

The Effect of Bleaching on the Elastic Modulus of Bovine Enamel

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The purpose of this study was to determine the elastic modulus of enamel during bleaching procedure with the use of an ultrasonic device. Enamel sections were obtained from freshly extracted bovine incisors. Specimens were exposed to 10% carbamide peroxide for two hours, followed by an application of a fluoride-containing toothpaste for five minutes and stored in artificial saliva (pH 7.0). An ultrasonic device was used to measure the sound velocities of longitudinal and shear waves as well as elastic modulus. The mean elastic modulus of bleached enamel decreased with time, from 15.5 GPa to 10.1 GPa. Conversely, the elastic modulus of bleached enamel followed by application of a fluoride-containing toothpaste increased with time, from 15.2 GPa to 20.2 GPa. Results of this study indicated that a decrease in elastic modulus associated with bleaching occurred, and that fluoride-containing toothpaste reversed this effect.

Keywords: Bleaching, Enamel, Fluoride

INTRODUCTION

Vital tooth bleaching includes direct contact of the bleaching agent on the tooth surface. The agent of choice for home bleaching is 10% carbamide peroxide, which has been suggested as a safe alternative to vital tooth bleaching with hydrogen peroxide¹. The effects of carbamide peroxide on enamel have been studied, whereby negligible impact in mineral loss² and microhardness³ have been reported. Though the alteration of enamel surfaces is less pronounced in *in vivo* situations⁴, slight surface alteration and decrease in surface microhardness have been reported⁵⁻⁷. On the other hand, it has been reported that fluoride application restored the changes in enamel microhardness, since fluoride incorporated in the tooth surface may lead to the formation of a calcium fluoride layer⁸.

To the end of improving clinical treatment planning, it is important to have a good knowledge and understanding about the mechanical properties of tooth substrates during bleaching. Elastic modulus, which is defined as the ratio of stress to corresponding strain in a material under load, is one of the basic properties that is of interest in many manufacturing and research applications. In particular, in the context of clinical applications, materials should have compatible moduli. On this note, elastic moduli of tooth substrates and restorative materials have been measured with compressive, flexural, and tensile tests⁹⁻¹³.

Owing to localized structural variations, reported

mechanical properties of teeth have run the whole gamut. It should be mentioned that a gradation exists in both morphology and mechanical properties from the dentin-enamel junction to the outer layer of enamel. When doing mechanical tests, such as the tensile strength test, stress distributions may be strongly influenced by factors such as loading characteristics and specimen size¹⁴. Thus, one must expect uneven stress distributions and acknowledge that the mechanical values reported are nominal values which require cautious interpretation. An indentation method has been used as an alternative to the flexural and tensile methods, and which may be especially useful when the specimen size is limited¹⁵⁻¹⁷.

To date, acoustic properties of enamel and dentin have been reported by several researchers¹⁸⁻²⁰. This is chiefly because it has been found that sonic velocity proportionally increases with the volumetric concentration of inorganic components²⁰. In other words, the degree of mineralization and the differences in histological structure result in different sonic velocities in each specimen. At the same time, ultrasonic imaging — which is used in many fields as a non-invasive technique — has emerged as a useful tool in diagnosis as well as research. As such, ultrasonic devices have been used to detect carious lesions²¹⁻²³ and evaluate the adhesive interface of dentin bonding systems²⁴.

It has been reported that hydrogen peroxide penetrates enamel and dentin, and reaches the pulp chamber²⁵. Though changes in hardness of bleached

enamel can be detected by microhardness tests, measurements are limited only on the surface of enamel specimens. Therefore, in this study, an ultrasonic pulse method was employed to assess the conditions of enamel during a bleaching procedure.

The purpose of this *in vitro* study was to evaluate the effect of 10% carbamide peroxide gel treatment, and that of a fluoride-containing toothpaste after bleaching, on the elastic modulus of bovine enamel with a non-destructive measurement method using an ultrasonic device.

MATERIALS AND METHODS

Preparation of specimens

A bleaching agent used in this study was 10% carbamide peroxide gel (HiLite Shade Up, Shofu Inc., Kyoto, Japan).

Twenty freshly extracted bovine incisors without cracks or erosion, cleaned and stored in physiological saline for up to two weeks, were selected. Labial surface of each tooth was sectioned along the central line into 1-mm sections in different directions with a low-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA). A tooth slab was obtained from each tooth, and a total of twenty slabs were thereby obtained. Each slab was carefully shaped into a rectangular form (4 mm × 4 mm × 1 mm) with a super-fine diamond point (ISO #021, Shofu Inc.) to make the specimen walls parallel to each other. Each surface of the specimen was ground on wet 600- to 2,000-grit SiC papers successively. Precise dimensions of the specimen were obtained by means of a dial gage micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan). The weight of each specimen was measured (AE 163, Mettler, Greifensee, Switzerland), and then the density of the specimen calculated.

The specimens were divided into two groups: one group received a post-bleach application of 950-ppm sodium fluoride-containing toothpaste (Merssage Cleargel, Shofu Inc.), and the other group a post-bleach application of a paste without fluoride. The toothpastes were adjusted to have similar viscosity and were intended to be different only in the presence or absence of fluoride. Both toothpastes were provided by Shofu Inc.

Bleaching was accomplished by covering the enamel specimen with the bleaching gel in a chamber of 100% relative humidity at 37 °C for two hours. After the bleaching procedure, the gel was removed under tap water with cotton pellets, followed by toothpaste application for five minutes. The specimens were washed and then stored in artificial saliva (pH 7.0; NaCl 14.4 mmol/L, KCl 16.1 mmol/L, MgCl₂·6H₂O 0.3 mmol/L, K₂HPO₄ 2.0 mmol/L, CaCl₂·2H₂O 1.0 mmol/L, sodium carboxymethyl cellulose (CMC-Na) 0.10 g/100 ml) at 37 °C. This procedure

was done everyday for the entire experimental period.

Measurement of the propagation time of ultrasonic waves

Prior to the bleaching procedure, the enamel surfaces were dried with cotton pellets. Then, ultrasonic measurements were done at 7, 14, 21, and 28 days after the start of bleaching. Specimens before the bleaching procedure were also measured as controls. The ultrasonic equipment employed in this study was composed of a Pulser-Receiver (Model 5900PR, Pamametrics, Waltham, MA, USA), a transducer for longitudinal waves (V112, Panametrics), a transducer for shear waves (V156, Panametrics), and an oscilloscope (Wave Runner LT584, LeCroy Corp., Chestnut Ridge, NY, USA)^{20,24}. The equipment with the transducer was calibrated for each use with a standard calibration procedure on a calibration block.

The transducer was oriented perpendicular to the contact surface of each specimen to receive the echo signal. Ultrasonic waves propagated in the transducer to the tooth, and were then reflected off the surface of the tooth or transmitted through the tooth. The reflected waves reached the probe as a surface echo (S-echo), while transmitted waves that were reflected off the interface between air and tooth specimen were received as a back echo (B-echo). The differential time between S-echo and B-echo then represented the time that it took the wave to propagate through the tooth. By means of the measured differential time (t), acoustic velocity (C) was calculated using the following equation:

$$C = 2T / t$$

where C : acoustic velocity

T : thickness of specimen

t : round-trip transit time

Three measurements were done for the central part of each specimen, and the average was used as the acoustic velocity of the specimen.

Round-trip transit time was measured through an area of known thickness with both longitudinal and shear wave transducers. Assuming the acoustic wave pathway in specimens as homogeneous, the elastic modulus of the specimens was calculated using the following equation:

$$E = \frac{4C_s^2 C_l^2}{C_s^2 C_l^2} \rho g$$

where E : elastic modulus (GPa)

C_s : shear velocity (m/sec)

C_l : longitudinal velocity (m/sec)

ρ : density (g/cm³)

g : acceleration due to gravity (9.8 m/s²)

Statistical analysis

Elastic moduli of the specimens were subjected to ANOVAs followed by Tukey's HSD test for comparison among the different treatments at a significance level of 0.05. Statistical analysis was carried out with Sigma Stat 3.1 software system (SPSS Inc., Chicago, IL, USA).

FE-SEM observation

For ultrastructural observation of enamel surfaces by FE-SEM, specimens stored in each condition were dehydrated in ascending concentrations of *tert*-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes, and 100% for two hours), and then transferred to a critical point dryer for 30 minutes. The surfaces were coated in a vacuum evaporator, Quick Coater Type SC-701 (Sanyu Denshi Inc., Tokyo, Japan), with a thin film of Au. The specimens were then observed using a field emission electron probe surface roughness analyzer (3D-SEM, ERA 8800FE, Elionix Ltd., Tokyo, Japan).

RESULTS

Table 1 summarizes the results of the acoustic velocities and elastic moduli obtained from the specimens with the use of the ultrasonic device. When specimens were treated with bleaching agent and fluoride-free toothpaste, the elastic modulus changed from

15.5 GPa to 10.1 GPa during the one-month experimental period. Indeed, significant decrease in elastic modulus was already found for specimens without fluoride at 14 days after the start of whitening. On the other hand, elastic modulus of specimens brushed with fluoride-containing toothpaste increased after seven days from the start of bleaching.

Figures 1 and 2 show the SEM images

Table 1 Densities, acoustic velocities, and elastic moduli of specimens

	(g/cm ³)	Cl (m/s)	Cs (m/s)	E (GPa)
Bleaching followed by no-fluoride-containing toothpaste application				
Control	1.89	3539 (59)	1752 (58)	15.5 (0.1)
7 Days	1.90	3428 (21)	1748 (31)	15.4 (0.2)
14 Days	1.90	3058 (41)	1559 (30)	12.3 (0.2)
21 Days	1.89	3042 (63)	1539 (65)	11.9 (0.2)
28 Days	1.91	2860 (50)	1406 (32)	10.1 (0.2)
Bleaching followed by fluoride-containing toothpaste application				
Control	1.86	3522 (37)	1751 (25)	15.2 (0.3)
7 Days	1.89	3844 (39)	2098 (27)	21.4 (0.3)
14 Days	1.89	3736 (37)	2044 (27)	20.3 (0.3)
21 Days	1.87	3782 (27)	2058 (24)	20.4 (0.3)
28 Days	1.85	3762 (24)	2060 (21)	20.2 (0.3)

Number of specimens for each group was 10. Values in parentheses indicate standard deviations.

= density, Cl = longitudinal velocity, Cs = shear velocity, E = elastic modulus

For elastic modulus, values connected by a vertical line indicate no significant differences (p>0.05).

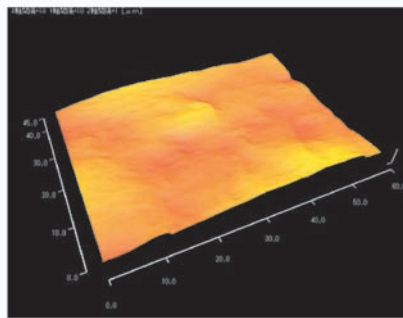
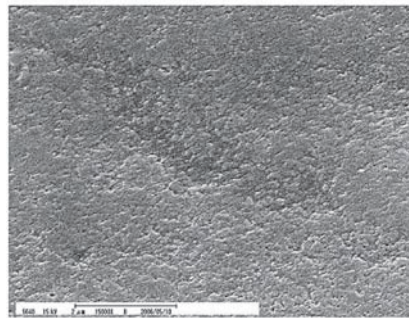


Fig. 1 SEM observation and corresponding 3D image of bleached enamel surface with the application of fluoride-free toothpaste. After 28 days from the start of bleaching treatment, slight surface alteration was observed.

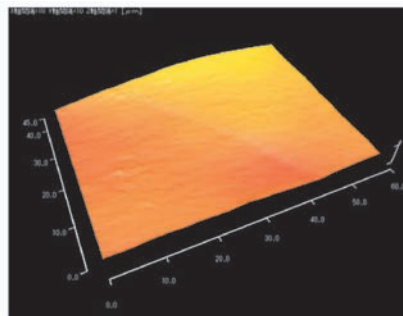
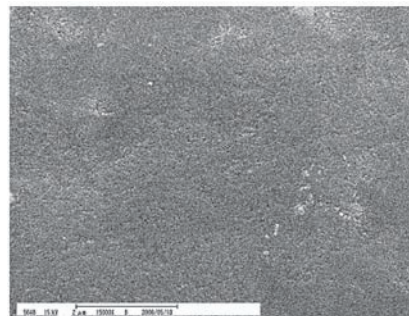


Fig. 2 SEM observation and corresponding 3D image of bleached enamel surface with the application of fluoride-containing toothpaste. After 28 days from the start of bleaching treatment, a smoother enamel surface compared to Fig. 1 was observed.

together with corresponding 3D images of representative enamel specimens. Different morphological features brought about by the different treatment conditions were revealed by SEM observation. With application of the bleaching agent on the enamel surface, different levels of surface change were shown. On the other hand, enamel specimens treated with fluoride-containing toothpaste after bleaching revealed slight or no changes in their morphological features.

DISCUSSION

Although there is a consensus that the use of human teeth is more relevant for conducting *in vitro* studies, bovine teeth were used in this study. The advantage of using bovine teeth instead of human teeth is that they are easy to obtain in large quantities in good condition, and have less composition variables than human enamel²⁶. By virtue of their large flat surface, bovine teeth also help to eliminate challenges posed by caries which might affect test results. It has been reported that mineral distribution in the carious lesions in bovine teeth was similar to that found in human teeth, and that structural changes in human and bovine teeth owing to carious lesions were similar²⁶. However, it should be mentioned that a threefold faster rate of caries progression was found for bovine enamel as compared to human enamel²⁷. Besides, it was necessary to polish enamel surfaces to produce specimens with flat surfaces for ultrasound measurement. Therefore, caution, discernment, and discretion should be exercised when drawing conclusions from the data as many factors are at play that may affect the results obtained *in vitro*.

When 10% carbamide peroxide gel was applied on the enamel slabs, elastic modulus chronologically decreased during the experimental period of 28 days. The bleaching agent used in this study had a neutral pH of 6.9 as measured with a pH meter, and was thus deemed to be safe for use on dental hard tissue.

The main ingredient of the bleaching agent was carbamide peroxide, which breaks down into urea, ammonia, carbon dioxide, and hydrogen peroxide. It has been described that urea released by carbamide peroxide has an ability to penetrate enamel and affect not only the surface, but also the interprismatic regions of enamel²⁸. On a further note about the effect of urea, it has been reported that the presence of urea in bleaching agents contributed to various surface morphological changes in enamel²⁹. One of which was enhanced permeability of enamel, leading to changes in surface structure and roughness of enamel. Urea was also able to attack proteins by dissociating H-bonds between CO and NH groups, leading to conformational changes.

Besides 10% carbamide peroxide, it has been reported that carbopol and its associations also caused alterations in microhardness of sound enamel and dentin⁷.

The present study was not done *in vivo*. Hence, it was difficult to estimate whether the observed enamel surface changes were reversible. At this juncture, it should be highlighted that alterations in surface morphology observed after 10% carbamide peroxide application in *in vitro* conditions are less pronounced than in *in vivo* situations⁴. It was speculated that these microstructural defects were repaired by the adsorption and precipitation of salivary components such as calcium and phosphate ions³⁰.

From the results of this study, it could be seen that a fluoride-containing toothpaste was effective in preventing a decrease in the elastic modulus of enamel. These results were consistent with previous investigations, whereby an increase in bleached enamel surface hardness was observed after application of fluoride treatment^{31,32}. This was attributed to the positive effect of fluoride in reducing demineralization due to erosion. By way of rationalization, it was concluded that decrease in elastic modulus of bleached enamel could be prevented by fluoride administration. This was chiefly because fluoride was speculated to enhance the repair of microstructural defects by remineralization.

From the SEM observations, the effect of carbamide peroxide on enamel surface structure resulted in an erosion-like roughened surface (Fig. 1). Indeed, slight surface alterations detected by SEM observation and decrease in microhardness of enamel surfaces after application of carbamide peroxide have been reported⁵⁻⁷. Such porous surfaces might allow for enhanced fluoride incorporation in the tooth surface. As a result, a calcium fluoride layer was formed, thereby increasing the elastic modulus as compared to the case where fluoride-free toothpaste was applied.

Under the conditions of this *in vitro* study, 10% carbamide peroxide bleaching gel application decreased the elastic modulus of bovine enamel. However, bleaching followed by fluoride toothpaste application led to an increase in the elastic modulus of bleached enamel.

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REFERENCES

- 1) Haywood VB, Heymann HO. Nightguard vital bleaching: how safe is it? *Quintessence Int* 1991; 22:515-523.
- 2) Goo DH, Kwon TY, Nam SH, Kim HJ, Kim KH, Kim YJ. The efficiency of 10% carbamide peroxide gel on dental enamel. *Dent Mater J* 2004; 23:522-527.
- 3) Park HJ, Kwon TY, Nam SH, Kim HJ, Kim KH, Kim YJ. Changes in bovine enamel after treatment with a 30% hydrogen peroxide bleaching agent. *Dent Mater J* 2004; 23:517-521.
- 4) Justino LM, Tames DR, Demarco FF. *In situ* and *in vitro* effects of bleaching with carbamide peroxide on human enamel. *Oper Dent* 2004; 29:219-225.
- 5) Basting RT, Rodrigues Junior AL, Serra MC. The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *in situ*. *Oper Dent* 2001; 26:531-539.
- 6) Rodrigues JA, Marchi GM, Ambrosano GM, Heymann HO, Pimenta LA. Microhardness evaluation of *in situ* vital bleaching on human dental enamel using a novel study design. *Dent Mater* 2005; 21:1059-1067.
- 7) Basting RT, Rodrigues AL Jr, Serra MC. The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness. *Oper Dent* 2005; 30:608-616.
- 8) Lewinstein I, Fuhrer N, Churaru N, Cardash H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin. *J Prosthet Dent* 2004; 92:337-342.
- 9) Watts DC, El Mowafy OM, Grant AA. Temperature-dependence of compressive properties of human dentin. *J Dent Res* 1987; 66:29-32.
- 10) Tonami K, Takahashi H, Nishimura F. Effect of frozen storage and boiling on tensile strength of bovine dentin. *Dent Mater J* 1996; 15:205-211.
- 11) Inoue T, Takahashi H, Nishimura F. Anisotropy of tensile strengths of bovine dentin regarding dentinal tubule orientation and location. *Dent Mater J* 2002; 21:32-43.
- 12) Liu J, Hattori M, Hasegawa K, Yoshinari M, Kawada E, Oda Y. Effect of tubule orientation and dentin location on the microtensile strength of bovine root dentin. *Dent Mater J* 2002; 21:73-82.
- 13) Inoue S, Pereira PN, Kawamoto C, Nakajima M, Koshiro K, Tagami J, Carvalho RM, Pashley DH, Sano H. Effect of depth and tubule direction on ultimate tensile strength of human coronal dentin. *Dent Mater J* 2003; 22:39-47.
- 14) Van Noort R, Cardew GE, Howard IC, Noroozi S. The effect of local interfacial geometry on the measurement of the tensile bond strength to dentin. *J Dent Res* 1991; 70:889-893.
- 15) Willems G, Celis JP, Lambrechts P, Braem M, Vanherle G. Hardness and Young's modulus determined by nanoindentation technique of filler particles of dental restorative materials compared with human enamel. *J Biomed Mater Res* 1993; 27:747-755.
- 16) Xu HHK, Smith DT, Jahanmir S, Romberg E, Kelly JR, Thompson VP, Rekow ED. Indentation damage and mechanical properties of human enamel and dentin. *J Dent Res* 1998; 77:472-480.
- 17) Kinney JH, Balooch M, Marshall GW, Marshall SJ. A micromechanics model of the elastic properties of human dentine. *Arch Oral Biol* 1999; 44:813-822.
- 18) Ng SY, Payne PA, Cartledge NA, Ferguson MWJ. Determination of ultrasonic velocity in human enamel and dentine. *Arch Oral Biol* 1989; 34:341-345.
- 19) Löst C, Irion KM, John C, Nüssle W. Two-dimensional distribution of sound velocity in ground sections of dentin. *Endod Dent Traumatol* 1992; 8:215-218.
- 20) Miyazaki M, Inage H, Onose H. Use of an ultrasonic device for the determination of elastic modulus of dentin. *J Oral Sci* 2002; 44:19-26.
- 21) Peck SD, Briggs GAD. A scanning acoustic microscope study of the small caries lesion in human enamel. *Caries Res* 1986; 20:356-360.
- 22) Ng SY, Ferguson MWJ. Ultrasonic studies of unblemished and artificially demineralized enamel in extracted human teeth: a new method for detecting early caries. *J Dent* 1988; 16:201-209.
- 23) Yanikoglu FÇ, Öztürk F, Hayran O, Analoui M, Stookey GK. Detection of natural white spot caries lesions by an ultrasonic system. *Caries Res* 2000; 34:225-232.
- 24) Watanabe T, Miyazaki M, Inage H, Kurokawa H. Determination of elastic modulus of the components at dentin-resin interface using the ultrasonic device. *Dent Mater J* 2004; 23:361-367.
- 25) Benetti AR, Valera MC, Mancini MNG, Miranda CB, Balducci I. *In vitro* penetration of bleaching agents into the pulp chamber. *Int Endod J* 2004; 37:120-124.
- 26) Edmunds DH, Whittaker DK, Green RM. Suitability of human, bovine, equine, and ovine tooth enamel for studies of artificial bacterial carious lesions. *Caries Res* 1988; 22:327-336.
- 27) Featherstone JD, Mellberg JR. Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. *Caries Res* 1981; 15:109-114.
- 28) Arends J, Jongebloed WL, Goldberg M, Schuthof J. Interaction of urea and human enamel. *Caries Res* 1984; 18:17-24.
- 29) Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent* 1999; 27:509-515.
- 30) Meyer-Luckel H, Tschoppe P, Kielbassa AM. Effect of various $\text{Ca}^{2+}/\text{PO}_4^{3-}$ concentrations of linseed-based saliva substitutes on enamel *in vitro*. *J Oral Rehabil* 2006; 33:760-766.
- 31) Attin T, Kielbassa AM, Schwanenberg M, Hellwig E. Effect of fluoride treatment on remineralization of bleached enamel. *J Oral Rehabil* 1997; 24:282-286.
- 32) Attin T, Kocabiyik M, Buchalla W, Hannig C, Becker K. Susceptibility of enamel surfaces to demineralization after application of fluoridated carbamide peroxide gels. *Caries Res* 2003; 37:93-99.