

## ORIGINAL RESEARCH

# Effect of Bleaching Agents on the Flexural Strength of Bovine Dentin

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## ABSTRACT

The aim of this study was to evaluate the effect of bleaching on the flexural strength of bovine dentin, using bleaching agents containing calcium (Whiteness HP Blue and Whiteness Class) and not containing calcium (Whiteness HP and Whiteness Perfect). Sixty bovine incisor tooth slices were obtained and divided into five experimental groups (n = 12), such as G1, untreated control group; G2, Whiteness HP Max [35% hydrogen peroxide (HP)]; G3, Whiteness HP Blue (35% HP); G4, Whiteness Class (7.5% HP) and G5, Whiteness Perfect (22% carbamide peroxide). Samples were submitted to bleaching treatment according to the manufacturers' instructions. The control group remained in artificial saliva during bleaching. After bleaching, the enamel was removed and dentin specimens (7 mm length × 1.7 mm width × 0.5 mm thickness) were prepared. Samples were tested in a universal testing machine (EMIC). Data were analyzed by analysis variance (ANOVA) and Tukey's test. Differences between the groups were identified by ANOVA. The mean values (± standard deviation) for the experimental groups, such as (in MPa) G1 = 19.05 ± 2.68 a; G2 = 12.69 ± 4.52 b; G3 = 16.39 ± 3.74 ab; G4 = 14.90 ± 5.60 ab; and G5 = 12.71 ± 2.25 b. Groups with the same lowercase letter were significantly different from each other. The presence of calcium in bleaching agents appeared to influence the flexural strength of bovine dentin after bleaching treatment, for both office bleaching (35% HP) and home bleaching (7.5% HP).

**Keywords:** Flexural strength, Carbamide peroxide, Hydrogen peroxide, Vital bleaching.

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## INTRODUCTION

Dental bleaching is achieved through an oxidation-reduction reaction. Reactive oxygen species and some free radicals are released from the process of bleaching agent degradation. These species attack the long chained, dark-colored chromophore molecules present in the dental tissues, breaking them down into smaller, less colored and more diffusible molecules, and producing the whitening effect.<sup>1,2</sup> Carbamide peroxide (CP) dissociates into hydrogen peroxide (HP) and urea when in contact with soft tissue or saliva at oral temperatures. Peroxide can diffuse through enamel and dentin due to its low molecular weight. HP further degrades into oxygen and water, whereas urea degrades into ammonia and carbon dioxide.<sup>3,4</sup>

The mechanical properties of dentin are of significant interest because dentin provides the bases for both enamel and cementum. Moreover, dentin is largely responsible for the structural integrity of the entire tooth. Slight changes in surface morphology and hardness have been identified in dentin after bleaching.<sup>5,6</sup> Several papers have hypothesized that HP and CP cause changes to the organic component of dentin by a mechanism of dentin denaturation or protein oxidation.<sup>7-9</sup> Protein degradation has been reported in nondental applications after treatment with HP or other antioxidants. Urea is an important component of CP and is commonly used in the laboratory as a protein-denaturing agent.<sup>10</sup>

Several studies have evaluated the mechanical properties of bleached dentin.<sup>2,11,12</sup> For example, Berger et al<sup>11</sup> evaluated the effect of bleaching agents used at different peroxide concentrations and compositions on the elastic modulus of demineralized bovine dentin. Tam et al<sup>13</sup> determined the effects of direct and indirect CP bleaching on the flexural strength and elastic modulus of dentin. However, the effect of calcium added by manufacturers to bleaching agents has not been reported. Therefore, the purpose of this study was to evaluate the effect of bleaching on the flexural strength of bovine dentin when bleaching was performed using agents containing calcium (Whiteness HP Blue and Whiteness Class) or not containing calcium (Whiteness HP and Whiteness Perfect).

## MATERIALS AND METHODS

### Specimen Preparation and Bleaching

About 12 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. The crowns were serially sectioned into thicknesses of  $0.5 \pm 0.1$  mm in the mesiodistal direction with a diamond blade saw (Series 15 HC Diamond, Buehler, Lake Bluff, IL, USA) under constant water irrigation to obtain 60 slices. The five slices from each tooth were allocated to one of the five experimental groups ( $n = 12$ ). Except for the buccal enamel surfaces, the surfaces of the dental slices were coated with two layers of nail varnish (Revlon Inc, New York, NY, USA).

The experimental groups, compositions of bleaching agents and application regimens are detailed in Table 1. Slices of the control group were kept in water and not subjected to any bleaching treatment. The remaining slices were exposed daily to bleaching agents, according to the manufacturers' instructions (refer Table 1). During the treatment period, slices were kept at 100% humidity and 37°C. After daily treatment, slices were thoroughly rinsed with deionized water. Immediately after bleaching, the enamel was removed and the slices were trimmed using a cylindrical diamond bur (#1095, KG Sorensen, Cotia, SP, Brazil) in a high-speed handpiece (Kavo, Joinville, SC, Brazil) to a final rectangular dimension of 0.5 mm thickness  $\times$  1.7 mm width  $\times$  7.0 mm length.

### Flexural Strength Test

For each sample, the dimensions were inputted into the software. Then, a three-point bending flexural test was

performed in a universal testing machine (DL2000, EMIC, Equipamentos e Sistemas de Ensaio LTDA, São José dos Pinhais, PR, Brazil). The load cell was applied perpendicular to the long axis of the specimen with a 0.5 mm/min crosshead speed until fracture. The dentin flexural strength (FS) was calculated using the equation  $FS = 3 F_{max} L / 2 bd^2$ , where  $F_{max}$  is the maximum load (in Newtons, N),  $L$  is the distance between support points (in mm), and  $b$  and  $d$  are the specimen width and thickness (in mm), respectively.

### Statistical Analysis

Means and standard deviations (SDs) of the dentin flexural strength were calculated. The Kolmogorov-Smirnov test was used to verify the normal distribution of the data. Then, data were compared by one-way analysis of variance (ANOVA) and Tukey's test. Differences with a  $p$ -value  $< 0.05$  were considered statistically significant.

## RESULTS

The means (in MPa) and SDs of dentin flexural strength after the different bleaching protocols are detailed in Table 2. ANOVA showed statistically significant differences ( $p = 0.0033$ ) among the bleaching agents. The mean flexural strength of the Control group (group 1) was significantly greater than the flexural strength of the group treated with Whiteness HP Maxx (group 2) or Whiteness Perfect (group 5) ( $p < 0.05$ , Tukey's test), but was not significantly different from the flexural strength of the group treated with Whiteness HP Blue (group 3) or Whiteness Class (group 4). The flexural strength values of all of the treated groups were statistically similar to each other.

**Table 1:** Material used in the study

Groups	Bleaching agent	Composition	Application time*	Manufacturer/Lot number
1	Control Group	No bleaching agent was used	The samples were maintained in distilled water until the test	—
2	Whiteness HP Maxx	35% hydrogen peroxide, thickeners, dye mixture, glycol, inorganic load and deionized water	Three applications of 15 minutes each	FGM Prod. Odont., Joinville, SC, Brazil/230211
3	Whiteness HP Blue	35% hydrogen peroxide, thickeners, inert violet pigment, neutralizing agent, calcium gluconate, glycol and deionized water	Three applications of 15 minutes each	FGM Prod. Odont., Joinville, SC, Brazil/150811
4	Whiteness Class	7.5% hydrogen peroxide gel, neutralized carbopol, potassium nitrate, sodium fluoride, aloe vera, calcium gluconate, stabilizer, deionized water and surfactant	Six hours daily for 14 days	FGM Prod. Odont., Joinville, SC, Brazil/270711
5	Whiteness Perfect	22% carbamide peroxide, neutralized carbopol, potassium nitrate, sodium fluoride, humectant (glycol), deionized water	Six hours daily for 14 days	FGM Prod. Odont., Joinville, SC, Brazil/280711

\*According to the manufacturer's instruction

**Table 2:** Means (standard deviation) of dentin flexural strength according experimental groups

Experimental groups	Flexural strength
Group 1: Control Group	19.05 (2.68) a
Group 2: Whiteness HP Maxx	12.69 (4.52) b
Group 3: Whiteness HP Blue	16.39 (3.74) ab
Group 4: Whiteness Class	14.90 (5.60) ab
Group 5: Whiteness Perfect	12.71 (2.25) b

Means followed by same letters indicate statistical similarity within columns ( $p < 0.05$ )

## DISCUSSION

Previous investigations into the effects of whitening agents have indicated changes with treatments.<sup>2,11,12,14,15</sup> According to Tam et al (2013),<sup>13</sup> bleaching procedures have a significant influence on the flexural strength and elastic modulus of intact dentin. When root exposure and occlusal attrition occur, the bleaching agent is directly applied to the dentin surface. Changes in mechanical properties indicate that structural alterations are likely to occur in the enamel-dentin complex exposed to bleaching agent.<sup>16</sup>

Natural enamel, as a hard and friable tissue, has a low flexural strength (high elastic modulus); however, enamel supported by dentin is naturally protected by tissue with a lower elastic modulus. Therefore, because dentin provides a supporting base for both enamel and cementum, it is largely responsible for the structural integrity of the entire tooth. When applied for 30 minutes, HP at different concentrations (10%, 35% and 50%) has been demonstrated to penetrate through the mineralized tissues of bovine teeth, increasing the dental permeability.<sup>17</sup> However, some factors, such as the enamel thickness, existence of enamel fractures and cracks, permeability, and direction of the dentinal tubules, influence the diffusion of bleaching agents through dentin.<sup>12</sup> Peroxide treatment may alter the chemical structure of dentin, reduce the potassium levels and affect the Calcium-potassium ratio.<sup>7,8</sup> Thus, some manufactures of bleaching agents have added calcium and fluoride to the agents. This approach reduces mineral loss during bleaching treatment, with little influence on the organic components of dental tissues.<sup>18</sup>

In the present study, four different bleaching agents, two for office use and two for home use, were evaluated. Calcium gluconate is present in the composition of Whiteness HP blue (group 3) and Whiteness class (group 4) (Table 1). The flexural strength results (Table 2) revealed no significant difference among the treated groups. However, groups treated with bleaching agents without calcium (groups 2 and 5) had flexural strength values that were significantly lower compared to the untreated Control Group (group 1), whereas the groups treated with bleaching agents containing calcium (groups 3 and 4) had values that were similar to those of the control group.

Ghavamsari et al<sup>16</sup> also evaluated the flexural strength of bovine dentin and found no statistical difference between the treated groups and control group. In contrast, Tam et al (2005)<sup>12</sup> demonstrated that the direct *in vitro* application of bleaching agent decreased the flexural strength of dentin after 2 months. In the present study, the groups that had the lowest flexural strength values and were different from the control group were treated with Whiteness HP Maxx and Whiteness Perfect (35% HP and 22% CP respectively). The urea present in CP is a common protein-denaturing agent.<sup>19</sup> Therefore, it was expected that the group treated with CP (Whiteness Perfect) would have low flexural strength. Tam et al (2005)<sup>12</sup> showed that CP had more deleterious effects on dentin flexural strength than HP agents.

Thus, as reported previously, the bleaching agent was capable of diffusing through enamel and dentin.<sup>17</sup> The groups treated with Whiteness HP Blue and Whiteness Class showed better results than the other experimental groups. It may be that the calcium present in these bleaching agents decreased the penetration of HP into the dentin, resulting in fewer effects on flexural strength. However, further studies are necessary to verify the effects of added calcium.

A limitation of this study is that it was performed *in vitro*. As reported by Khoroushi et al (2011),<sup>15</sup> the effects of bleaching agents on the tooth structure *in vitro* are generally more than those observed clinically. This difference is due to the presence of biological factors, such as the buffering properties of saliva and the outward flow of intratubular fluids, which decreases the diffusion of bleaching agent through dentin.

## CONCLUSION

The presence of calcium in bleaching agents influenced the flexural strength of bovine dentin after bleaching treatment, for both office bleaching (35% HP) and home bleaching (7.5% HP).

## CLINICAL SIGNIFICANCE

The presence of calcium in bleaching agents decreases the effects of bleaching agents on flexural strength.

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