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Effect of direct peroxide bleach application to bovine dentin on flexural strength and modulus in vitro☆

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Summary *Objectives*. The objective of this study was to determine the effects of carbamide peroxide (CP) and hydrogen peroxide (HP) bleaching on the flexural strength (FS) and flexural modulus (FM) of dentin.

Methods. $2\times2\times20$ mm bovine dentin specimens were immersed in the bleaching agents to simulate overnight (10 or 15% CP, 6 h daily, 2 weeks), exaggerated overnight (10% CP, 6 h/day, 5 days/week, 2 months), daytime (6.5 or 7.5% HP, 1 h daily, 3 weeks) and in-office (35% HP, 1 h/day, 2 days/week, 3 weeks) treatment protocols. Distilled water (DW) and a placebo gel acted as control immersion materials. After immersion, the specimens were rinsed and stored in DW. Mechanical testing was performed 24 h after the last treatment using an Instron Universal Testing Machine with a crosshead speed of 0.75 mm/min. The results were analyzed by ANOVA and Tukey's tests ($p<0.05$).

Results. There were significant reductions in the FS and FM of dentin after 2-week and 2-month exposures to CP. There were no significant differences in the FS or the FM of the dentin among the HP treatment and control groups.

Conclusions. Direct in vitro application of CP bleaches caused significant decreases in dentin FS and FM. Similar decreases were not observed among the HP-treated dentin groups, which were exposed to shorter treatment times. Further research is needed to determine the effect of CP and HP on dentin in vivo.

Introduction

Successful vital bleaching requires good whitening efficacy without tooth damage. Scanning electron microscopy (SEM) studies of the bleached enamel are equivocal with regard to surface morphology changes. Some SEM studies have shown no changes in the enamel morphology, but others have demonstrated increased surface porosity or roughness.[1-7]

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A fracture toughness assessment on the enamel surface, hardness assessments and abrasion studies on enamel imply further possible surface enamel changes that could occur as a result of bleaching.[8-13] The clinical significance of the reported enamel surface changes, however, is considered to be minimal and such effects may be reversed after the cessation of bleaching.[1,7]

Fewer studies have evaluated the biomechanical properties of the tooth beyond the enamel surface. The mechanical properties of dentin are of significant interest because dentin provides the base for both enamel and cementum and 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.[10,14] Significant decreases in the ultimate tensile and micropunch shear strength of dentin were reported after an intracoronal bleach application of 30% hydrogen peroxide (HP).[15] This led the authors to conclude that the 30% HP bleach could weaken dentin enough to cause the tooth to fracture in function when there is extensive tooth structure loss. It is unknown whether this reported decrease in dentin mechanical properties is relevant to patient-applied bleaching techniques that use lower HP concentrations or carbamide peroxide (CP).

The patient-applied bleaching treatment protocol generally involves the application of a 10% CP (or HP equivalent) product by the patient to the surfaces of the teeth for several hours daily over several weeks. There has been a recent increase in the number of products available which contain a higher concentration of CP. Furthermore, it has been recommended that tetracycline-stained teeth should be bleached for a period of months as opposed to weeks.[16-18] The availability of over-the-counter bleach products increases the opportunity for patients to bleach their teeth in an unsupervised manner for a protracted period of time. The safety and efficacy of higher concentration CP products or the prolonged used of bleaching products have not been fully evaluated. It is important to determine the physical effects of normal bleaching regimens and of prolonged or higher concentration peroxide bleaching treatments on bulk tooth structure.

The objective of this study is to determine the effects of CP and HP bleaching on the flexural strength (FS) and flexural modulus (FM) of dentin. The null hypothesis for this study is CP and HP materials have no effect on the FS and FM of dentin.

Materials and methods

Dentin rectangular beams measuring 20 mm in length by 2 mm in width by 2 mm height were cut from the roots of previously frozen extracted bovine incisors. The thickest aspects of each root were selected for specimen preparation. The depth of the dentin in every specimen ranged from deep to superficial because the 2 mm by 2 mm thick specimen section utilized the bulk of the dentin circumferential to the canal space. The length of each specimen section ran parallel to the long axis of the root. A single root usually provided one or two dentin specimens. The mean height and width of the specimens were measured using a digital micrometer to an accuracy of 0.01 mm at three locations along the specimen length. The specimens were randomly divided into treatment groups ($n=8$) and subjected to immersion in bleach or control treatments (minimum one millimeter of gel or liquid surrounding the specimen) as outlined in Tables 1 and 2. To simulate an overnight wear

Table 1 Materials used for the carbamide peroxide treatment groups.

CP treatment group ($n=8$)	Bleach product/manufacturer	Treatment period	Immersion time (h/day)
Distilled water	-	2 weeks (daily)	6
Placebo gel	Ultradent Products Inc., South Jordan, UT, 84095	2 weeks (daily)	6
10% Carbamide peroxide	Opalescence/Ultradent Products Inc.	2 weeks (daily)	6
15% Carbamide peroxide	Opalescence/Ultradent Products Inc.	2 weeks (daily)	6
Distilled water	-	2 months (5 days/week)	6
Placebo gel	Opalescence/Ultradent Products Inc.	2 months (5 days/week)	6
10% Carbamide peroxide	Opalescence/Ultradent Products Inc.	2 months (5 days/week)	6

The immersion protocols are provided for the simulated overnight (2-week) and exaggerated overnight (2-month) treatments.

Table 2 Materials used for the hydrogen peroxide treatments groups.

HP treatment group ($n=8$)	Bleach product/ manufacturer	Treatment period	Immersion time
Distilled water	-	3 weeks (daily)	One 60 min applications/ day
Placebo gel	Opalescence Ultradent Products Inc.	3 weeks (daily)	Two sequential 30 min applications/day
6.5% hydrogen peroxide	Crest Professional White-strips, Procter & Gamble, Cincinnati, OH, 45202	3 weeks (daily)	Two sequential 30 min applications/day
7.5% hydrogen peroxide	Day White 2, Discus Dental Culver City, CA 90232	3 weeks (daily)	Two sequential 30 min applications/day
35% hydrogen peroxide	Opalescence Xtra Ultradent Products Inc.	3 weeks (2 days/week)	Four sequential 15 min applications/day

The immersion protocols are provided for the simulated daytime (daily) and in-office (2 days/week) treatments.

pattern of bleaching, the specimens were treated for 6 h daily with 10 or 15% CP for a period of 2 weeks. To simulate an exaggerated or prolonged use of overnight bleach, the specimens were treated for 6 h (5 days/week) with 10% CP for a period of 2 months. To simulate a daytime wear pattern of bleaching, the specimens were treated for 1 h (two sequential 30 min applications) daily with 6.5 or 7.5% hydrogen peroxide for a period of 3 weeks. To simulate a series of in-office bleaching applications, the specimens were treated for 1 h (four sequential 15 min applications) with 35% hydrogen peroxide twice/week for 3 weeks. Distilled water (DW) and a placebo gel acted as control immersion materials. The conditions for storage during immersion were 37 °C and >80% RH. After immersion, the specimens were rinsed thoroughly with distilled water and stored in 37 °C DW until the time of the next immersion.

Twenty four hours after the last bleach or control treatment, the specimens were subjected to a three-point-bend test on a Model 4301 universal uniaxial servo mechanical testing machine (Instron Corporation, Canton, Mass.) at a crosshead speed of 0.75 mm/min. The mounting apparatus consisted of two rods (2 mm in diameter), mounted parallel with 20 mm between the centers. During testing, the dentin specimen and mounting apparatus were immersed in distilled water at 37 °C to simulate clinical conditions.

The maximum load supported by the specimen prior to failure and the load-deformation profile were used, respectively, to determine FS and FM. FS was determined using the following equation:

$$FS = 3P_f L/2WH^2$$

where p_f is the measured maximum load at the time of specimen fracture, L is the distance between the supports on the tension surface (20 mm), W is the mean specimen width, and H is the mean height of the specimen between the tension and compression surfaces.

FM was determined using the following equation:

$$FM = (\Delta F/\Delta Y)(L^3/4WH^3)$$

where $\Delta F/\Delta Y$ is the change in force (ΔF) per unit change in deflection of the center of the specimen (ΔY), L is the distance between the supports on the tension surface (20 mm), W is the mean specimen width, and H is the mean height of the specimen between the tension and compression surfaces.

The results were subjected to statistical analyses using ANOVA and Tukey's tests ($p<0.05$) (SPSS for Windows 10.0.7, SPSS Inc., Chicago, IL 60606).

Table 3 Flexural strength (FS) (MPa) of bovine dentin after carbamide peroxide (CP) bleach immersion using simulated overnight wear pattern of bleaching.

CP treatment group	2 weeks (mean ± SD)	2 months (mean ± SD)
Distilled water	232.4 ± 12.8[a]	211.3 ± 20.4
Placebo gel	221.9 ± 11.0[a]	177.6 ± 23.2
10% Carbamide peroxide	186.8 ± 21.7[b]	116.3 ± 27.5
15% Carbamide peroxide	184.8 ± 18.3[b]	-

Same letters indicate no significant difference in mean FS among groups; the FS of bleached dentin (10% CP and 15% CP) was significantly lower than the FS of dentin controls for the 2-week bleaching period. The FS of 10% CP bleached dentin further decreased after 2 months.

Table 4 Flexural modulus (FM) (GPa) of bovine dentin after carbamide peroxide (CP) bleach immersion using simulated overnight wear pattern of bleaching.

CP treatment group	2 Weeks (mean ± SD)	2 Month (mean ± SD)
Distilled water	14.4 ± 2.7^{a}	13.0 ± 1.1
Placebo gel	$12.4 \pm 1.4^{a,b}$	10.0 ± 1.4^{c}
10% Carbamide peroxide	11.5 ± 1.5^{b}	10.9 ± 1.8^{c}
15% Carbamide peroxide	$11.7 \pm 2.2^{a,b}$	-

Same letters indicate no significant difference in mean FM among groups; the FM of dentin exposed to 10% CP was significantly lower than the FM of dentin exposed to distilled water for the 2-week and 2-month tests. The FM of the dentin exposed to the placebo was also significantly lower after 2 months.

The pH of the control and bleach materials was taken using an Accumet 620 pH/mV meter (Fisher, catalog # 13-627-620, Pittsburgh, PA 15238) ($n=3$).

Results

The FS and FM results for the CP treatment groups are depicted in Tables 3 and 4. The FS and FM results for the hydrogen peroxide treatment groups are depicted in Tables 5 and 6. The ANOVA tables from the statistical analyses are presented in Appendices A-F.

The FS of bleached dentin (10% CP and 15% CP) was significantly lower than the FS of unbleached dentin (DW and placebo) for the 2-week bleaching period. The FS of bleached dentin (10% CP) decreased further after 2-months and was significantly lower than the 2-month FS for unbleached dentin (DW and placebo). Compared with the DW group, the placebo did not significantly affect the FS of dentin treated for 2 weeks but there was a significant reduction in FS of dentin treated with placebo for 2 months.

Table 5 Flexural strength (FS) (MPa) of bovine dentin after hydrogen peroxide (HP) bleach immersion using a simulated daytime wear pattern of bleaching or a series of in-office bleaching applications.

HP treatment group	3 Weeks (mean ± SD)
Distilled water	214.1 ± 28.8^{a}
Placebo gel	207.0 ± 25.9^{a}
6.5% Hydrogen peroxide	188.3 ± 15.08^{a}
7.5% Hydrogen peroxide	182.0 ± 10.0^{a}
35% Hydrogen peroxide	186.9 ± 35.2^{a}

Same letters indicate no significant difference in mean FS among groups; there were no significant differences in the FS of the dentin among the HP groups.

Table 6 Flexural modulus (FM) (GPa) of bovine dentin after HP bleach immersion using a simulated daytime wear pattern of bleaching or a series of in-office bleaching applications.

HP treatment group	3 Weeks (mean ± SD)
Distilled water	13.7 ± 2.3^{a}
Placebo gel	13.0 ± 2.5^{a}
6.5% Hydrogen peroxide	12.6 ± 1.0^{a}
7.5% Hydrogen peroxide	14.4 ± 1.5^{a}
35% Hydrogen peroxide	13.8 ± 1.7^{a}

Same letters indicate no significant difference in mean FM among groups; there were no significant differences in the FM of the dentin among the HP groups.

The FM of dentin exposed to 10% CP was significantly lower than the FM of dentin exposed to DW for the 2-week and 2-month tests. The FM of the dentin exposed to the placebo was significantly lower than the FM of the dentin exposed to DW for the 2-month test.

There were no significant differences in the FS or the FM of the dentin among the hydrogen peroxide and their respective control (placebo and DW) groups.

The pH results are reported in Table 7. DW had significantly the highest pH. The pH values for the HP bleaches were generally lower than the pH values for the CP bleach materials.

Discussion

Significant decreases in the FS and FM of dentin following direct CP bleach application for 2 weeks and 2 months were observed in vitro. The lowest FS results occurred in the 10% CP 2-month group. The results of this study did not show a significant

Table 7 Mean pH results (±SD) for the peroxide control and bleach materials.

Material ($n=3$)	pH
Distilled water	7.4 ± 0.3
Placebo gel	6.8 ± 0.04
10% Carbamide peroxide	6.4 ± 0.01
15% Carbamide peroxide	6.6 ± 0.04
6.5% Hydrogen peroxide	5.1 ± 0.04
7.5% Hydrogen peroxide	6.3 ± 0.3
35% Hydrogen peroxide	4.4 ± 0.04

dose-related response because the higher 15% CP did not significantly reduce the dentin FS and FM more than the 10% CP at the 2-week time period. A dose-related response might have become apparent after a longer exposure time. However, the effect of the higher 15% CP for the longer 2-month application time was not tested due to the limited number of bovine incisors available.

The placebo gel likely had an effect on dentin FS and FM but the decrease in the FS and FM did not become significant until the long-term (2-month) exposure. The composition of the placebo gel included glycerine, water and carbopol. Its pH was slightly lower than the pH for DW. The decrease in the 2-month FS and FM for the placebo groups could have been caused by demineralization or some other effect such as dehydration. The greater reduction in FS observed for the 10 and 15% CP groups compared with the placebo groups, however, suggested that much of the dentin weakening was directly attributable to the CP.

Although the effective hydrogen peroxide concentrations were higher for all the HP treatment groups than 10 and 15% CP, the FS and the FM of the dentin for the HP treatment groups were not significantly lower when compared with the control groups. The total treatment times for the HP groups, however, ranged from 6 to 20 h and were much less than for the CP groups (84-240 h). It is possible that the treatment times for the HP groups, which simulated daytime or in-office applications, were not long enough to cause a significant reduction in dentin mechanical properties. Color changes, although not specifically measured, were not as easily observed in the HP groups compared with the CP groups.

The greater reduction in the 2-month FS data compared with the 2 week data for the CP groups suggested that the decrease in dentin strength was related to the length of bleach application. Although surface changes to enamel and dentin could be reversed after exposure to saliva,[1,7] potential changes to the physical structure beyond the dentin and enamel surface after bleaching may not be readily reversible over time. The greater decrease in the dentin FS in groups exposed to bleach for longer periods of time suggested that the changes to the physical structure of dentin could very well be cumulative.

One limitation of this study was the use of the three-point bend test. The flexural properties of rigid or semi-rigid materials that break at small deflection could be determined by the use of a three-point loading system utilizing central loading on a simply supported beam.[19] However, only a relatively small volume of the three-point bend specimen is exposed to high tensile stresses and the specimens are very sensitive to edge or surface damage.[20] Dentin from bovine incisors was also used in this study because of the specimen size requirements for the three-point bend test. The authors are currently studying the effects of peroxide bleaching materials on human dentin using a fracture mechanics approach to better quantify the fracture resistance of dentin.

Another limitation of this study was that the findings were based upon direct application of bleach to the dentin surface. Although there would be many clinical situations in which tooth bleach is applied directly to the dentin (cases of root exposure, occlusal attrition and in cases of non-vital tooth bleaching), the more common, intended application of tooth bleach is onto the enamel surface. That being said, enamel is not considered an impermeable barrier to HP or CP.[21-23] Fluid penetrates through enamel.[24] The ability of HP and CP bleaching agents to readily penetrate through intact enamel and dentin has been documented and is evident from both the significant amounts of bleach measured in the pulp chambers after bleach application in vitro[25-27] and from many clinical reports of tooth sensitivity during indirect bleach application in vivo. The diffusion data for HP and CP suggested that bleaching agents were capable of diffusing through enamel and dentin within 15 and 25 min.[25,26]

Whether or not the direct application of bleach to dentin is an appropriate model for patient-applied bleaching is a legitimate question. However, it is still important to know if there are adverse effects on dentin strength, even if they are only observed after direct bleach application and not after indirect bleach application. At present, gingival recession and occlusal attrition are not considered contraindications for bleaching. There is a critical need to determine if a recommendation to avoid bleaching dentin directly in cases of gingival recession or occlusal attrition should be advised based on reduced dentin strength after direct bleach application. It is also important to determine if the cumulative length of time of bleach application should be limited. The 2-month bleaching test is relevant for patients who undergo prolonged bleaching treatments, as indicated for tetracycline stains, for patients who re-bleach their teeth after regression of tooth whitening has occurred, and for patients who overuse the bleach in an ever-increasing desire to whiten their teeth.

It is necessary to characterize the nature of the dentin microstructural changes after bleach treatment in order to determine the cause of the reported dentin structural weakening. Changes to both the inorganic and organic components of dentin were considered. The lower pH of the placebo and bleaching agents compared with DW could have contributed to reduced dentin strength and stiffness by causing demineralization of the inorganic component of dentin. Studies have reported chemical and physical evidence of tooth structure demineralization to some degree following bleach application.[28–30] However, it is unlikely (based on the small degree of dentin demineralization that is generally observed) that dentin demineralization could have accounted for the total observed decrease in dentin flexural strength. The lowest pH values were reported for the group that had the shortest treatment times, 35% HP.

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.[29–31] Protein degradation following treatment with hydrogen peroxide or other oxidants has been reported in non-dental applications.[32–34] Urea is a significant component of CP and is commonly used in the laboratory as a protein denaturing agent. It was postulated that the addition of urea to a chemomechanical caries removal solution significantly improved the caries removal efficacy by disrupting the hydrogen bonds in deciduous dentin collagen and further breaking down the partially degraded collagen in carious dentin.[35] In this study, the urea-containing CP had greater deleterious effects on dentin flexural strength than non-urea HP bleaching agents. However, the different modes of bleach application for the CP and HP bleaches did not allow direct comparisons of the materials' effects.

The observed reductions in the FS and FM of dentin after direct CP bleach application raises questions regarding the structural integrity of the tooth after bleach treatment, especially for prolonged periods of time. There are no clinical reports of fractured teeth attributable to bleaching to date. The history of CP bleaching as a treatment modality is still relatively new but growing. The results of this in vitro study suggest the possibility of an increased potential for tooth fractures, which could manifest later as the tooth ages, as a result of CP tooth bleaching applied directly to the dentin for prolonged periods. Further studies are planned. A test will be conducted to better represent the clinical situation, i.e. the fracture toughness of dentin will be measured after application of CP to overlying enamel. Secondly, the potential for a reversal in the FS and FM decrease will be investigated. Finally, studies to characterize the microstructural changes to the dentin structure are needed. These include SEM, TEM and LM studies.

Conclusions

Direct in vitro applications of 10 and 15% CP bleaches for 2 weeks caused significant decreases in dentin FS and FM. The reduction in dentin mechanical properties was even greater after an exaggerated prolonged use of 10% CP bleach. Similar decreases in dentin FS and FM were not observed among the HP-treated dentin groups, which were exposed to shorter treatment times. Further research is needed to determine the effect of CP and HP on dentin in vivo.

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Appendix A. ANOVA table for flexural strength results of 2 week carbamide peroxide treatment groups

	Sum of squares	df	Mean square	*F*	Sig.
Between groups	14113.361	3	4704.454	17.278	0.000
Within groups	7624.047	28	272.287		
Total	21737.408	31			

Appendix B. ANOVA table for flexural strength results of 2 month carbamide peroxide treatment groups

	Sum of squares	df	Mean square	*F*	Sig.
Between groups	37068.013	2	18534.006	32.488	0.000
Within groups	11980.079	21	570.480		
Total	49048.092	23			

Appendix C. ANOVA table for flexural modulus results of 2 week carbamide peroxide treatment groups

	Sum of squares	df	Mean square	*F*	Sig.
Between groups	41.773	3	13.924	3.451	0.030
Within groups	112.974	28	4.035		
Total	154.747	31			

Appendix D. ANOVA table for flexural modulus results of 2 month carbamide peroxide treatment groups

	Sum of squares	df	Mean square	*F*	Sig.
Between groups	37.632	2	18.816	10.704	0.001
Within groups	36.915	21	1.758		
Total	74.548	23			

Appendix E. ANOVA table for flexural strength results of 3 week hydrogen peroxide treatment groups

	Sum of squares	df	Mean square	*F*	Sig.
Between groups	6293.559	4	1573.390	2.570	0.055
Within groups	21428.871	35	612.253		
Total	27722.430	39			

Appendix F. ANOVA table for flexural modulus results of 3 week hydrogen peroxide treatment groups

	Sum of squares	df	Mean square	*F*	Sig.
Between groups	15.767	4	3.942	1.133	0.357
Within groups	121.766	35	3.479		
Total	137.533	39			

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