Flexural Strength and Modulus Properties of Carbamide Peroxide–Treated Bovine Dentin

LAURA EVA TAM, DDS, MSC*
RYAN ABDOOL, BSC*
WAFA EL-BADRAWY, DDS, MSC*

ABSTRACT

Background: The effects of carbamide peroxide bleach on tooth structure are uncertain.

Purpose: The purpose of this study was to investigate the effects of direct or indirect carbamide peroxide application on dentin flexural strength and modulus in vitro.

Materials and Methods: Dentin bars were machined from bovine incisors and treated with 10% carbamide peroxide for 6 h/d for 14 days. Four groups were created, with eight bars in each group: group 1—direct bleach application only; group 2—indirect bleach application only; group 3—direct bleach application followed by a 2-week storage in artificial saliva; group 4—direct bleach application followed by a 2-week storage in artificial saliva and daily topical fluoride treatments. The specimens' flexural strength and modulus were tested after 24 hours, and the results were compared with water controls using independent *t*-tests ($p < .05$).

Results: Flexural strength results (in megapascals) for bleached versus control dentin (1 = 205 ± 26 vs 215 ± 14, 2 = 257 ± 25 vs 261 ± 14, 3 = 180 ± 22 vs 193 ± 36, 4 = 157 ± 18 vs 184 ± 11) were significantly lower in group 4 ($p = .005$). Modulus results (in gigapascals) for bleached versus control dentin (1 = 10.7 ± 1.4 vs 12.2 ± 0.6, 2 = 14.2 ± 1.7 vs 14.4 ± 1.6, 3 = 10.0 ± 2.0 vs 10.9 ± 1.3, 4 = 9.0 ± 1.2 vs 11.1 ± 1.0) were significantly lower in groups 1 and 4 ($p = .013$ and $p = .003$, respectively). There were no significant differences in strength and modulus results between the bleached and control dentin in groups 2 and 3. A direct application of carbamide peroxide to bovine dentin significantly decreased some dentin mechanical properties in vitro. An indirect application of carbamide peroxide to dentin did not significantly decrease dentin strength and stiffness.

CLINICAL SIGNIFICANCE

This in vitro study suggests that a bleaching treatment, when applied to the enamel of intact teeth, does not significantly affect the mechanical properties of the underlying dentin. However, when applied directly to dentin in clinical situations such as root exposure or occlusal attrition, the bleaching treatment may result in altered mechanical properties of dentin. The clinical consequences of this observation are, however, unknown.

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**Restorative Dentistry, Faculty of Dentistry, University of Toronto, Toronto, ON, Canada*

Vital tooth bleaching is an increasingly popular cosmetic procedure in dentistry. The at-home bleaching procedure generally involves the application of a 10% carbamide peroxide (or hydrogen peroxide equivalent) product by the patient to the surfaces of the teeth for a period of 0.5 to 8.0 hours daily over several weeks under the supervision of a dentist. Clinical studies have evaluated the at-home bleaching procedure for adverse effects and effectiveness.[1–3] Laboratory studies have reported that hydrogen peroxide and carbamide peroxide bleaching agents cause changes to the morphology and hardness of the enamel surface, but these studies are conflicting and suggest changes primarily to the outer few microns of enamel only.[4–16] Hence, it is generally presumed that the elective tooth-bleaching procedure causes no significant long-term adverse effects to tooth structure.

Material strength is a measure of a material's resistance to fracture and can be represented by the flexural strength parameter. Modulus parameters are a measure of the stiffness of a material. Both strength and modulus parameters are affected by the stress field and flaw distribution within the structure, as well as by environmental factors. It is important to determine the effects of carbamide peroxide bleach on the structural integrity of the dentin beyond the surface that is subjected to patient-applied bleach. Changes to dentin beyond the surface, represented partially by changes in flexural strength and modulus of elasticity, would be less reversible than changes to the outer few microns of enamel and dentin. Reported values for flexural strength for dentin range from 245 to 280 MPa.[17] Reported values for modulus of elasticity for dentin range from 7 to 30 GPa.[17,18] A previous study showed a significant reduction in bovine dentin flexural strength and modulus of elasticity after a 2-week direct application of 10% and 15% carbamide peroxide.[19] Chng and colleagues have previously shown significant decreases in the ultimate tensile and micropunch shear strengths of dentin after an intracoronal bleach application of 30% hydrogen peroxide.[20]

The aim of this study was to determine, in vitro, the effects of direct and indirect carbamide peroxide bleaching on the flexural strength and modulus of elasticity of dentin. This study also investigated the effects of artificial saliva and a topical fluoride gel on bleached dentin to determine whether any reductions in dentin mechanical properties caused by the bleach treatment were reversible. This study should provide new information relevant to the long-term effects of vital tooth bleaching on tooth structural integrity.

MATERIALS AND METHODS

Dentin rectangular bar specimens (n = 8/group) measuring approximately 20 × 2 × 2 mm were machined from previously extracted, cleaned, and frozen bovine incisors using an Accutom low-speed saw (Struers, Westlake, OH, USA). Previous studies have shown that frozen storage did not significantly affect the tensile strength of or the shear bond strengths to bovine dentin.[21,22] The incisors were extracted from cows that were at a minimum age of 2 years old to ensure that the incisors were large enough to obtain specimens of the required dimension. The machined specimens were stored in distilled water at 4°C until the start of the immersion protocol.

Opalescence (Ultradent Products Inc, South Jordan, UT, USA; lot no. 5496; pH 6.4), a commercially available at-home bleaching product containing 10% carbamide peroxide, was used for all bleach immersion protocols in this investigation. Four immersion protocols were followed and are shown in Table 1. During the bleach immersion period, the specimens were completely surrounded by bleach (minimum 1 mm thickness) and were stored at 37°C in an incubator with ≥ 80% relative humidity for 6 continuous hours. Following each bleach immersion period, the specimens were rinsed thoroughly with distilled water to remove all external traces of the bleach and were then stored at 37°C in a container of distilled water (pH 7.4) until the following bleach immersion. The bleach immersion protocol was repeated daily for 14 days.

TABLE 1. PROTOCOLS FOR SPECIMEN AND POST-BLEACH TREATMENT.

Immersion Protocol Group No.	Treatment Application Method	Material Treatment	Post-Bleach Treatment	Post-Bleach Storage Solution
1	Directly onto dentin	10% CP 6 h/d for 2 wk	Nil	Nil
	Directly onto dentin	DW 6 h/d for 2 wk	Nil	Nil
2	Indirectly onto dentin through enamel	10% CP 6 h/d for 2 wk	Nil	Nil
	Indirectly onto dentin through enamel	DW 6 h/d for 2 wk	Nil	Nil
3	Directly onto dentin	10% CP 6 h/d for 2 wk	Nil	Artificial saliva
	Directly onto dentin	DW 6 h/d for 2 wk	Nil	Artificial saliva
4	Directly onto dentin	10% CP 6 h/d for 2 wk	1.1% NaF 30 min/d for 2 wk	Artificial saliva
	Directly onto dentin	DW 6 h/d or 2 wk	1.1% NaF 30 min/d for 2 wk	Artificial saliva

CP = carbamide peroxide; DW = distilled water (control).

To investigate the effect of indirect bleach application to dentin (as opposed to the direct placement of bleach on the dentin surface) and to mimic a more clinically relevant situation, bleach was applied to the enamel of intact incisors prior to specimen preparation in group 2. Intact incisors were placed in storage bottles filled with distilled water. The necks of each bottle were fitted with wax so that the root of the specimen, up to the cementoenamel junction, was immersed in water while bleach was applied to the enamel surface (minimum 1 mm thickness). The specimens were stored at 37°C in an incubator with ≥ 80% relative humidity for 6 continuous hours. Following each bleach immersion, the crowns of the specimens were rinsed thoroughly with distilled water to remove the bleach and the teeth were then stored at 37°C in a container of distilled water until the next bleach immersion. The rectangular dentin bar specimens were machined 24 hours after the last bleach immersion.

To investigate the remineralization potential of dentin in saliva and a topically applied fluoride, specimens from groups 3 and 4 were subjected to a post-bleach treatment. Following the initial 14-day bleach immersion protocol, group 3 and 4 specimens were stored for 2 more weeks in an artificial saliva solution (pH 7.0) in an incubator at 37°C with ≥ 80% relative humidity, without or with a daily topical fluoride treatment, respectively.[23] The fluoride treatment consisted of immersion into a plastic container of fluoride (Neutragel, Germiphene Corporation, Brantford, ON, Canada; 1.1% NaF stabilized gel base providing 0.5% fluoride ion; pH 7) for 30 minutes daily for 2 weeks. The fluoridation time was chosen to simulate the clinical application of fluoride in bleaching trays to treat tooth sensitivity during bleach treatment. Following each fluoride immersion, specimens were rinsed thoroughly with distilled water to remove all external traces of the fluoride gel. The artificial saliva solution that was used for storage was changed once daily.

Control specimens (n = 8/group), for which distilled water was used instead of bleach, were included for each immersion protocol (groups 1–4).

The specimens' flexural strength and modulus of elasticity were tested 24 hours after the last bleach or post-bleach treatment. The precise dimensions of the rectangular dentin bar specimens were measured using a digital micrometer to an accuracy of 0.01 mm at three locations along the specimens' width and height. The specimens were then subjected to a three-point-bend test on a Model 4301 Universal uniaxial servo mechanical testing machine (Instron Corporation, Canton, MA, USA) that applied force at the center of the specimen at a crosshead speed of 0.75 mm/min (Figure 1). The specimens were loaded in a parallel direction relative to the dentinal tubules in either an "in-plane" or "antiplane" direction as described by Nalla and colleagues.[24] The effect of the two different directions of loading was considered small since Nalla and colleagues reported no significant differences between the in-plane parallel and antiplane parallel fracture toughness of dentin.[24]

The mounting apparatus consisted of two rods (2 mm in diameter), mounted parallel with 17 mm between the centers. During testing, the mounting apparatus was immersed in a 37 ± 1°C water bath. The maximum load supported by the specimen prior to failure was used to calculate flexural strength. The straight line portion of the load-deformation profile was used to calculate the modulus of elasticity. The flexural strength and modulus of elasticity results of the bleached specimens were compared with those of the distilled water control specimens were using independent *t*-tests ($p \leq .05$). Analysis of variance was additionally used to test for the significance and interaction of the factors, material and application method for groups 1 and 2, and material and post-bleach treatment method for groups 3 and 4.

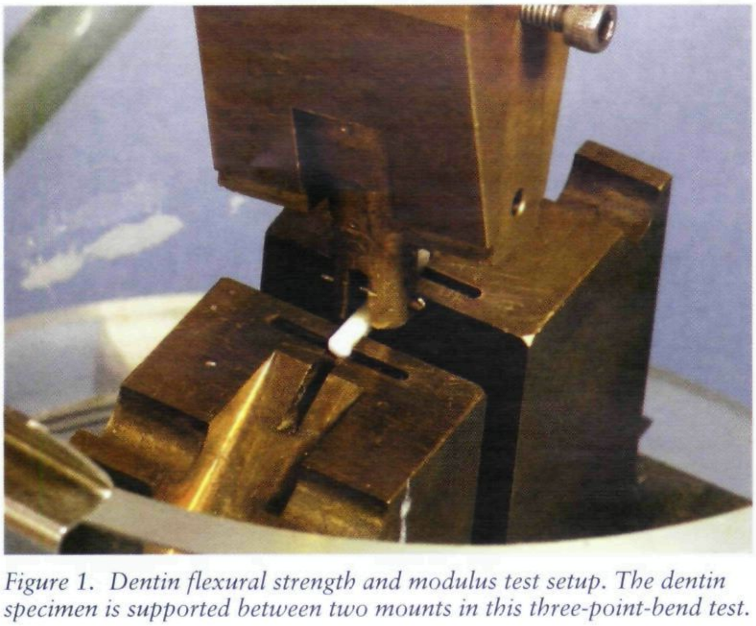

Figure 1. Dentin flexural strength and modulus test setup. The dentin specimen is supported between two mounts in this three-point-bend test.

RESULTS

Figures 2 to 5 show the mean flexural strength and modulus results for groups 1 to 4. The flexural modulus of elasticity was significantly lower for the bleached specimens compared with control specimens in group 1 ($p = .013$). When the bleach treatment was applied indirectly through intact enamel in group 2, there were no significant differences in the mean flexural strength and modulus results between the bleach and control specimens ($p = .721$ and $p = .802$, respectively). There were no significant differences in the mean flexural strength and modulus results between the bleach and control specimens after artificial saliva storage ($p = .384$ and $p = .361$, respectively). The flexural strength and modulus of elasticity for the bleached specimens were both significantly lower than the control specimens in group 4 ($p = .005$ and $p = .003$, respectively).

The application method (direct vs indirect) was a significant factor without interaction for both flexural strength and modulus results for groups 1 and 2 ($p < .0001$). The material (bleach vs control) was a significant factor without interaction for both flexural strength and modulus results for groups 3 and 4 ($p = .010$ and $p = .028$, respectively).

DISCUSSION

The results of this in vitro study indicated some reduction in dentin mechanical properties after the direct application of 10% carbamide peroxide to dentin but not after the indirect application of 10% carbamide peroxide to dentin.

The observed reduction in dentin mechanical properties could also be attributed to changes caused by carbamide peroxide in the inorganic or organic component of dentin. Collagen fibrils comprise 30% by volume of dentin and act as the scaffold for hydroxyapatite mineral. It is possible that some dentin demineralization occurred as a result of the exposure to the carbamide peroxide bleach with a pH of 6.4. Studies

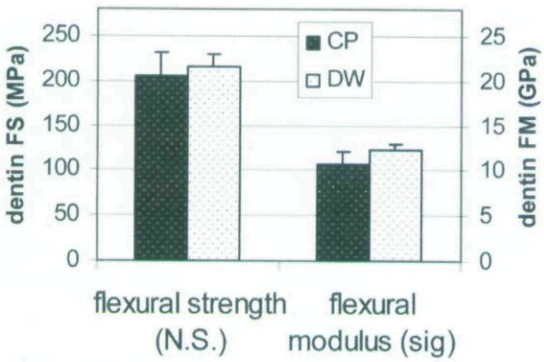

Figure 2. Dentin flexural strength (FS) and modulus (FM) results (mean and SD) comparing bleached versus control for group 1 (direct application of bleach for 2 weeks). The modulus of elasticity was significantly lower for the bleached specimens compared with control specimens ("sig," p = . 013). "N.S." denotes no significant difference (p < .05) between the bleach and control groups. CP = 10% carbamide peroxide; DW = distilled water (control).

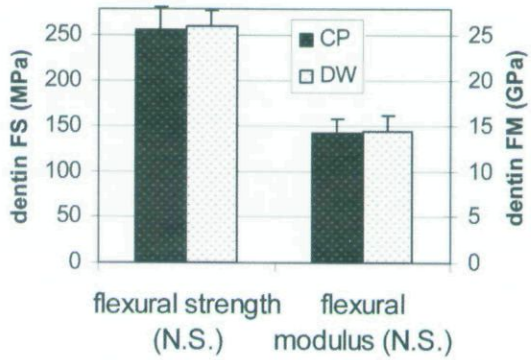

Figure 3. Dentin flexural strength (FS) and modulus (FM) results (mean and SD) comparing bleached versus control for group 2 (indirect application of bleach for 2 weeks). There were no significant differences in the flexural strength and modulus results between the bleach and control dentin specimens (p = .721 and p = .802, respectively). "N.S." denotes no significant difference (p < .05) between the bleach and control groups. CP = 10% carbamide peroxide; DW = distilled water (control).

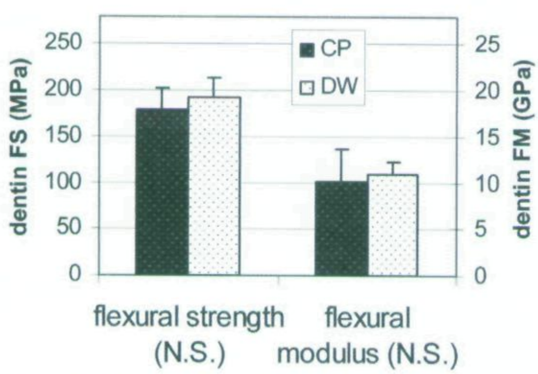

Figure 4. Dentin flexural strength (FS) and modulus (FM) results (mean and SD) comparing bleached versus control for group 3 (direct application of bleach for 2 weeks followed by storage in artificial saliva for 2 weeks). There were no significant differences in the flexural strength and modulus results between the bleach and control dentin specimens (p = .384 and p = .361, respectively). "N.S." denotes no significant difference (p < .05) between the bleach and control groups. CP = 10% carbamide peroxide; DW = distilled water (control).

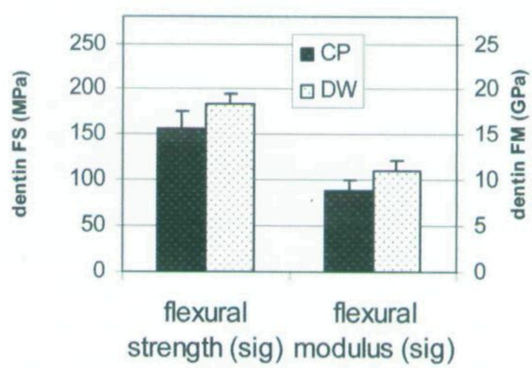

Figure 5. Flexural strength (FS) and modulus (FM) results (mean and SD) comparing bleached versus control for group 4 (direct application of bleach for 2 weeks followed by storage in artificial saliva for 2 weeks with a daily topical fluoride treatment). The flexural strength and modulus results were significantly lower for the bleached specimens compared with control specimens ("sig," p = .005 and p = .003, respectively). CP = 10% carbamide peroxide; DW = distilled water (control).

have reported chemical and physical evidence of enamel and dentin demineralization to some degree following bleach application.[25–27]

Carbamide peroxide contains both urea and hydrogen peroxide, and both urea and hydrogen peroxide (as an oxidant) have been associated with proteolytic activity.[28,29] With regard to enamel, it was hypothesized that bleaching agents containing urea and peroxide affected the organic phase of enamel.[30,31] Little is known about the effects of hydrogen peroxide on dentinal collagen. An attempt to determine the effects of both hydrogen peroxide and carbamide peroxide on collagen is presently underway in our laboratory. Changes to dentinal collagen would be of concern because these changes would not be easily reversible.

The application of bleach to enamel, as opposed to dentin, is the common clinical practice. When the bleach treatment was applied to dentin indirectly through intact enamel, there were no significant differences between the bleach and control groups in dentin flexural strength and modulus of elasticity results. Within the limitations of this study, the results indicate that a 2-week bleaching regimen using 10% carbamide peroxide causes insignificant changes to dentin mechanical properties when the bleach is applied to the outer intact enamel surface. This suggests that, although it has been shown that peroxide penetrates through enamel and dentin through a capillary rise in enamel interprismatic spaces, convective mass transfer, or classic molecular diffusion based on a random molecular path, and forms measurable amounts of bleach within the tooth pulp, enamel can reduce the effects of hydrogen peroxide on dentin.[32–34]

It was interesting to note that the dentin flexural strength and modulus of elasticity results were highest in group 2 and lowest in groups 3 and 4. The results for flexural strength for the dentin in group 2 in this study are similar to those reported elsewhere.[17] The results for modulus of elasticity for the dentin in group 2 in this study are lower than that reported for human dentin by Craig and colleagues but are similar to those reported for bovine dentin by Sano and colleagues.[18,35] In a review of the mechanical properties of human dentin, similar low values of modulus were attributed to dentin mineral dissolution as a result of specimen storage in water.[36] There is the potential for some dentin demineralization to occur during water storage because distilled water does not contain calcium and phosphate ions.[36,37] It appears that the length of specimen storage time after specimen preparation affected the dentin flexural strength and modulus results in this study. The length of specimen storage time after specimen preparation was longest in groups 3 and 4 and shortest in group 2. The higher mean strength and modulus results for both the bleach and control specimens in group 2 are likely related to an effect of reduced water storage.

The treatments for both groups 3 and 4 were instituted to address the question of whether the dentin could be remineralized, on the assumption that some demineralization occurred during bleach exposure, and whether any reduction in dentin flexural strength and modulus of elasticity caused by bleach treatment could be reversed. The additional 2-week storage period without bleach treatment also provided time for residual bleach to diffuse out of the dentin. Although the effects of residual bleach have been shown to affect subsequent bonding for approximately 1 week,[38,39] the effect of residual bleach on dentin mechanical properties is unknown.

Saliva is normally supersaturated with respect to hydroxyapatite at neutral pH.[37] Precipitates were observed on enamel specimens exposed to natural saliva after tooth bleaching in vitro, and these precipitates were presumed to occur as a result of remineralization in saliva.[40] The remineralization potential of saliva on bleached teeth has been demonstrated by the restoration of enamel microhardness following the cessation of bleach treatment.[41,42] A similar effect of saliva storage was also shown on dentin microhardness 14 days after the cessation of bleach treatment.[43] The results from this study do not suggest a similar effect from a post-bleach treatment of artificial saliva. The "no significant difference" result in the mean modulus of elasticity between the bleached and control groups in the specimens exposed to artificial saliva in group 3 (compared with the significantly lower modulus in the bleached group than in the control group without artificial saliva in group 1) alone cannot suggest mechanical property restoration owing to artificial saliva storage. The significantly reduced flexural strength and modulus of the bleach specimens compared with the control specimens in group 4 do not indicate mechanical property restoration owing to artificial saliva storage in conjunction with daily fluoride treatments.

Specimens from group 4 were further given a daily neutral fluoride treatment to form calcium fluoride precipitates and facilitate remineralization.[44] In one study, it was shown that fluoride treatment prior to storage in artificial saliva decreased the observed reduction in enamel surface hardness following bleaching.[45] However, in other studies, fluoride treatment could not restore the structurally bound fluoride or the erosion resistance of bleached enamel to the levels of unbleached enamel,[46] or had no significant effect on the hardness of bleached enamel and dentin.[47] Higher concentrations of fluoride in solution are generally needed to induce remineralization in dentin than in enamel.[48] In this study, daily fluoride treatments did not restore the flexural strength and modulus of the bleached dentin. This result could partially be explained by the timing of the placement of the teeth into the fluoride and artificial saliva. Because the teeth were not immersed into the artificial saliva until after the fluoride application, there was little calcium available for remineralization during the time of fluoride application. A better experimental design to study the effect of fluoride on bleached dentin would have included the application of fluoride (such as a fluoride varnish) in conjunction with the immersion into artificial saliva. The fluoride treatment on the bleached dentin may also have the effect of hampering ionic diffusion during artificial saliva storage. A similar impaired ionic diffusion was speculated as a result of dentin demineralization by Kleter and colleagues.[49]

Since these data were collected from groups of specimens with different storage times and mediums, a comparison of means among the different groups with different storage conditions should be made cautiously. A better match of the storage conditions among the different groups would have provided better information to assess the effects of fluoride or artificial saliva storage. Storage of the prepared dentin specimens in artificial saliva on a daily basis between bleach applications also would have provided a more clinically relevant situation.

Previous studies and this study have used strength tests to assess the effects of bleach in dentin structural integrity.[19,20] Strength tests, however, are nominal values that are highly influenced by specimen geometry and loading variables. The fracture toughness parameter measures a material's resistance to crack propagation and better quantifies the tooth's resistance to fracture than does a conventional strength test. The fracture toughness test has been used on enamel and a decrease in the fracture toughness of enamel was reported after bleaching.[50,51] We plan to use the fracture toughness test to assess the effects of bleach on tooth structure using human dentin and standardized specimen bleach and storage conditions in future studies.

CONCLUSIONS

A direct application of 10% carbamide peroxide to bovine dentin significantly decreased the dentin's flexural strength and modulus of elasticity under specific in vitro test conditions for some groups. An indirect application of 10% carbamide peroxide onto the enamel of intact bovine incisors did not significantly decrease the flexural strength and modulus of elasticity of bovine dentin.

This in vitro study suggests that a 2-week 10% carbamide peroxide bleaching treatment does not significantly affect the flexural strength and modulus of dentin when the carbamide peroxide is applied to the enamel of intact teeth. However, the effect of a 2-week 10% carbamide peroxide bleaching treatment on dentin mechanical properties after direct application in clinical situations such as root exposure or occlusal attrition requires further study.

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Reprint requests: Laura Tam, DDS, MSc, Restorative Dentistry, Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, Ontario, Canada M5G 1G6; e-mail: laura.tam@utoronto.ca

COMMENTARY

FLEXURAL STRENGTH AND MODULUS PROPERTIES OF CARBAMIDE PEROXIDE-TREATED BOVINE DENTIN

Stephen C. Bayne, MS, PhD*

Tam and colleagues deserve special credit for trying to sort out some of the potential problems of bleaching regimes on the structural integrity of tooth structure. This study is well-organized, diligent in including appropriate controls, and carefully conducted. That said, it also begs a couple of other unresolved questions that should be emphasized: (1) What is the best model for testing undesirable bleaching effects on tooth structure? (2) What is the best laboratory test for detecting these changes?

Dentin is a wonderfully complex and resilient tissue often distinguished from enamel as being connected to the pulp through odontoblastic processes. As such, it is capable of responding to challenges by remodeling itself. In a practical sense, this means that structural problems that arise during function or exposure to challenges such as bleaching might be repaired. That is good. Since we do not understand the way that might happen or how fast it might happen, one might be tempted to overreact to any negative changes that arise in laboratory tests that would not include considerations of actual physiologic recovery.

Professor, Department of Operative Dentistry, UNC School of Dentistry, Chapel Hill, NC, USA