

ORIGINAL ARTICLE

Changes in the stiffness of demineralized dentin following application of tooth whitening agents

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Abstract

Objective. The purpose of this study was to evaluate the effect of the bleaching agents on the elastic modulus of bovine demineralized dentin matrix (EMDM). **Materials and methods.** Eighty-five slices were obtained from 17 bovine teeth. The slices were divided randomly into five experimental groups ($n = 17$): unbleached control group (CG), 4% hydrogen peroxide (HP4), 4% hydrogen peroxide + 0.05% Ca (HP4 + Ca), 7.5% hydrogen peroxide + ACP (HP7.5) and 10% carbamide peroxide (CP10). The HP4, HP4 + Ca and CP10 groups were treated with the bleaching agents for 8 h/day (14 days), while the samples of HP7.5 group were exposed to bleaching agent for 30 min twice a day (14 days). The CG was kept in 100% humidity. After bleaching treatments, the enamel of the samples was removed and 85 dentin beams ($0.5 \times 1.7 \times 7.0$ mm) were prepared. Afterwards, the beams were immersed in 10% phosphoric acid solution (5 h) and rinsed with water (10 min). The beams were tested after 24 h, 7 and 14 days of storage in distilled water, using three-point bend method. Data were statistically analyzed using ANOVA and Fisher's test. **Results.** All bleaching treatments reduced the EMDM. After 14 days post-bleaching, the EMDM increased for HP4 and HP4+Ca groups. **Conclusions.** The use of bleaching agents promoted a decrease in EMDM, which indicates that the bleaching treatment interacts with the dentin organic matrix. The EMDM measurement for the specimens of the 7.5% hydrogen peroxide group that were immersed in water at 14 days post-bleaching did not recover the EMDM values when compared to the control group.

Key Words: elastic modulus, dentin, whitening

Introduction

Since its introduction, the nightguard whitening with low concentration of peroxides has been accepted as an efficient and simple aesthetic procedure for removing intrinsic and extrinsic tooth stain and to treat discolored teeth [1]. Moreover, the nightguard tooth whitening has gained popularity in recent years because it is an affordable and conservative technique to bleach teeth [2,3].

Changes in the mechanical properties of dental tissues have been reported after whitening treatment [4–8] and alterations to the enamel surfaces such as increased porosities, erosion and demineralization [9,10] has been reported in *in vitro* scanning electron microscopy (SEM) studies. The mechanism by which bleaching agents affect dentin is not well understood [11], however studies reported that hydrogen peroxide may cause dissolution of inorganic content with decreased calcium-phosphorus ratio and reduction in

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the organic components of dentin by protein oxidation or dentin denaturation [5,12,13,14].

While a negative effect of bleaching agents on the mechanical properties of undemineralized dentin has been reported using tensile strength tests, the influence of these agents in the mechanical properties of the organic matrix has not been reported. This study evaluated the effect of four whitening products used at different peroxides concentrations and compositions on the elastic modulus of demineralized coronal bovine dentin at 24 h, 7 and 14 days post-bleaching. The null hypothesis tested in this study was that the whitening would not affect the elastic properties of demineralized dentin when compared to an untreated group, regardless of the type of bleaching agent and post-bleaching evaluation time.

Materials and methods

Specimen preparation and bleaching

Seventeen extracted bovine incisor teeth were used in this study. After pumicing, they were stored in 0.1% thymol solution at 4°C for 30 days. The root portion was sectioned 1 mm below the cement-enamel-junction and discarded (Figure 1A). The crowns were serially sectioned into 0.5 mm ± 0.1 mm thick in the mesio-distal direction with a diamond blade saw (Series 15HC Diamond, Buehler, Lake Bluff, IL) under constant water irrigation (Figures 1B and C) to obtain 85 slices (five slices per tooth). The five slices from each tooth were allocated to one of the five experimental groups ($n = 17$). Except the buccal

enamel surfaces, other surfaces of dental slices were coated with two layers of nail varnish, with the exception of the buccal enamel surfaces (Revlon Inc., New York, NY).

The composition of the whitening products and the application regimens for the bleached groups were described in Table I. The slices of the control group were kept in water and were not subjected to any whitening treatment. The remained slices were daily exposed to a mixture of 0.1 mL of a bleaching agent with 0.05 mL of artificial saliva. During the treatment period, the slices were placed in 100% humidity at 37°C. After daily treatment, the slices were thoroughly rinsed with deionized water and stored in artificial saliva at 37°C.

Immediately after whitening, the enamel was removed and the slices were further trimmed using a cylindrical diamond bur (#557D, Brasseler, Savannah, GA) in a high speed handpiece (Impact Air 45tm, Palidases Dental, Englewood, NJ) to a final rectangular dimension of 0.5 mm thickness × 1.7 mm width × 7.0 mm length (Figure 1E). A dimple was made at one end of the surfaces to allow for repeated measurements to be performed on the same surface. Dentin specimens were immersed in 10% phosphoric acid solution (LabChem, Pittsburgh, PA) for a period of 5 h and thoroughly rinsed with distilled water for 10 min (Figure 1F) [15].

Three-point bend microflexural test

Specimens were tested in compression while immersed in distilled water using a universal testing

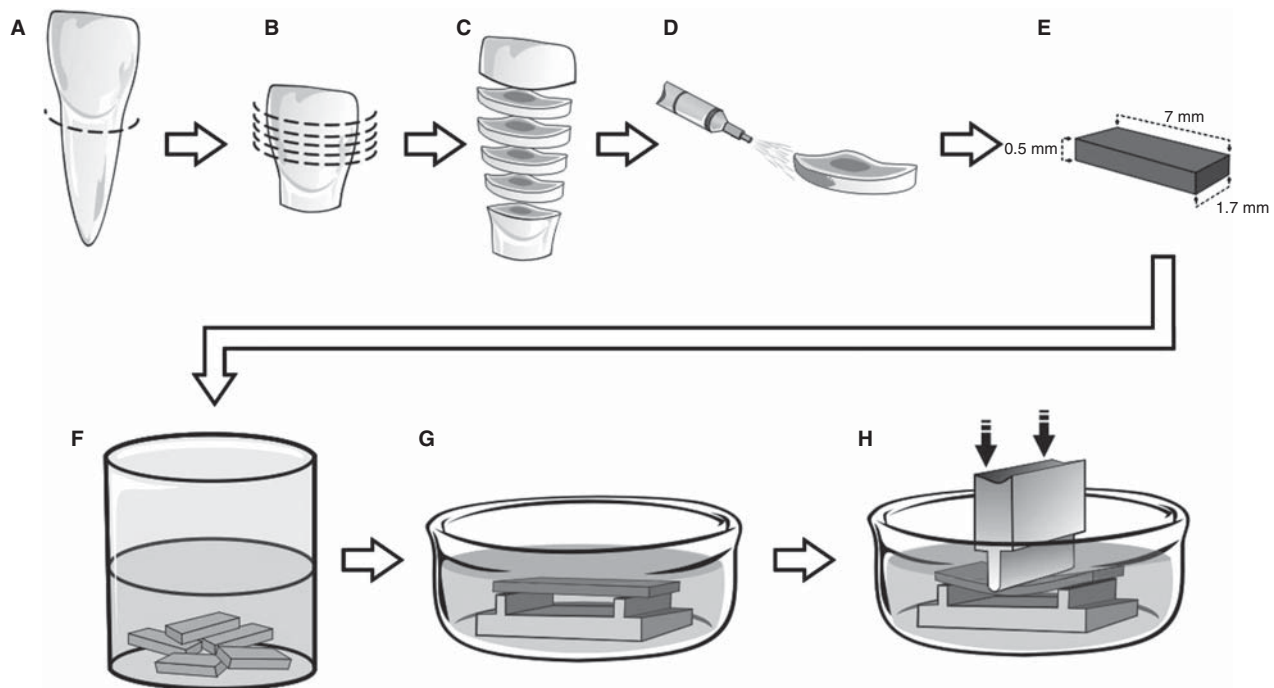


Figure 1. Specimen preparation (A, B, C), whitening treatments (D), final sample (E), specimens immersion in 10% phosphoric acid solution (F), three point bending testing (G, H).

Table I. Materials used in the study.

Bleaching agent	Main ingredient and concentration	Application time	Manufacturer
Experimental bleaching	4% Hydrogen Peroxide	8 hours daily (14 days)	FGM Prod. Odont. (Joinville, SC, Brazil)
Experimental bleaching	4% Hydrogen Peroxide (+ 0.05% Ca)	8 hours daily (14 days)	FGM Prod. Odont. (Joinville, SC, Brazil)
DayWhite ACP	7.5% Hydrogen Peroxide (+ ACP)	30 min, twice daily (14 days)	Discus Dental (Culver City, CA)
Opalescence	10% Carbamide Peroxide	8 hours daily (14 days)	Ultradent Products Inc, (South Jordan, UT)
No Bleaching	—	—	—

machine (EZ Graph, Shimadzu, Tokyo, Japan), with a 100 g load cell at crosshead speed of 0.5 mm/min (Figures 1G and H). Load–displacement curves were converted to stress–strain curves and the apparent elastic modulus calculated at 3% strain. Displacement (D) during compression was displayed in millimeters and calculated at a maximum strain of 3% using the following formula [16]:

$$D = \epsilon L^2 / 6T$$

where ϵ is strain, L is support span and T is thickness of the specimen. Then the modulus of elasticity (E) of the specimens was expressed in MPa (Mega Pascal) and calculated using the following formula [16]:

$$E = PL^3 / 4DbT$$

where P is the maximum load, L is the support span, D is the displacement, b is the width of the specimen and T is the thickness of the specimen.

The specimens were tested at three post-bleaching times: 24 h, 7 days and 14 days. Between the measurements the samples remained in distilled water at 37°C. The data were collected and statistically analyzed using two-way ANOVA (evaluation time \times treatments) repeated measurements at a 95% confidence interval and Fisher's PLSD test.

Results

The elastic modulus mean values and standard deviations (MPa \pm SD) are shown in Table II. Two-way ANOVA revealed that there were statistically significant differences for the factor 'treatments' ($p = 0.0008$), factor 'evaluation time' ($p = 0.0059$) and for the interaction between factors ($p = 0.0092$).

Fisher's PLSD test showed a higher elastic modulus for the control group when compared to the bleached groups tested at 24 h and 7 days post-bleaching ($p < 0.05$). At 24 h and 7 days post-bleaching, no statistically significant differences were observed among the bleached groups ($p > 0.05$). After 14 days of water storage, only the specimens treated with 7.5% hydrogen peroxide presented a lower elastic modulus when compared to the control group ($p < 0.05$). No statistically significant differences were observed among the bleached groups at 14 days post-bleaching ($p > 0.05$).

The control group, bleached with 7.5% hydrogen peroxide and 10% carbamide peroxide, had their elastic modulus constant between 24 h and 14 days ($p < 0.05$). Conversely, the dentin's elastic modulus for the groups bleached using 4% hydrogen peroxide with or without 0.05% calcium increased after 14 days of water storage ($p > 0.05$).

Discussion

Studies have shown that whitening treatments with peroxides can reduce the flexural strength and

Table II. Means (standard deviation) of the elastic modulus (MPa) of bovine demineralized dentin matrix.

Treatments	Time		
	24 h	7 days	14 days
4% Hydrogen Peroxide	2.0 (0.5) ^{B b}	2.0 (0.4) ^{B b}	2.5 (0.6) ^{AB a}
7.5% Hydrogen Peroxide +ACP	2.3 (0.8) ^{B a}	2.2 (0.7) ^{B a}	2.3 (1.0) ^{B a}
4% Hydrogen Peroxide + 0.05%Ca	2.5 (0.7) ^{B b}	2.1 (0.4) ^{B c}	2.7 (0.6) ^{AB a}
10% Carbamide Peroxide	2.5 (0.9) ^{B a}	2.5 (0.7) ^{B a}	2.5 (0.6) ^{AB a}
Control Group	3.3 (0.8) ^{A a}	3.0 (0.8) ^{A a}	3.0 (1.0) ^{A a}

Means followed by different letters, capital letters in columns and lower case letters in rows are statistically different (Fisher test, $p < 0.05$).

modulus of mineralized dentin. In those studies, mineralized and sound bovine dentin presented ~ 140–220 MPa of flexural strength, while the flexural modulus was 12–14 MPa [8,17,18]. Maciel et al. [19] and Bedran-Russo et al. [15] indicate that the elastic modulus of decalcified human dentin matrix in water can be varied between 5–7 MPa. This current study used bovine teeth and the elastic modulus of untreated demineralized dentin (control group) was ~ 3 MPa, which represented half of that for human dentin value. The results may partially reflect the compositional and morphological differences between human and bovine teeth. In this study, all bleaching agents reduced the dentin elastic modulus until 7 days post-bleaching when compared to an unbleached control group; however, at 14 days post-bleaching, the bleaching agents did not differ from the control group, except for the group treated with 7.5% hydrogen peroxide. Since the elastic properties of demineralized dentin changed after whitening for the four tested materials and most groups recovered or had similar values to the control group at 14 days of water storage, the null hypothesis tested in this study was rejected.

Studies have suggested that bleaching agents are capable of diffusing through enamel and dentin [20–22]. This study confirms the ability of peroxides to diffuse and generate free radicals, which interact with organic structures, since the enamel was kept intact during whitening regimens. Free radical reactions are not specific and can potentially react with other organic structures [4]. The peroxides may cause alteration in the chemical structure of the dentin and reduction in potassium levels and in the Ca/P ratio [13,14]. Moreover, compromised mechanical properties [5,23] and increased dentine permeability [24] following whitening treatment have been reported.

Although the peroxide's compositions and concentrations differed among the whitening products, the elastic modulus of dentin at 24 h, 7 and 14 days post-bleaching were similar among bleached groups. The two materials that contained calcium (7.5% hydrogen peroxide + ACP (1–5% amorphous calcium phosphate and 4% hydrogen peroxide + 0.05% calcium) had different application modes, but presented similar results. The elastic modulus for the group treated with the bleaching agent containing 7.5% hydrogen peroxide plus ACP was lower than the control group 14 days post-bleaching. The higher peroxide concentration in the gel composition may be accountable for the effect observed in demineralized dentin. The addition of calcium and fluoride to bleaching agents have been proposed to reduce the mineral loss during whitening with little influence on the organic components of dental tissues [25,26]. The application of 10% carbamide peroxide releases 3% hydrogen peroxide and ~ 7% urea. This bleaching agent contains the lowest peroxide concentration among the materials

and the urea released may provide beneficial effects due to the rise of pH that reduces the demineralization level [4,27]. Urea is a protein destabilizer with the ability to break covalent and hydrogen bonds [17,18,28], leading to proteoglycans and type I collagen structural changes and alterations on the physical properties of dental structures.

It has been reported that the color of teeth comes from the organic content of dentin [29,30], which can be attacked by components of the bleaching products. The results of this study showed that the alterations on elasticity modulus of dentin post-bleaching are due to the effects on the organic matrix, as a secondary or adverse effect produced by the whitening products. The weakening of dental structures has been considered as one of the main causes of bond strength reduction reported immediately after bleaching [31,32]. The recurrence of stains or tooth discoloration may occur in time as a function of the reversal effect on the dentin organic matrix promoted by bleaching agent, as well as the residual oxygen from the peroxides may impair the recovery on the elasticity modulus values of dentin until 7 days post-bleaching. After 14 days of storage in water, the concentration of oxygen was reduced or eliminated, which could favor the reversal of compromised elastic modulus values of dentin. This study evaluated a mechanical property of dentin post-bleaching; however, biochemistry analysis would be necessary to confirm the 'reversible' effect.

While studies [8,17,18] have analyzed the effect of whitening on the flexural modulus of mineralized and intact dentin, the present study removed the mineral content and evaluated the effects of different bleaching agents on the elastic modulus of only the decalcified dentin matrix. The present studies highlight the importance of the mechanical properties of the organic dentin matrix on the overall properties of dentin.

Conclusions

In conclusion, when the specimens were tested immediately after whitening, a reduction of the elastic modulus of demineralized dentin matrices was observed. However, after 14 days storage in water, most of the bleached groups had a similar elastic modulus to the unbleached control group, demonstrating that the effects produced by some bleaching agents can be reversible by storage in water.

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