The mechanical properties of dentin are changed after bleaching, although the effects of the bleaching conditions on the tensile strength of dentin have not been determined. In the current study, a tensile test of bovine dentin was conducted after bleaching and the effects of the bleaching conditions were investigated, including the number of bleaching times, the location where the bleaching agent was applied, and the kind of illumination. The weight of organic content in dentin before and after application of the agent was also measured. The results showed that the tensile strengths did not change after three times office bleaching procedure, and the location where the bleaching agent was applied, the kind of bleaching agent and the kind of illumination did not have influence on the tensile strength. On the other hand, organic component in dentin significantly decreased after application of the agent (p<0.05). Since the bleaching agent would potentially affect tensile strength with deterioration of collagen matrix, the frequency of bleaching treatment should be minimized in clinical use.

**Key words**: tensile strength, dentin, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), free radicals, matrix collagen

**Introduction**

In recent years, public interest in esthetics has grown in the West and in Japan, and vital and non-vital tooth bleaching has become common. Bleaching is useful because tooth color is modified without cutting of teeth, although the effect of bleaching agents on the physical properties of tooth substances is of concern. Bleaching agents are believed to reduce discoloration of teeth through decomposition of peroxides into unstable free radicals which acted upon molecules of colored matter. These effects occur at the tooth surface and also in the pulp chamber through the enamel and dentin. Moreover, free radicals are non-specific and may influence both inorganic and organic substances in dentin. For example, Rotstein et al. reported changes in the solubility and Ca/P ratio of dentin following application of a bleaching agent, and SEM observations show a change in surface morphology of teeth, including increased roughness and deformation of organic constituents following bleaching.
Dissolution of dentin by 35% H₂O₂ has been reported and free radicals may also influence the mechanical properties of dentin, since Toko et al. showed that bleaching increases the hardness of dentin. In contrast, Unlü et al. reported that 10 or 15% carbamide peroxide does not significantly affect enamel and dentin surface hardness, and Faraoni-Romano et al. found that 10%, 15%, and 22% carbamide peroxide bleaching has little influence on microhardness in bovine root dentin, although Tam et al. showed a significant decrease in dentin fracture toughness (K₁c) after 8 weeks of direct bleach treatment. In addition, direct exposure to 10% carbamide peroxide was found to cause a significant decrease in the flexural modulus of elasticity of bovine dentin. Piemjai et al. and Chng et al. have also shown that the tensile strength of dentin decreases after bleaching.

The above studies indicate that the mechanical properties of dentin change after bleaching, although the effects of the bleaching conditions on the tensile strength of dentin have not been established; such conditions include the bleaching times, the location of application of the bleaching agent, and the kind of illuminant. For example, a phenomenon referred to as shade rebound has been reported to occur after bleaching and patients may insist on retreatment. However, it is unclear if such repeated bleaching is safe. We have studied the tensile strength of dentin under various conditions and shown the procedure to be reproducible.

In the current study, a tensile test of bovine dentin after bleaching conditions include the bleaching times, the location of application of the bleaching agent, and the kind of illuminant. For example, a phenomenon referred to as shade rebound has been reported to occur after bleaching and patients may insist on retreatment. However, it is unclear if such repeated bleaching is safe. We have studied the tensile strength of dentin under various conditions and shown the procedure to be reproducible.

The tensile strength of dentin was investigated based on the experimental results.

Materials and Methods

1. Chemicals
The bleaching agents were 30% hydrogen peroxide (pH 4.27; Wako Pure Chemical Industries, Japan) and 10% carbamide peroxide (Merck, Japan). The following agents were used for SDS-PAGE: acetic acid, Coomassie brilliant blue, bromophenol blue, EDTA, glycine, 2-mercaptoethanol, methanol, sodium chloride, tris (hydroxymethyl) aminomethane (Merck, Darmstadt, Germany); glycerol, sodium dodecyl sulfate (Eastman Kodak, Rochester, NY, USA); ammonium persulfate, N,N,N,N'-tetramethylethylenediamine, 30% acrylamide/bis-sodium, SDS-PAGE broad range molecular weight standards (Bio-Rad Laboratories, Richmond, CA, USA); and pepsin (3443F, 10,900 unit/mg; ICN Biomedicals, Aurora, OH, USA). All reagents and solutions were of analytical grade.

2. Analysis of the organic component in dentin after bleaching
Eleven human teeth, consisting of one lower canine, three upper premolars, three lower premolars, one upper molar and three upper wisdom teeth, were extracted from the eleven patients aged from 16 to 66 years old. The teeth were stored in a freezer of -15°C immediately after extraction until their use. The cervical part of each tooth was powdered using round steel burs. Three 20-mg samples of the dentin powder from each tooth were put in different tubes. One of the samples was put in 1.5 ml of distilled water for 15 minutes as a control (CO treatment); the second sample was immersed in 1.5 ml of 30% hydrogen peroxide for 15 minutes and irradiated with light (Zoom!Lamp, Discus Dental, CA, USA) during the last 10 minutes (WP treatment); and the third sample was immersed in 1.5 ml of 10% carbamide peroxide for 2 hours (WU treatment). The CO, WP and WU samples were washed three times in 1.5 ml of distilled water, and then the tubes were centrifuged at 12,000g for 10 min. The resulting precipitate (dentin powder after bleaching) was freeze-dried (Freeze Dryer DC800, Yamato, Tokyo, Japan) and weighed. Next, the precipitate was demineralized in 1.5 ml of 10% EDTA (pH 7.0) for 7 days, and then washed three times in 1.5 ml of distilled water before a final centrifugation step at 12,000g for 10 min. The precipitate (organic component of dentin) was freeze-dried and weighed. The amount of organic component (weight %) of each CO, WP and WU sample was calculated by dividing the weight of the organic component by the weight of dentin powder after bleaching treatment, and these data were averaged over the 11 teeth for each treatment method. The organic component (4-mg pellet) from each of the CO, WP and WU samples was digested by 2 mg pepsin in

\[ H_2O_2 \rightarrow H_2O + O_2 \]

The precipitate (organic component of dentin) was freeze-dried and weighed. The amount of organic component (weight %) of each CO, WP and WU sample was calculated by dividing the weight of the organic component by the weight of dentin powder after bleaching treatment, and these data were averaged over the 11 teeth for each treatment method. The organic component (4-mg pellet) from each of the CO, WP and WU samples was digested by 2 mg pepsin in
0.5 ml acetic acid (pH 2) at 4°C and analyzed using SDS-PAGE24,25. The protein profiles were compared among CO, WP and WU treatments for each tooth.

3. Tensile strength of bovine dentin

The surfaces of 60 bovine incisors were varnished with nail enamel. The teeth were divided into 6 groups of 10 incisors each. In four groups, the corona of each tooth was sliced parallel to the tooth axis at a depth of 0.5 mm from the labial surface using a low speed-cutting machine (Isomet5, Buehler, Lake Bluff, IL, USA) to make a flat enamel surface for application of the bleaching agent under the conditions for each group indicated in Table 1. After bleaching agent application, the corona of each tooth was sliced, a dentin sheet located at 2.0-3.5 mm from the labial surface was obtained. Dumbbell-shaped specimens with a 1 x 1 x 1.5 mm central narrow portion were prepared from the dentin sheets, using a previously reported method19-23. In the fifth group, the corona of each tooth was sliced parallel to the tooth axis at a depth of 2.0 mm from the labial surface using a low speed-cutting machine to make a flat dentin surface for bleaching agent application using the conditions indicated in Table 2. After bleaching agent application, the corona of each tooth was sliced, a dentin sheet located 2.0-3.5 mm from the labial surface was obtained, and dumbbell-shaped specimens were prepared as described above. From the sixth group of bovine incisors, similar dumbbell-shaped specimens were prepared as controls without application of bleaching agent (hereafter, CON). The tensile test was performed immediately after preparation of the specimens using a universal test machine (1123, Instron, Canton, MA, USA) fitted with special jigs (crosshead speed 0.5 mm/min) and a 37°C constant temperature water bath. The average tensile strength of the ten specimens in each treatment group was determined.

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<tr>
<th>Table 1. Conditions for bleaching of the enamel surface</th>
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<td>Illuminant</td>
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<td>Product</td>
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<td>Manufacturer</td>
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<td>Kind of lamp</td>
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<td>Range of wavelength</td>
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<tr>
<td>Procedure</td>
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<tr>
<td>Application of agent for 15 minutes; illumination in the last 10 minutes</td>
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<th>Table 2. Conditions for bleaching of the dentin surface</th>
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<td>Code</td>
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<td>Bleaching agent</td>
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4. Statistical analysis
The statistical significances among the amount of organic component and among the tensile strengths were analyzed by one-way ANOVA, followed by Tukey’s test using statistical software (JMP 6, SAS, Cary NC, USA). P<0.05 was considered statistically significant.

Results

1. Amount of organic component in dentin after bleaching
The amount of organic component (weight %) and the standard deviation for CO, WP and WU was 20.0 and 3.2, 15.7 and 3.1, 19.5 and 4.3, respectively (Figure 1). Statistically significant differences were found between CO and WP, and WP and WU treatment (P<0.05)

2. SDS-PAGE
Bands corresponding to the α band and pepsin were observed at 100 kDa and 40 kDa, respectively, for all experimental conditions. There were no differences in the protein profile among the CO, WP, and WU samples (Figure 2).

3. Tensile strength
The bleaching effect was observed by the naked eye through visualization of the color of the specimen surfaces for all conditions except the control. The tensile strength and the standard deviation of CON, U1E, U1D, V1E, V3E and URE were 74.9 and 7.5, 70.1 and 8.4, 68.8 and 9.0, 71.9 and 10.1, 68.9 and 5.5, 70.2 and

![Figure 1](image1.png)
Fig. 1. Amount of organic component of decalcified samples (n=11). CO: control, WP: application of 30% hydrogen peroxide for 15 minutes with illumination using mercury metal halide lamp in the last 10 minutes, WU: application of 10% carbamide peroxide for 2 hours without illumination. The asterisks denote a significant difference (p<0.05).

![Figure 2](image2.png)
Fig. 2. Protein profiles of organic component in dentin after bleaching treatment examined with SDS-PAGE. Lane a: molecular weight marker, b: CO control, c: WP: application of 30% hydrogen peroxide for 15 minutes with illumination using mercury metal halide lamp in the last 10 minutes, d: WU application of 10% carbamide peroxide for 2 hours without illumination. The asterisks denote a significant difference (p<0.05).
There were no significant differences between any experimental conditions.

Discussion

1. Weight of organic component in dentin after bleaching, and SDS-PAGE analysis

The teeth bleach is generally practiced for anterior teeth. However, the teeth used in this experiment include posterior teeth because chemical compositions of teeth were reported to be constant\(^26\). The decrease in the amount of organic component relative to control after bleaching with 10% carbamide peroxide was 1/8 of that with 30% hydrogen peroxide. The free radical content of 10% carbamide peroxide corresponds approximately to that of 3.6% hydrogen peroxide (that is, approximately 1/8 of the free radical content of 30% hydrogen peroxide)\(^27\). Therefore, the concentration of radicals may be correlated with the change in organic content, although differences in treatment time should also be considered. The decrease in the amount of organic component may be caused by decomposition of intermolecular and intramolecular bonds in the matrix collagen by non-specific reactions with free radicals, which may lead to a change of the higher-order structure of collagen in aqueous solution after decalcification; as a result, the solubility of collagen in water might increase. SDS-PAGE gave a similar protein profile to that reported previously; that is, bands were present for the \(\alpha\) chain and pepsin that are found in normal dentin\(^24\). Moreover, no difference was found in the profile among the CO, WP, and WU samples. The residual organic fraction for which solubility did not change after decalcification was used as the sample for SDS-PAGE.

2. Tensile strength

Many reports have shown the changes of mechanical properties after bleaching such as hardness\(^4,15-17\), although these results have been inconsistent. The hardness depends on inorganic components in dentin, hydroxyapatite\(^21-24\). The bleaching effect of an agent depends on the action of free radicals in decomposing coloring matter\(^4\) and this may influence both inorganic and organic substances in dentin\(^9,10\). In addition, 30% hydrogen peroxide has a pH of 4\(^16\) and such an acidic solution may dissolve the inorganic component. On the other hand, as a result of the current study, we found that the bleaching agent has influence on the organic component as well as on the inorganic component, in agreement with Rotstein \textit{et al}\(^9\). The tensile strength of dentin has been suggested to depend on matrix collagen\(^19-22\). Therefore, the bleaching procedure using free radicals could potentially affect the tensile strength of dentin, which is revealed by Piemjai \textit{et al}\(^12\) and Chng \textit{et al}\(^18\). Since the deterioration of collagen matrix would less concern with the hardness, the tensile strength of dentin after bleaching should be examined in detail in addition to hardness and other mechanical properties.

In this study, we examined the effects of the number of bleaching times, the location of application of the bleaching agent, the kind of the bleaching agent and the kind of illumination on the tensile strength of dentin. As a result, no significant difference in the tensile strength was found among all the conditions. With the same experimental system, the authors revealed that the tensile strength of dentin decreased by the dental laser irradiation under the condition of clinical use and the change were statistically significant\(^23\). Therefore, the bleaching procedures conducted in this study affected tensile strength of dentin less than some of the dental laser application in clinic.

The weight of organic component changed by bleaching while the tensile strength not. The considerable reasons were that the bleaching agent affects powdered dentin sample for solubility measurement more than dentin mass used in tensile test. Tam \textit{et al}\(^16\) reported that the dentin K\(_c\) depends on the distance from the location where the bleaching agent was

![Fig. 3. Tensile strength. CON: control, U1E: application of 30% hydrogen peroxide from enamel for 15 minutes with illumination using mercury metal halide lamp in the last 10 minutes, U1D: application of 30% hydrogen peroxide from dentin for 15 minutes with illumination using mercury metal halide lamp in the last 10 minutes, V1E: application of 30% hydrogen peroxide from enamel for 15 minutes with illumination using halogen lamp in the last 10 minutes, V3E: application of 30% hydrogen peroxide from enamel for 15 minutes with illumination using halogen lamp in the last 10 minutes; the procedure was repeated three times., URE: application of 10% carbamide peroxide for 2 hours without illumination.](image-url)
applied, with the $K_{1C}$ decreasing with a shorter distance. $K_{1C}$ would possibly be more sensitive to the action of free radical than the tensile strength. The experimental conditions using 30% hydrogen peroxide are similar to those in an in-office bleaching procedure, whereas the URE treatment is similar to that in home bleaching using a 2-hour treatment with 10% carbamide peroxide. As mentioned above, the concentration of free radicals for 10% carbamide peroxide corresponds to that for 3.6% hydrogen peroxide. However, the URE treatment showed similar effects on tensile strength compared to those with 30% hydrogen peroxide, which may be due to the long application time in the URE group. Although the tensile strength did not change after three times agent application in this study, as mentioned above, the bleaching agent would potentially affect tensile strength with deterioration of collagen matrix. Thus, the frequency of bleaching treatment should be minimized in clinical use.

References