	346.1771 ± 27.66453	A
	1 day		338.3333 ± 27.94593	B
	7 days		342.2188 ± 27.88579	AB
	14 days		344.1771 ± 27.38181	A
OX	Baseline	32	342.2188 ± 25.46550	A
	1 day		334.0833 ± 25.56025	B
	7 days		339.2917 ± 25.52653	A
	14 days		341.9688 ± 24.32978	A
CO	Baseline	32	343.7917 ± 24.24897	A
	1 day		347.6771 ± 23.74876	A
	7 days		353.0417 ± 23.24280	B
	14 days		356.7188 ± 24.58561	B

Similar letters within groups indicate statistical similarity $p<0.05$).

Table 3: *Percent Surface Microhardness Change (%SMC) and Results of Tukey's Multiple Comparison for Means after 1, 7 and 14 days (5% significance level, same letter indicates similar behavior) According to the Periods and Groups*

Period	Group	N	Mean	SD	Tukey
1 day	LA	32	-2.9203	1.8070	B
	HA	32	-5.812	3.9477	A
	LED	32	-2.281	0.9388	B
	OX	32	-2.378	1.5126	B
	CO	32	1.149	0.9343	C
	Total	160	-2.449	3.0652	–
7 days	LA	32	-1.841	1.9229	AB
	HA	32	-2.3523	2.6746	A
	LED	32	-1.1523	0.7816	B
	OX	32	-0.853	1.3082	AB
	CO	32	2.7365	1.6764	C
	Total	160	-0.6924	2.5200	–
14 days	LA	32	-0.481	2.4092	A
	HA	32	-0.9117	2.2587	A
	LED	32	-0.5736	0.6721	A
	OX	32	-0.0354	1.7633	A
	CO	32	3.7786	1.2278	B
	Total	160	0.3554	2.4781	–

Similar letters within groups indicate statistical similarity $p<0.05$).

associated with light-emitting diodes (LEDs).[1,3,10,26-29] This in-office dental bleaching with light activation provides reduced treatment time.[9-10] Conversely, it has been suggested that the effect of a single in-office application of hydrogen peroxide gel is virtually imperceptible, raising doubts about the efficacy of several commercially available systems.[28,30-31]

The three light-curing units used in the current study accelerate the bleaching process by increasing the release of oxygen—the ion responsible for the bleaching effect.[9,32-33] Laser and high-output LEDs have been used for in-office bleaching,[34] despite discouraging results obtained by some studies.[10,35] These light-curing units emit photons—non-ionizing concentrated radiation that is quickly absorbed when contacting tissues and bleaching agents. The bleaching gel used in the current research (Opalescence Xtra) contains beta-carotene, an orange hydrosoluble organic dye capable of absorbing blue light.[29,36] Therefore, the light-sources are not responsible for tooth bleaching. Essentially, their function seems to activate the light-sensitive gel, whether by heat or light.[37-38]

The use of an intraoral appliance allowed for direct contact of the specimens with saliva, which plays a relevant role in the remineralization of bleached enamel.[39] The *in vitro* and *in situ* comparisons of the effect of 10% carbamide peroxide on human enamel based on microhardness, SEM and calcium ion release analyses have shown that the final microhardness was similar to the baseline values for the *in situ* groups and higher for the *in vitro* groups.[14] SEM analysis revealed *in vitro* changes, but not *in situ*. The *in vitro* release of calcium ions was 2.5 times higher than the *in situ* condition. Earlier reports found that it is not realistic to evaluate the effects of bleaching on the microhardness of tooth structure without subjecting the specimens to saliva.[6,13-15,40-41]

Microhardness values were significantly lower for the LA, HA, LED and OX groups when compared to the CO group (p=.0051) and were significantly different between periods (p<.0001). The microhardness of the HA group after one day was lower than the other groups, but no difference was found after 14 days. The inclusion of a control group (CO) could be responsible for these statistical differences. It has been shown that a decrease in microhardness of bleached enamel can be reversed after a period of remineralization after bleaching through absorption and precipitation of salivary components, such as calcium and phosphate.[14,21,39,41-42] It can be hypothesized that the 14-day period after-bleaching was sufficient to allow for salivary components to increase the microhardness values found after one and seven days and to reinforce the surface resistance of non-bleached enamel, according to other studies.[14,41]

Notwithstanding, it was believed that activation with laser units would be more effective than other light sources.[43] This hypothesis was not confirmed by the results of the LA group. The greater the number of wavelengths from the light-source that coincide with the dye absorption spectrum, the greater the potential to absorb light and the resulting heat.[3] This could explain the significantly higher decrease in microhardness of the HA group after one- and seven-day periods. A previous study showed that an argon laser produced the lowest temperature increase for the bleaching gel.[3] Therefore, it seems that using this type of light to increase the temperature of the bleaching gel would be less effective than using other types of light.

The %SMC revealed similar behavior among all periods (2.28% to 5.81%), except for the CO group. Mineral loss could be expected after bleaching therapy, but it seemed to be insignificant.[44] This transient mineral loss is expected to be reversed a couple of days after bleaching through remineralization, especially when a fluoridated dentifrice or other substance is used. Thus, microhardness testing can be used not only as a comparative measure, but also as a direct measurement of mineral loss or gain resulting from demineralization and potential remineralization.[18]

CONCLUSIONS

According to the results, it is possible to conclude that:

There was an initial difference in microhardness of human enamel when all groups were considered. However, no statistically significant differences in microhardness were observed after 14 days. Hence, the different light-activation sources associated with 35% hydrogen peroxide did not influence the microhardness of human enamel subjected to in-office bleaching.

Acknowledgements

The authors have no financial interest in any of the companies or products mentioned in this article.

The authors express their gratitude to Dr Fábio Andretti and Jeremy Matis for helping with the manuscript revision.

(Received 8 October 2008)

References

1. Barghi N (1998) Making a clinical decision for vital tooth bleaching: At-home or in-office? *Compendium of Continuing Education in Dentistry* **19(8)** 831-838.
2. Qualtrough AJE & Burke FJT (1994) A look at dental esthetics *Quintessence International* **25(1)** 7-14.
3. Baik JW, Rueggeberg FA & Liewehr FR (2001) Effect of light-enhanced bleaching on *in vitro* surface and intrapulpal temperature rise *Journal of Esthetic Restorative Dentistry* **13(6)** 370-378.

4. Sarret DC (2002) Tooth whitening today *Journal of the American Dental Association* **133** 1535-1538.
5. Shannon H, Spencer P, Gross K & Tira D (1993) Characterization of enamel exposed to 10% carbamide peroxide bleaching agents *Quintessence International* **24(1)** 39-44.
6. Ernst CP, Marroquin BB & Willershausen-Zonnchen B (1996) Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel *Quintessence International* **27(1)** 53-56.
7. Hairul Nizam BR, Lim CT, Chng HK & Yap AUJ (2005) Nanoindentation study of human premolars subjected to bleaching agent *Journal of Biomechanics* **38(11)** 2204-2211.
8. Kugel G & Ferreira S (2005) The art and science of tooth whitening *Journal of Massachusetts Dental Society* **53(4)** 34-37.
9. Reyto R (1998) Laser tooth whitening *Dental Clinics of North America* **42(4)** 755-762.
10. Jones AJ, Diaz-Arnold ANAM, Vargas MA & Cobb DS (1999) Colorimetric assessment of laser and home bleaching techniques *Journal of Esthetic Dentistry* **11(2)** 87-94.
11. Sun G (2000) The role of lasers in cosmetic dentistry *Dental Clinics of North America* **44(4)** 831-850.
12. Basting RT, Rodrigues AL Jr & Serra MC (2001) The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *in situ* *Operative Dentistry* **26(6)** 531-539.
13. Araujo EM Jr, Baratieri LN, Vieira LCC & Ritter AV (2003) *In situ* effect of 10% carbamide peroxide on microhardness of human enamel: Function of time *Journal of Esthetic Restorative Dentistry* **15(3)** 166-173.
14. Justino LM, Tames DR & Demarco FF (2004) *In situ* and *in vitro* effects of bleaching with carbamide peroxide on human enamel *Operative Dentistry* **29(2)** 219-225.
15. Arcari GM, Baratieri LN, Maia HP & Freitas SFT (2005) Influence of the duration of treatment using a 10% carbamide peroxide bleaching gel on dentin surface microhardness: An *in situ* study *Quintessence International* **36(1)** 15-24.
16. Mair LH, Stolarski TA, Vowles RW & Lloyd CH (1996) Wear: Mechanisms, manifestations and measurement. Report of a workshop *Journal of Dentistry* **24(1-2)** 141-148.
17. Ryge G, Foley DE & Fairhurst CW (1961) Micro-indentation hardness *Journal of Dental Research* **40(6)** 1116-1127.
18. Featherstone JDB, ten Cate M, Shariati M & Arends J (1983) Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles *Caries Research* **17(5)** 385-391.
19. Attin T, Hannig C, Wiegand A & Attin R (2004) Effect of bleaching on restorative materials and restorations—A systematic review *Dental Materials* **20(9)** 852-861.
20. Dietschi D, Rossier S & Krejci I (2006) *In vitro* colorimetric evaluation of the efficacy of various bleaching methods and products *Quintessence International* **37(7)** 515-526.
21. Maltz M, Scherer SC, Parolo CCF & Jardim JJ (2006) Acid susceptibility of arrested enamel lesions: *In situ* study *Caries Research* **40(3)** 251-255.
22. Mahoney E, Holt A, Swain M & Kilpatrick N (2000) The hardness and modulus of elasticity of primary molar teeth: An ultra micro-indentation study *Journal of Dentistry* **28(8)** 589-594.
23. Potocnik L, Kosec L & Gaspersic D (2000) Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content *Journal of Endodontics* **26(4)** 203-206.
24. Lopes GC, Bonissoni L, Baratieri LN, Vieira LCC & Monteiro S Jr (2002) Effect of bleaching agents on the hardness and morphology of enamel *Journal of Esthetic Restorative Dentistry* **14(1)** 24-30.
25. Swift EJ Jr & Perdigão J (1998) Effect of bleaching on teeth and restorations *Compendium of Continuing Education in Dentistry* **19(8)** 815-820.
26. Goldstein RE & Garber DA (1995) *Complete Dental Bleaching* Chicago, Quintessence Publishing p 165.
27. Haywood VB (2000) Current status of nightguard vital bleaching *Compendium of Continuing Education in Dentistry* **28** S10-S17.
28. Papathanasiou A, Kastali S, Perry RD & Kugel G (2002) Clinical evaluation of a 35% hydrogen peroxide in-office whitening system *Compendium of Continuing Education in Dentistry* **23(4)** 335-338.
29. Stabholz A, Zeltser R, Sela M, Peretz B, Moshonov J & Ziskind D (2003) The use of lasers in dentistry: Principles of operation and clinical applications *Compendium of Continuing Education in Dentistry* **24(12)** 935-948.
30. Luk K, Tam L & Hubert M (2004) Effect of light energy on peroxide tooth bleaching *Journal of the American Dental Association* **135(2)** 194-201.
31. Al Shethri S, Matis BA, Cochran MA, Zekonis R & Stropes M (2003) A clinical evaluation of two in-office bleaching products *Operative Dentistry* **28(5)** 488-495.
32. Garber DA (1997) Dentist-monitored bleaching: A discussion of combination and laser bleaching *Journal of the American Dental Association* **128** 26S-30S.
33. Goldstein RE (1997) In-office bleaching: Where we came from, where we are today *Journal of the American Dental Association* **128** 11S-15S.
34. Lu AC, Margiotta A & Nathoo SA (2001) In-office tooth whitening: Current procedures *Compendium of Continuing Education in Dentistry* **22(9)** 798-800.
35. Weesner BW Jr (1998) Lasers in medicine and dentistry: Where are we now? *Journal of the Tennessee Dental Association* **78(1)** 20-25.
36. Smigel I (1996) Laser tooth whitening *Dentistry Today* **15(8)** 32-36.
37. Powell LV & Bales DJ (1997) Tooth bleaching: Its effect on oral tissues *Journal of the American Dental Association* **122(11)** 50-54.
38. Buchalla W & Attin T (2007) External bleaching therapy with activation by heat, light or laser—A systematic review *Dental Materials* **23(5)** 586-596.
39. Attin T, Kielbassa AM, Schwanenberg M & Hellwig E (1997) Effect of fluoride treatment on remineralization of bleached enamel *Journal of Oral Rehabilitation* **24(4)** 282-286.
40. Zero DT (1995) *In situ* caries models *Advances in Dental Research* **9** 214-230.

41. Rodrigues JA, Basting RT, Serra MC & Rodrigues JAL (2001) Effects of 10% carbamide peroxide bleaching material on enamel microhardness *American Journal of Dentistry* **14(2)** 67-71.

42. Teixeira ECN, Ritter AV, Thompson JY, Leonard RH Jr & Swift EJ Jr (2004) Effect of tray-based and trayless tooth whitening systems on microhardness of enamel surface and subsurface *American Journal of Dentistry* **17(6)** 433-436.

43. Dederich DN & Bushick RD (2004) Lasers in dentistry—Separating science from hype *Journal of the American Dental Association* **135(2)** 204-212.

44. Rodrigues JA, Marchi GM, Ambrosano GMB, Heymann HO & Pimenta LAF (2005) Microhardness evaluation of *in situ* vital bleaching on human dental enamel using a novel study design *Dental Materials* **21(11)** 1059-1067.