In vitro Analysis of Human Enamel Microhardness as Subjected to Prolonged Use of External Bleaching Agents

Análise *in vitro* da Microdureza do Esmalte Humano Submetido ao uso Prolongado de Agentes Clareadores Externos

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Abstract

The aim of this study was to evaluate the effects of external bleaching agents on the microhardness of human enamel after its prolonged use. Twenty intact human third molars were submitted to mesio-distal crosscut and embedded in polystyrene resin. The specimens were submitted to finish, and half of the enamel surface of each specimen was covered with cosmetic varnish, representing the control group (G0 – did not receive bleaching agents). The sample was divided into four groups (n=10): G1 – one bleaching session with 16% carbamide peroxide; G2 – three bleaching sessions with 16% carbamide peroxide; G3 – one bleaching session with 22% carbamide peroxide; and G4 – three bleaching sessions with 22% carbamide peroxide. Each session lasted 8 hours a day over the course of two weeks, with 45 days interval between G2 and G4 sessions. During this period, the specimens were kept in artificial saliva at 37°C. Then, the Knoop hardness test was done on the middle third of each bleached and non-bleached surface. The data were submitted to analysis of variance (two-way ANOVA), Tukey's test ($p < 0.05$) and Dunnet test for comparison to G0, which showed the highest superficial hardness averages, differing statistically from the other groups (306.69 KHN). Group 4 showed the lowest average (135.37 KHN). It was concluded that bleaching reduced enamel hardness. Furthermore, increasing of carbamide peroxide (22%) associated with an increased number of sessions (3 sessions) enhanced the decrease in microhardness.

Keywords: Dental Enamel. Peroxides. Tooth Bleaching.

Resumo

O objetivo deste estudo foi avaliar os efeitos de agentes clareadores externos sobre a microdureza do esmalte dental humano após clareamento caseiro prolongado. Vinte terceiros molares humanos hígidos foram seccionados no sentido mesio-distal e incluídos em resina de poliestireno. Os espécimes foram submetidos a acabamento, e metade da superfície do esmalte de cada espécime foi coberta com verniz cosmético, representando o grupo controle (G0 – não recebeu agente clareador). A amostra foi dividida em quatro grupos (n = 10): G1 - uma sessão de clareamento com peróxido de carbamida 16%; G2 - três sessões de clareamento com peróxido de carbamida 16%, G3 - uma sessão de clareamento com peróxido de carbamida 22% e G4 - três sessões de clareamento com peróxido de carbamida a 22%. Cada sessão durou 8 horas por dia ao longo de duas semanas; para G2 e G4 houve 45 dias de intervalo entre as sessões. Durante este período, os espécimes foram mantidos em saliva artificial a 37°C, sendo posteriormente realizado o teste de microdureza Knoop, no terço médio de cada superfície clareada e não clareada. Os dados foram submetidos à análise de variância (dois fatores) e teste de Tukey ($p <0,05$), além do teste de Dunnet para comparação com G0, que mostrou as maiores médias de dureza superficial, diferindo estatisticamente dos demais grupos (306,69 KHN). O grupo 4 apresentou a menor média (135,37 KHN). Concluiu-se que o clareamento diminuiu a dureza do esmalte. Além disso, o aumento da concentração do peróxido de carbamida (22%), associado ao aumento do número de sessões (3 sessões) potencializou a diminuição da microdureza superficial.

Palavras-chave: *Esmalte Dentário. Peróxidos. Clareamento Dental.*

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1 Introduction

The relentless pursuit of overly bleached teeth has been making tooth bleaching treatment widely accepted by patients[1,2]. The aesthetic appeal and ease of obtaining these agents lead many people to use these products inadvertently, ignoring their adverse effects.

The dental market offers bleaching materials with different compositions and concentrations. Carbamide peroxide is often used for homemade dental bleaching, and it can be found in concentrations ranging from 10% to 22%. In contact with saliva and oral tissues, carbamide peroxide decomposes into hydrogen peroxide (active agent) and urea[3]. The lower molecular weight of hydrogen peroxide allows it to freely transit by interprismatic spaces, through enamel and dentin, causing pigments oxidation of these structures[4]. Therefore, compounds with pigmented carbon rings are converted in clearer chains, resulting in dental bleaching effect[5.]

Studies have shown that there is a possibility of subclinical changes in superficial microhardness of the enamel[6-11], although this reversing possibility has not been established yet.

Dental bleaching using carbamide peroxide for long periods also destroys different layers of enamel and produces minerals loss[7]. Changes may occur to the enamel, dentin and cementum after bleaching[5]. Bleaching agents probably lead to cell destruction of the pulp due to enzymatic inactivation and rupture of normal cellular activity[12,13]. Nevertheless, the sodium ascorbate, a component of these agents, was able to protect cultured cells against cytotoxic effects of carbamide peroxide[14].

The aesthetic appeal of whiter teeth leads people to pursue it. Ease of access to bleaching agents makes us alert to the growing use of these materials. Considering these facts, the aim of this study was to evaluate *in vitro* the effects of bleaching agents on enamel microhardness after different number of sessions and concentrations of homemade dental bleaching.

2 Material and Methods

2.1 Preparation of specimens

This research project was approved by the Ethics Committee in Human Research of the University under report n. 113/2008.

Twenty intact human third molars extracted for clinical reasons were cleaned and frozen in saline solution. After being defrosted at 25 ^{o}C, the roots were removed and the teeth were submitted to mesio-distal crosscut using diamond disk (Labcut 1010 Low Speed Diamond Saw-EXTEC, Chicago, IL, USA) under water refrigeration. Vestibular and lingual surfaces were used in this study. Forty specimens were obtained and embedded in polystyrene resin. They were submitted to finishing by using wet sandpaper (Block Stone Waterproof – Bosch, Campinas, SP, Brazil) with decreasing granulation (400, 600, 800, 1200, 1500, and 2000) under refrigeration until flat enamel areas were exposed. Half of the enamel surface of each specimen was covered with cosmetic varnish and did not receive bleaching treatments (Revlon Incorporated, New York, NY, USA) in order to create the control group (G0). The other half was later submitted to the bleaching agent, defining experimental groups described below.

2.2 Experimental groups

The specimens were divided into four groups, as shown in Table 1. Each session lasted 8 hours a day over the course of two weeks, as suggested by the manufacturer. During the intervals between sessions, the specimens were kept at 37 °C in artificial saliva, switched daily.

Table 1: Experimental groups with respectives used bleaching agents, number, duration and interval between sessions.

Group (N=10)	Bleaching Agent	Number of Sessions	Interval Between Sessions
G1	Carbamide Peroxide 16%*	1	-
G2	Carbamide Peroxide 16%*	3	45 days
G3	Carbamide Peroxide 22%*	1	-
G4	Carbamide Peroxide 22%*	3	45 days

*Whiteness Perfect®-FGM Dental Products, Joinvile, SC, Brazil.

2.3 Knoop Microhardness Test

After bleaching sessions, specimens were submitted to the Koop Hardness Test. They were positioned perpendicularly along the long axis of the edentator (Shimadzu Corporation, Model HNZ2, Kyoto, Japan). Three edentations were made (50g for 15 seconds) in the middle third of each surface, obtaining the average values of Knoop Hardness Number (KHN). The results were submitted to statistical analysis by two way-ANOVA and the averages were compared by the Tukey's test (5% significance). Dunnet test was applied to compare the experimental groups with the control group.

3 Results and Discussion

There was a significant reduction ($P<.05$) of hardness in all groups when each was compared to non-bleached specimens (G0: 306.69 KHN; SD = 44.9). The comparisons between bleached specimens (G1, G2, G3, and G4) are shown in Table 2.

Table 2: Comparison between bleached specimens, varying time and concentration (Values in KHN)

Time	Concentration 16%	Concentration 22%
1 week	202,20 (55,40) Aa	172,10 (35,36) Aa
3 weeks	201,50 (57,40) Aa	135,10 (36,14) Ab

*Capital letters compare lines (time). Lowercase letters compare columns (concentration), 5% significance level by the Tukey's test

When 16% carbamide peroxide was used (G1 and G2), there was no significant hardness reduction between one and three bleaching sessions. The same occurred with 22% carbamide peroxide (G3 and G4). However, while comparing different peroxides concentrations, there was a significant hardness reduction when 22% carbamide peroxide was used in three sessions (G4).

The results of this study show that either 16% or 22% carbamide peroxide used for homemade dental bleaching reduced human enamel hardness and are in accordance to those of several authors[4,6,7,15-19].

However, some studies have not showed significant statistical reduction on enamel hardness using low concentration of bleaching agents (10%). These studies considered its use safe up to five weeks[20-25]. No studies were found using the same concentration of peroxide (22% for six weeks) as in our study.

Analyzing the concentrations of bleaching agents used in the present research, the results showed that 22% carbamide peroxide significantly impacted the hardness reduction only when used for prolonged periods (three sessions-G4). When used for 14 days (G3), there were no statistically significant changes other than those produced in specimens bleached by 16% carbamide peroxide (G1 and G2). This fact is supported by the changes on surface morphology, indicated by the loss of the aprismatic layer, depressions, erosion, and increase in the depth of the irregularities and pores as reported by Junqueira *et al.*[26]. Such changes, although microscopic, can make the enamel more fragile and the teeth more susceptible to sensitivity[27].

When 16% carbamide peroxide was used, there was no significant statistical difference due to the different number of bleaching sessions, since G1 and G2 did not differ. Studies corroborate this fact, showing that at low concentrations (up to 15%) the hardness reduction is not significant, but there is a change in the morphology of enamel[15,16,18,19].

Another fact to be considered is the influence of the storage solution. The artificial saliva, in which specimens are kept during the period between the applications of the bleaching agent, leads to remineralization of the bleached enamel surface[7], which could increase microhardness. However, this fact was not observed in our study, since there was microhardness reduction compared with the non-bleached enamel, which was also kept in the same condition.

4 Conclusion

Within the limitations of this *in vitro* study, we concluded that prolonged use of bleaching agents, especially in higher concentration, may reduce microhardness of enamel, thus possible alternatives to reverse this situation should be investigated.

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