UNIVERSIDADE DE UBERABA DORCA LUIZA DE FREITAS SALOMÃO

EFEITO DE DIFERENTES AGENTES CLAREADORES E DE DUAS TÉCNICAS DE FLUORTERAPIA NA SUSCEPTIBILIDADE À DESMINERALIZAÇÃO DO ESMALTE DENTAL

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DORCA LUIZA DE FREITAS SALOMÃO

EFEITO DE DIFERENTES AGENTES CLAREADORES E DE DUAS TÉCNICAS DE FLUORTERAPIA NA SUSCEPTIBILIDADE À DESMINERALIZAÇÃO DO ESMALTE DENTAL

Dissertação apresentada ao Programa de Mestrado em Odontologia da Universidade de Uberaba, para a obtenção do Título de Mestre em Odontologia, área de Concentração em Biomateriais.

Orientador: Prof. Dr. Vinícius Rangel Geraldo-Martins Catalogação elaborada pelo Setor de Referência da Biblioteca Central UNIUBE

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RESUMO

O objetivo desta pesquisa foi avaliar a susceptibilidade à desmineralização ácida do esmalte dental clareado e submetido a diferentes técnicas de fluorterapia. Cem amostras de esmalte dental bovino (6x6 mm) foram aleatoriamente divididas em 10 grupos (n= 10). Os grupos 1 e 2 não receberam clareamento. As amostras dos grupos 3 a 6 foram submetidas à técnica de clareamento caseiro utilizando peróxido de hidrogênio a 6% (HP; G3 e G4) ou peróxido de carbamida 10% (PC; G5 e G6). As amostras dos grupos 7 a 10 foram submetidas à técnica de clareamento em consultório utilizando peróxido de hidrogênio a 35% (HP; G7 e G8) ou peróxido de carbamida a 35% (CP; G9 e G10). Durante o processo de clareamento, os grupos 3, 5, 7 e 9 receberam fluorterapia diária com solução de fluoreto de sódio (NaF) 0,05%, enquanto os grupos 4, 6, 8 e 10 receberam fluorterapia semanal com NaF a 2%. Depois, as amostras dos grupos 2 a 10 foram submetidos a um desafio ácido (ciclagem de pH) durante 14 dias consecutivos. Após, foi realizada a análise da microdureza Knoop das amostras em diferentes profundidades (20, 40, 60, 80, 100, 120 e 200 µm) a partir da superfície externa do esmalte. Os resultados foram submetidos à Análise de Variância de 1 critério, seguido pelo teste de Tukey (α = 0,05). A comparação entre os grupos 1 e 2 mostrou que o método de desmineralização foi eficaz. Os grupos que receberam o clareamento caseiro mostraram a mesma susceptibilidade à desmineralização ácida que o grupo 2, independentemente da fluorterapia utilizada. No entanto, as amostras submetidas a clareamento de consultório que receberam um regime semanal de fluorterapia (grupos 8 e 10) demonstraram maior susceptibilidade à desmineralização ácida que o grupo 2 (p< 0,05). Os grupos 7 e 9 mostraram resultados semelhantes ao grupo 2, mas diferentes dos grupos 8 e 10. Pode-se concluir que a utilização de PH a 6% e PC a 10%, associado a um regime diário ou semanal, não aumenta a susceptibilidade do esmalte à desmineralização ácida. No entanto, o uso de PH a 35% e PC a 35% devem ser associados com um regime diário de fluorterapia, caso contrário o clareamento de consultório pode deixar o esmalte clareado mais susceptível à desmineralização ácida.

Palavras-chave: clareamento, flúor, dureza, esmalte, peróxido de carbamida, peróxido de hidrogênio.

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Laboratory research

Susceptibility to acid demineralization of dental enamel submitted to different bleaching techniques and fluoridation regimes

Running title: Susceptibility of bleached enamel to acid demineralization

Clinical Relevance: The in-office bleaching technique must be done in conjunction with a daily fluoridation regime to minimize the damages produced by that procedure in dental enamel.

Dorca Luiza de Freitas Salomão DDS, Uberaba University – Uberaba-MG, Brazil

Diones Machado dos Santos DDS Graduate Student, Uberaba University – Uberaba-MG, Brazil

Ruchele Dias Nogueira MSc, PhD, Adjunct Professor, Uberaba University – Uberaba-MG, Brazil

Regina Guenka Palma-Dibb DDS, PhD, Associate Professor, Ribeirao Preto School of Dentistry, São Paulo University, Ribeirão Preto-SP, Brazil

Vinícius Rangel Geraldo-Martins MSc, PhD, Adjunct Professor, Uberaba University – Uberaba-MG, Brazil

Corresponding Author Prof. Dr. Vinícius Rangel Geraldo-Martins Universidade de Uberaba – Campus Aeroporto Av. Nenê Sabino, 1801 Room# 2C03 and 2C04 Bairro Universitário. ZIP Code 38.055-500 Uberaba – MG, Brazil Phone/Fax: +55 (34) 3319-8958 e-mail: vinicius.martins@uniube.br

ABSTRACT

The aim was to assess the susceptibility to acid demineralization of bleached dental enamel submitted to different fluoridation regimes. One hundred bovine enamel blocks (6x6x3 mm) were randomly divided in 10 groups (n=10). Groups 1 and 2 received no bleaching. Groups 3 to 6 were submitted to the at-home bleaching technique using 6% hydrogen peroxide (HP; G3 and G4) or 10% carbamide peroxide (CP; G5 and G6). Groups 7 to 10 were submitted to the in-office bleaching technique using 35% HP (G7 and G8) or 35% CP (G9 and G10). During bleaching, a daily fluoridation regime with 0.05% of sodium fluoride (NaF) solution was done in groups 3, 5, 7 and 9, while a weekly fluoridation with a 2% NaF gel was applied in groups 4, 6, 8 and 10. The samples of the groups 2 to 10 were pH cycled during 14 consecutive days. Afterwards, the samples of all groups were assessed by cross-sectional Knoop microhardness at different depths from the outer enamel surface. The average Knoop hardness numbers (KHN) were compared by Two-Way Anova and Tukey's tests (α= 0.05). The comparison between groups 1 and 2 showed that the demineralization method was effective. The comparison among the groups 2 to 6 showed the same susceptibility to acid demineralization, whatever the fluoridation method used. However, the samples of the groups 8 and 10 showed more susceptibility to acid demineralization then group 2 (p< 0.05). Groups 7 and 9 promoted similar results then group 2, but different then groups 8 and 10. The use of HP 6% and CP 10% associated with daily or weekly fluoridation regime do not increase the susceptibility of enamel to acid demineralization. However, the use of HP 35% and CP 35% must be associated with a daily fluoridation regime, otherwise the in-office bleaching can let the bleached enamel more susceptible to acid demineralization.

Keywords: Dental Bleaching, Fluoride, Hardness, Enamel, Carbamide Peroxide, Hydrogen Peroxide.

INTRODUCTION

Teeth discoloration is one of the main reasons that lead patients to look for dental treatment. For that reason, tooth bleaching has become very popular and, sometimes, it is done indiscriminately. The success of the treatment depends, among other factors, on the dentist, who must know the dental materials used, the techniques of application and the possible side effects that the treatment may result in the patient.

The effectiveness of tooth bleaching by applying carbamide or hydrogen peroxide to discolored vital teeth has been previously reported.¹ The application of carbamide and hydrogen peroxide gels at a concentration of 10%-15% and of 6%-10%, respectively, for 4 to 8 hours per day, during 3 to 6 weeks, are still the most popular at-home bleaching techniques and are suggested as efficient and simple procedures for tooth whitening.^{2,3} The in-office bleaching treatment was introduced to shorten the treatment time. In this technique the teeth can be bleached in 2 or 3 sessions since it uses the same bleaching agents cited before, but in higher concentrations.³

Both at-home and in-office techniques of tooth bleaching can lead to tooth sensitivity and this effect may occur during or after treatment.⁴ Several approaches to reduce tooth sensitivity have been used, such as reduction in wearing time and frequency of application, temporary interruption of whitening and use of an active ingredient such as potassium nitrate have been used.⁵ The use of fluoride compounds, such as gels, solutions or dentifrices, have also been used to prevent tooth sensibility, to enhance the remineralization of the bleached enamel and to increase the abrasion resistance of softened enamel.⁶ It is known that the fluoride uptake is higher in demineralized enamel compared to a sound tissue.⁷ Furthermore, bleaching might render enamel porous, which might allow a better diffusion and penetration of the best fluoride compound to prevent enamel demineralization during the bleaching are still not stated.

Previous studies showed that the bleaching agents can promote alterations on enamel surface.^{9,10} Scanning electronic microscopic analysis showed that morphological alterations of the superficial enamel surface, such as erosion, decalcification and porosity, might occur following exposure to carbamide or hydrogen peroxide at different concentrations.¹¹ Additionally, the application of

bleaching gels can also affect calcium and phosphate content and, consequently, may decrease the surface and the subsurface microhardness of the enamel. ¹⁰

Despite the protective and remineralizing potential of human saliva, the mineral loss is sometimes evident under in situ conditions.¹² Saliva increases the microhardness of bleached enamel by providing phosphate and calcium ions, but sometimes the rehardening is not complete.¹³ In this way, fluoride might also contribute to the repair of the microstructural defects of bleached enamel.

According to the described above, it was observed that the bleaching gel might change the structure of the dental enamel, but the literature is unclear about the correct fluoridation method to impair an excessive demineralization of dental enamel. The purpose of this study was to assess the susceptibility to acid demineralization of bleached dental enamel submitted to different fluoridation regimes. The null hypothesis is that the susceptibility of dental enamel to acid demineralization is not altered when exposed to the at-home and to the in-office bleaching techniques in conjunction with different fluoridation regimes.

METHODS AND MATERIALS

Preparation of Specimens

One hundred freshly extracted bovine incisors were stored in in 0.1% thymol solution (pH 7.0) for at least 1 month. The crowns were separated from the roots using a water-cooled diamond disc (Isomet; 10.2cm×0.3mm, arbour size 1/2 in., series 15HC diamond; Buehler Ltd., Lake Bluff, IL, the USA) mounted in a section machine (Minitom, Struers Inc., Westlake-OH, USA). The crowns were sectioned to obtain 100 enamel blocks (6x6x3 mm). The labial surfaces were ground flat and polished with water-cooled sandpapers (#600, 800, 1200, 2400 grit, Saint-Gobain Abrasivos Ltda, Sao Paulo-SP, Brazil) to standardize the substrate. Prior to the experiment, the specimens were stored in distilled water.

Bleaching and Fluoridation Regime

The enamel samples were randomly divided in 10 experimental groups (n=10). In each sample, a 16 mm² area was delimited at the buccal surface. Around this demarcated area, two layers of varnish sealer (Colorama Maybelline Ltda, São Paulo, Brazil) were applied.

The samples of group 1 were kept in artificial saliva (pH=7.0) and no experiment was done in those samples. The samples of group 2 were submitted only to the pH cycling (see below). The treatments done in the other samples are described in Table 1. The at-home an in-office bleaching were simulated in each respective group.

The at-home bleaching technique was performed by applying 6 % hydrogen peroxide gel (HP) (groups 3 and 4; Mix Day, Dentalville do Brasil Ltda, Joinville-SC, Brasil; pH=7.0) or 10 % carbamide peroxide gel (CP) (groups 5 and 6; grupo 3-Whitness Perfect 10%, FGM Produtos Odontológicos, Joinville, SC, Brasil; pH=6.5) on the delimited enamel surface, according to the manufacturers' instructions, for six hours per day in a humidified atmosphere at 37°C. This process was done daily during 21 days.

Daily, just after the removal of the bleaching gel, the samples of groups 3 and 5 were washed in distilled water and they were immersed in a colorless neutral 0.05 % sodium fluoride solution (NaF - Fluor Sol Clear, Dentsply Indústria e Comércio Ltda, Petrópolis-RJ, Brasil) during 5 minutes. After, the samples were kept in artificial saliva at 37 °C.

The samples of groups 4 and 6 were covered, once a week, by a 2 % NaF (pH= 6.5) gel (Nuprogel, Dentsply) during 4 minutes. After, the excess of gel was removed and the samples were kept in artificial saliva at 37 °C.

The in-office bleaching technique was performed by applying 35 % HP gel (groups 7 and 8; Mix One Supreme, Dentalville; pH=6.4) or 37 % CP gel (groups 9 and 10; Whiteness Super, FGM; pH 6.5) on the delimited enamel surface, according to the manufacturers' instructions. Three in-office bleaching sessions were done in each group, in days 0, 7 and 14. In each session, the bleaching gel was applied on the enamel surface during 20 minutes. After that period, the gel was replaced over the enamel surface. So, each session had three gel applications.

The fluoridation regime started at the end of each session. The samples of the groups 7 and 9 had the same fluoridation regime and duration described for groups 3 and 5. The fluoridation regime for groups 4, 6, 8 and 10 was the same. After the third bleaching session (day14) specimens were stored in artificial saliva for additional 7 days.

The pH of the bleaching agents was verified by a pH meter (Digimed DM-20-Digicrom Analítica Ltda., São Paulo, Brazil) fitted with an electrode (DME-Digimed CV8).

pH cycling

For the acid challenge, samples were submitted to a pH-cycling procedure, modified from a previously described protocol.¹⁴ First of all, the samples were impermeabilized, except the bleached enamel surface. Groups 2 to 9 were submitted to this acid challenge. The demineralization solution (pH = 5.0) consisted of 2.0 mmol/l of Ca, 2.0 mmol/l of phosphate in buffer solution of acetate 0.075mol/l, and the remineralization solution (pH = 7.0) consisted of 1.5 mmol/l of Ca, 0.9 mmol/l of phosphate, 150 mmol/l of potassium chloride. Each specimen was cycled in 5.0 mL of both solutions for 6 h in the demineralizing solution and 18 h in the remineralizing solution. This procedure was carried out for 14 days at 37°C. At the end of each 5 consecutive days of cycling, the samples were immersed in remineralizing solution for 2 days.

Microhardness test

At the end of the pH cycling, the samples of all ten groups were individually embedded in self-curing acrylic resin, with the bleached surface exposed. The samples were longitudinally sectioned to make possible the cross-section microhardness analysis. After serially polishing the embedded teeth, each sample was assessed with a microhardness examination of the enamel, starting at 20 μ m from the outer enamel surface, with indents at 20 μ m intervals between 20 μ m and 120 μ m, and a last indentation was done at 200 μ m from the outer surface. In each area, 3 measurements of knoop microhardness were done, and the distance between measurements was 500 μ m, to prevent that the marks overlap each other. A static load of 25 g/ 10 s was applied.

Statistical Analysis

The data were submitted to the D'Agostino normality test. After, two-way ANOVA followed by Tukey's test, when necessary, were used to analyze the differences in Knoop hardness numbers (KHN) of all Groups. The level of statistical

significance was set at 0.05. All analyses were done using Bioestat 5.3 (Instituto Mamirauá, Tefe-AM, Brazil).

RESULTS

The mean KHN found in groups 1 (no treatment) and 2 (unbleached and pH cycled) are represented in Figure 1. The KHN were compared at each depth. The graph shows that the demineralization occurred at depths of up to 120 μ m, and the hardness decrease was higher on the superficial layers of the dental enamel. At 200 μ m, no differences were observed between those two groups.

The results obtained for at-home bleaching groups were compared to those obtained in group 2, and this analysis is showed on table 2. The KHN were compared at each depth. The analysis of variance showed that no statistically significant differences were found (p>0.05).

Table 3 shows the KHN found for the in-office bleaching groups, as well as the data obtained for group 2. For 20 μ m, groups 2, 7 (Hydrogen Peroxide 35% and daily fluoridation with NaF 0.05%) and 9 (Carbamide Peroxide 35% and daily fluoridation with NaF 0.05%) showed similar KHN, but statistically different from groups 8 (Hydrogen Peroxide 35% and weekly fluoridation with NaF 2%) and 10 (Carbamide Peroxide 35% and weekly fluoridation with NaF 2%). The same situation occurred for the depths of 40, 60 and 80 μ m. The experimental groups showed no statistically significant differences in the deeper layers (100, 120 and 200 μ m) of the bovine dental enamel.

DISCUSSION

The null hypothesis of the present investigation was partially rejected, since groups treated with CP 35% and HP 35% which received weekly fluoridation were more susceptible to acid demineralization.

The bleaching agents used today are composed, mainly, by carbamide peroxide (CP) or hydrogen peroxide (HP).³ The HP has low molecular mass and this

facilitate its rapid diffusion into enamel prisms and interprismatic spaces.¹⁵ The bleaching agent is capable to remain entrapped, exerting a prolonged effect in structures that do not necessarily need to be bleached. This also applies to CP, which when in contact with dental structures, it dissociates into urea and HP. Thus, it is possible to believe that bleaching causes alterations in dental hard tissues, such as erosion, porosity and increase in enamel's roughness.¹¹ Although these alterations are not clinically or macroscopically visible, past studies found microstructural changes of enamel induced by bleaching agents, particularly when peroxides are used in high concentrations.^{10, 16,17} In fact, the present study showed that enamel treated with high concentrated bleaching agents was more susceptible to acid demineralization, even when a 2 % NaF weekly fluoridation regime is used.

Some bleaching agents can lead to erosive effects on enamel due to their low pH, and even tooth brushing during bleaching can increase the roughness of the enamel surface.¹⁸ The chemical analysis of enamel after the application of CP and HP in concentrations between 10 and 30 % revealed a reduction in Calcium (Ca) levels, as well as the average Ca:P value of bleached dental enamel.^{10,19} The reduction of mineral element (Ca) is attributed to the dissolution of this element by bleaching agents. In addition to reducing the surface hardness, loss of minerals increases the enamel permeability to acids produced by cariogenic bacteria, leading to the formation of deeper carious lesions.²⁰

Similarly, these changes allow the penetration of bleaching gel into the deeper layers of the dental hard tissue, which may promote effects in dentin and pulp.²¹ Clinically, the presence of these changes may be suggested by the occurrence of tooth sensitivity during bleaching. Therefore, the use of fluoridated compounds throughout treatment has been proposed to avoid the occurrence of side effects.

The acid challenge used here was based on previous studies and it was effective to demineralize deeper enamel layers.¹⁴ According to Figure 1, layers distant up to 120 μ m from enamel outer surface were affected by the pH cycling used. The bleaching treatments and fluoridation regimens used here, as described before, are usually applied in dental offices. The data obtained in the present study suggests that in-office bleaching reduced the mineral content of the dental enamel in an extent that weekly fluoride application could not restore its initial condition of mineralization. According to the results obtained by the in-office bleaching groups that were submitted to a daily fluoridation regime, it can be concluded that fluoridation must be

present throughout the treatment, at low and constant concentrations to reduce mineral loss and stimulate remineralization, and not only indicated after each bleaching session.

The use of fluoride compounds is effective in increasing the hardness of enamel samples and preventing mineral loss during at-home bleaching.²² The fluoride incorporation into the demineralized tooth surface creates a calcium fluoride layer that increases enamel hardness.²³ As described before, bleaching makes the enamel surface porous and rough, and it is accepted that fluoride uptake into demineralized enamel is higher when compared to sound enamel since the porous and permeable structure of the demineralized tissue allows deeper diffusion and penetration of the fluoride applied and that the porosity increases the retention sites for the fluoride.²⁴ Small amounts of fluoride in solution around the tooth effectively inhibit demineralization more than incorporated fluoride and have a much greater caries-protective potential than a large proportion of fluoride in enamel mineral.²⁴

In the present research, the excess of fluoride gel was aspirated after 4 minutes of its contact with the hard dental tissue and the sample was immersed in artificial saliva. Thus, the only contact between the samples and the 2% NaF was at the day of its application. It is accepted that topical fluorides promote remineralization and inhibit demineralization of dental hard tissues.²⁴ In fact the weekly application of fluoride was enough for the samples submitted to the at-home bleaching treatment, probably because the bleaching agents used are less concentrated and less aggressive to tooth enamel. Nevertheless, this same fluoridation regime was not effective for samples bleached with agents in high concentrations, which promote more severe changes in dental tissue.²⁵ In addition, the results obtained here suggested that fluoridation does not leave the bleached enamel more acid resistant when compared to the sound enamel and, therefore, fluoridation does not reinforce the tooth structure during treatment, but probably help to keep the levels of mineralization of bleached enamel to return to its initial situation.

Some bleaching gels incorporated the ions Ca^{2+} and F^{-} in their formulations.²⁶ That was thought to be a possible alternative to overcome the adverse effects of bleaching gels on enamel surface, since the ions could diffuse into the enamel structure along with the CP and HP. The deposit of those ions in the tooth structures may act as a physical barrier, minimizing the contact of the acid to enamel, or providing additional mineral to be dissolved during the acid challenge before the

underlying enamel is attacked. This effect is not completely confirmed, but recent research has shown promising results for those bleaching agents.²⁶

According to some studies, the potential for demineralization depends on the pH of the bleaching agents.^{27,28} In the present study, the pH of the bleaching gels were between 6.4 and 7.0. As they are near to the neutral value, their pH had no influence in the present results. Nevertheless, as previous studies have defined that bleaching agents are also able to demineralize tooth enamel, it is possible that the enamel demineralization is a combination between the action of the concentration of bleaching agent and the low pH of the gel.^{25,28}

However, there are also past studies which have shown that bleaching agents do not change the enamel surface and, consequently, do not alter the susceptibility of that tissue to acid demineralization. ^{29,30} This agrees with the results obtained here for the at-home treatment and for the in-office bleaching with a daily fluoridation regimen. The divergence among the various researches is natural, because there are several variables involved in each study, as the pH of bleaching gels, the HP gel concentration, the contact period between the tooth and the bleaching gel, the full treatment time, among others. Nevertheless, the common sense is that additional fluoridation is essential during the treatment.

What should be taken into consideration is that this study was performed in vitro. It is not known whether these findings would be the same if the study was conducted in vivo or in situ, using exactly the same products and the same methodology applied here. What is known is that saliva plays an important role in enamel remineralization. ³¹ Additionally, tooth brushing with fluoride dentifrices is essential to increase the acid resistance of enamel and to minimize the effects of the bleaching gels over that tissue. ²⁴ These factors could affect the results found on groups 8 and 10, but clinical studies are needed to confirm or reject the findings obtained here.

CONCLUSION

With the limitations of this in vitro study, it may be concluded that the use of low concentrated bleaching agents (HP 6% and CP 10%) used in the at-home bleaching technique, associated with daily or weekly fluoridation regime, do not increase the susceptibility of enamel to acid demineralization. However, the use of high concentrated bleaching agents (HP 35% and CP35%) may increase susceptibility

to dental enamel demineralisation acid. To minimize this side effect, it is necessary to use a daily fluoridation regime, since weekly fluoridation is not capable of inhibiting that adverse effect.

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Group	Bleaching	Bleaching Agent	Fluoride	Fluoridation	pH
	Technique		Concentration	Regime	Cycling
1	None	None	None	None	No
2	None	None	None	None	Yes
3	At-Home	Hydrogen Peroxide 6%	NaF 0.05%	Daily	Yes
4	At-Home	Hydrogen Peroxide 6%	NaF 2%	Once a Week	Yes
5	At-Home	Carbamide Peroxide 10%	NaF 0.05%	Daily	Yes
6	At-Home	Carbamide Peroxide 10%	NaF 2%	Once a Week	Yes
7	In-Office	Hydrogen Peroxide 35%	NaF 0.05%	Daily	Yes
8	In-Office	Hydrogen Peroxide 35%	NaF 2%	Once a Week	Yes
9	In-Office	Carbamide Peroxide 35%	NaF 0.05%	Daily	Yes
10	In-Office	Carbamide Peroxide 35%	NaF 2%	Once a Week	Yes

 Table 1- Description of the experimental groups according to the bleaching technique and to the fluoridation regime

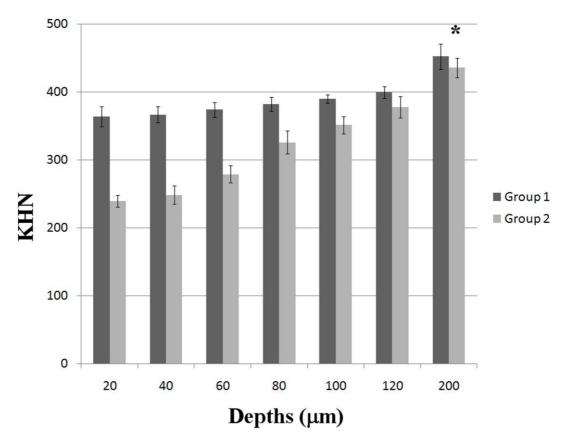


Figure 1. Comparison among the mean KHN obtained in each depth for groups 1 and 2. The * symbol means no statistical differences (p>0.05).

Depth (µm)	Group 2	Group 3	Group 4	Group 5	Group 6
20	239(8.8)	249(13.6)	266(18.6)	263(15.4)	259(21.0)
40	248(13.7)	256(9.4)	275(23.5)	270(19.9)	269(22.2)
60	279(12.3)	282(13.0)	291(17.3)	295(13.9)	286(20.6)
80	326(17.3)	323(22.8)	336(29.0)	325(27.4)	321(21.2)
100	351(12.9)	352(25.0)	371(26.6)	361(23.2)	353(26.9)
120	377(15.4)	380(19.1)	388(17.2)	385(24.9)	386(21.5)
200	435(14.4)	432(21.8)	445(15.6)	450(21.0)	439(24.4)

Table 2. Mean KHN (\pm standard deviation) found at different depths for unbleached and at-home bleached samples. No statistically differences were found (p>0.05).

Group 2	Group 7	Group 8	Group 9	Group 10
239(8.8)A	260(17.2)A	203(11.3)B	259(24.0)A	208(6.3)B
248(13.7)C	268(24.6)C	217(8.23)D	265(22.9)C	219(10.3)D
279(12.3)E	289(17.5)E	249(7.16)F	288(19.3)E	246(12.2)F
326(17.3)G	323(26.57)G	292(10.19)H	326(29.6)G	287(16.4)H
351(12.9)I	357(28.6)I	342(7.16)I	368(22.9)I	335(37.4)I
377(15.4)J	387(14.49)J	367(7.94)J	399(21.1)J	379(26.4)J
435(14.4)K	438(21.13)K	431(15.8)K	445(15.3)K	432(13.14)]
	239(8.8)A 248(13.7)C 279(12.3)E 326(17.3)G 351(12.9)I 377(15.4)J	239(8.8)A 260(17.2)A 248(13.7)C 268(24.6)C 279(12.3)E 289(17.5)E 326(17.3)G 323(26.57)G 351(12.9)I 357(28.6)I 377(15.4)J 387(14.49)J	239(8.8)A260(17.2)A203(11.3)B248(13.7)C268(24.6)C217(8.23)D279(12.3)E289(17.5)E249(7.16)F326(17.3)G323(26.57)G292(10.19)H351(12.9)I357(28.6)I342(7.16)I377(15.4)J387(14.49)J367(7.94)J	239(8.8)A260(17.2)A203(11.3)B259(24.0)A248(13.7)C268(24.6)C217(8.23)D265(22.9)C279(12.3)E289(17.5)E249(7.16)F288(19.3)E326(17.3)G323(26.57)G292(10.19)H326(29.6)G351(12.9)I357(28.6)I342(7.16)I368(22.9)I377(15.4)J387(14.49)J367(7.94)J399(21.1)J

Table 3. Mean KHN (\pm standard deviation) found at different depths for unbleached and in-office bleached samples. Similar capital letters in row mean no statistical differences (p> 0.05)

Legends

Figure 1- Comparison among the mean KHN obtained in each depth for groups 1 and 2. The * symbol means no statistical differences (p>0.05).

Figure 2

Table 1- Description of the experimental groups according to the bleaching technique and to the fluoridation regime

Table 2. Mean KHN (\pm standard deviation) found at different depths for unbleached and at-home bleached samples. No statistically differences were found (p>0.05).

Table 3. Mean KHN (\pm standard deviation) found at different depths for unbleached and in-office bleached samples. Similar capital letters in row mean no statistical differences (p> 0.05)

ANEXO

OPERATIVE DENTISTRY

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Journal article: multiple authors

Eick JD, Gwinnett AJ, Pashley DH & Robinson SJ (1997) Current concepts on adhesion to dentin Critical Review of Oral and Biological Medicine 8(3) 306-335.

Journal article: special issue/supplement

Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry Operative Dentistry (Supplement 6) 119-144.

Abstract:

Yoshida Y, Van Meerbeek B, Okazaki M, Shintani H & Suzuki K (2003) Comparative study on adhesive performance of functional monomers Journal of Dental Research 82(Special Issue B) Abstract #0051 p B-19.

Corporate publication:

ISO-Standards (1997) ISO 4287 Geometrical Product Specifications Surface texture: Profile method – Terms, definitions and surface texture parameters Geneve: International Organization for Standardization 1st edition 1-25.

Book: single author

Mount GJ (1990) An Atlas of Glass-ionomer Cements Martin Duntz Ltd, London.

Book: two authors

Nakabayashi N & Pashley DH (1998) Hybridization of Dental Hard Tissues Quintessence Publishing, Tokyo.

Book: chapter

Hilton TJ (1996) Direct posterior composite restorations In: Schwarts RS, Summitt JB, Robbins JW (eds) Fundamentals of Operative Dentistry Quintessence, Chicago 207-228.

Website: single author

Carlson L (2003) Web site evolution; Retrieved online July 23, 2003 from: http://www.d.umn.edu/~lcarlson/cms/evolution.html

Website: corporate publication

National Association of Social Workers (2000) NASW Practice research survey 2000. NASW Practice Research Network, 1. 3. Retrieved online September 8, 2003 from: http://www.socialworkers.org/naswprn/default

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