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Original

Changes in elastic modulus of adhesive and adhesive-infiltrated dentin during storage in water

Genta Yasuda[1)], Hirohiko Inage[1)], Ryo Kawamoto[1)], Yutaka Shimamura[1)], Chikako Takubo[1)], Yukie Tamura[1)], Kensaku Koga[1)] and Masashi Miyazaki[1,2)]

[1)]Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan
[2)]Division of Biomaterials Science, Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan

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Abstract: The purpose of this study was to determine the elastic modulus of components at the resin-dentin interface with the use of an ultrasound device. Dentin slabs were obtained from freshly extracted bovine incisors shaped into a rectangular form. After demineralization, the dentin specimens were immersed in adhesives and polymerized. Adhesives were also polymerized and trimmed into the same shape as the dentin slabs. The specimens were then immersed in distilled water at 37°C for up to one year. The ultrasound equipment employed in this study was a Pulser-Receiver, transducers and an oscilloscope. By measuring the longitudinal and shear wave sound velocities, the elastic modulus was determined. When the elastic modulus of adhesive resin-infiltrated demineralized dentin was compared with that of adhesives, slightly but significantly lower values were found for adhesives used in a self-etching primer system. On the other hand, a higher elastic modulus was observed for resin-infiltrated dentin than for an adhesive used in an etch and rinse system. The elastic modulus of the resin-infiltrated dentin prepared with the etch and rinse system was affected by long-term storage in distilled water. (J. Oral Sci. 50, 481-486, 2008)

Correspondence to Dr. Masashi Miyazaki, Department of Operative Dentistry, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai, Chiyoda-Ku, Tokyo 101-8310, Japan
Tel: +81-3-3219-8141
Fax: +81-3-3219-8347
E-mail: miyazaki-m@dent.nihon-u.ac.jp

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Introduction

Advances in adhesive systems have been significant since the introduction of methods that create a resin-dentin interdiffusion zone (1). The dentin-resin interface is composed of several layers of materials with differing mechanical properties, and this is thought to be one of the important factors that determine the bond strength of adhesive systems (2-4). An understanding of the basic mechanism of dentin bonding relies on knowledge about the dentin substrate, its demineralization characteristics, and the approaches used to create a resin-dentin interdiffusion zone. Dentin is a mineralized tissue located between the enamel and the pulp, and consists of a hydrated matrix of type I collagen reinforced with hydroxyapatite crystallites (5). The microstructure of dentin is dominated by the presence of dentinal tubules that transverse the entire thickness of the dentin and occupy around 10% of total dentin volume (6).

It has been reported that the bonding to a dentin substrate created by currently available adhesive systems might degrade severely over time (7). The most important consideration for creating an authentic resin-dentin interdiffusion zone is how well resinous components are able to infiltrate into demineralized dentin. Although the interaction of etching agents with dentin is limited by the buffering effect of the mineral component of the tooth substrate (8), there is often a discrepancy between the depth of dentin demineralization and monomer penetration.

The remaining unprotected demineralized dentin layer at the base of the resin/dentin interface permits nanoleakage (9), which may lead to bonding failure. It has been suggested that the exposed collagen fibrils are susceptible to hydrolytic degradation over a long period, leading to reduction of bond strength (10).

Knowledge about the mechanical properties of tooth substrates and restorative materials could be helpful for devising and improving adhesive systems (11-13). Since the mechanical properties of the various resin-dentin components are expected to play important roles, the durability of resin-dentin bonds depends on the stability of their components over time. Catastrophic failure of resin-dentin bonds may be initiated in one specific component of the interface. By testing their physical properties, it might be possible to determine which is least stable during storage under various conditions.

Elastic modulus, which is defined as the ratio of stress to the corresponding strain in a material under loads below the elastic limit, is a one of the basic properties that are of interest in many manufacturing and research applications. The purpose of this study was to determine the elastic modulus of adhesive-infiltrated dentin and polymerized adhesive by non-destructive measurement using an ultrasound device. The null hypothesis tested was that there would be no significant changes in elastic properties of these components, regardless of the storage period.

Materials and Methods

The adhesive systems used in this study are listed in Table 1. The light intensity of the curing unit (Optilux 500, sds Kerr, Danbury, CT, USA) was adjusted to 600 mW/cm^2 as measured with a dental radiometer (Model 100, Demetron/ Kerr).

Freshly extracted bovine incisors without cracks or erosion were cleaned and stored in physiological saline for up to two weeks. The teeth were then sliced into 1-mm-thick sections in different directions with a low-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA). The dentin slab was carefully shaped into a rectangular form ($5 \times 5 \times 1$ mm) with a super-fine diamond point (ISO #021, Shofu Inc., Kyoto, Japan) to make the dentin walls parallel to each other. Each surface of the specimen was ground successively on wet #600- to #2,000-grit silicon carbide

Table 1 Materials tested

Adhesive system (Code)	Primer/ etchant (Lot No.) Main component	Adhesive (Lot No.) Main component	Resin composite (Lot No.)	Manufacturer
Clearfil Mega Bond (CB)	Primer (00206A) MDP, HEMA, water, PI, ethanol	Bond (00211A) MDP, HEMA, PI, bis-GMA, micro filler	Clearfil AP-X (00933)	Kuraray Medical
Fluoro Bond (FB)	FB Primer (A:129957, B:129934) A: Catalyst, water B: 4-AET, 4-AETA, HEMA	FB Bond (129972) 4-AET, HEMA, filler, UDMA, TEGDMA, CQ, PI	Lite-Fil IIA (099902)	Shoufu Inc.
Mac-Bond II (MB)	Primer (A:030, B:015) A: MAC-10, HEMA, acetone, isopropyl alcohol, phosphate monomer B: Ethanol , water	Bonding Agent (022) MAC-10, HEMA , PI, bis-GMA, TEGDMA	Palfique Estelite (062130)	Tokuyama Dental
Single Bond (SB)	Etchant (7EC) 35% phosphoric acid	Single Bond (9DE) Vitrabond copolymer , HEMA, bis-GMA , ethanol, CQ	Filtek Z250 (9J03)	3M ESPE

MDP: 10-methacryloxydecyl di-hydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate, PI: photo initiator, bis-GMA: 2, 2bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]phenyl, 4-AET: 4-acryloyloxyethyl trimellitic acid, 4-AETA: 4-acryloyloxyethyl trimellitate anhydride, UDMA: urethane dimethacrylate, CQ: *dl*-camphorquinone, TEGDMA: triethylene glycol di-methacrylate, MAC-10: 11-methacryloxy-1,1-undecandicarboxylic acid

(SiC) paper. Rectangular (5 × 5 × 1 mm) specimens of adhesives used in a two-step self-etching primer system were also made in the same manner as the dentin specimens.

The dentin specimens were immersed in 0.5 M EDTA (pH 7.4, Showa Yakuhin Kako Co., Tokyo, Japan) solution for 3 days at 37°C. The demineralized specimens were then rinsed thoroughly in distilled water for 1 h, and excess water was blotted off with Kimwipes S-200 (Nippon Paper Crecia Co., Tokyo, Japan). These specimens were treated in accordance with the manufacturer's instructions prior to application of the bonding agent. For the self-etching primer systems CB and MB, primer liquids were applied to the dentin surface for 20 s, and in the case of FB for 10 s. For SB, the dentin surfaces were etched with 35% H_3PO_4 for 15 s, rinsed thoroughly with distilled water, and lightly air-dried without desiccation. The treated specimens were then placed in adhesives for 48 h of resin infiltration under dark conditions. After wiping the excess bonding agent from the specimens, each side was light-irradiated for 60 s. Each surface of the specimens was polished on wet 1000-grit SiC paper to limit the surface roughness. The specimens were then immersed in distilled water at 37°C for up to 18 months.

The ultrasound equipment employed in this study comprised 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). The equipment with the transducer was calibrated each time using a standard calibration procedure on a calibration block.

The central part of each specimen was used for measurements of ultrasonic velocity. The transducer was oriented perpendicular to the contact surface of each specimen, in order to obtain the echo signal. The ultrasonic waves propagated in the transducer were then transmitted through the tooth. After the ultrasonic wave had travelled through the specimen, it was received by a second transducer. The registered time delay for the signal to travel from the first transducer to the second was displayed on the oscilloscope, and measured with the use of cursors.

To measure the E value, the thickness and size of each set cement were measured using a dial gauge micrometer. Precise thickness and size of the specimen were measured using a dial gauge micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan). Then the weight of each specimen was measured (AE 163, Mettler, Greifensee, Switzerland), and the density of the specimen calculated. We recorded the round-trip transit time through an area of known thickness using both longitudinal-wave and shear-wave transducers. The following equation was used to calculate the elastic modulus of the specimens:

$$E = \rho/g \cdot (3\,Cs^2 \cdot Cl^2 - 4\,Cs^4) \div (Cl^2 - Cs^2)$$

where E = elastic modulus (GPa)

Cs: shear velocity (m/s)

Cl: longitudinal velocity (m/s) ρ: density (g/cm^3)

g: gravity (9.8 m/s^2)

Data on the elastic modulus of the specimens were subjected to repeated measures analysis of variance (ANOVA) followed by Dunnet's test, in order to compare them among the different storage times (at a significance level of $P < 0.05$). The statistical analysis was carried out with the Sigma Stat 3.1 software system (SPSS Inc., Chicago, IL, USA).

Results

Data for the change in elastic moduli obtained from the specimens during storage in distilled water at 37°C with the use of the ultrasound device are summarized in Table 2. When the dentin was demineralized with EDTA solution, the elastic modulus of the specimens was reduced from 17.4 GPa to 1.4 GPa. When the demineralized specimens were infiltrated with adhesives, significant increases in elastic modulus were found (3.7 - 4.7 GPa). The elastic modulus of resin-infiltrated dentin was significantly lower than that of each adhesive except for SB, which showed a significantly higher value. Storage in water for 18 months had a significant effect on the elastic modulus of both mineralized and demineralized dentin specimens. Reduction in the elastic modulus of CB, FB and MB after 18 months of storage in water was lower than that of SB.

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 enamel (14). Bovine teeth have large flat surfaces and would not have had any prior caries that might have affected the test results. Mineral distribution in the carious lesions of bovine teeth is reportedly similar to that in human teeth, and structural changes in human and bovine teeth are similar (14). On the other hand, a three-fold faster rate of caries progression has been found for bovine enamel as compared to human enamel (15). In addition, polishing of the enamel was necessary to create flat specimen surfaces for ultrasound measurement. Care should be taken when drawing conclusions from the data, as many factors can affect the results obtained *in vitro*.

The degree of polymerization and differences in

Table 2 Changes in elastic modulus (in GPa) of the specimens during storage in water

Code	24 h	1 month	3 months	6 months	12 months	18 months
Dentin						
Mineralized	17.4 (1.1)	17.4 (1.2)	17.8 (1.8)	20.3 (2.1)*	24.6 (3.2)*	25.6 (3.7)*
Demineralized	1.4 (0.3)	1.4 (0.1)	1.3 (0.2)	1.2 (0.6)	um	um
CB						
Adhesive	6.4 (0.4)	4.5 (0.2)*	4.8 (0.2)*	4.8 (0.2)*	4.8 (0.2)*	4.6 (0.3)*
Resin-infiltrated dentin	4.7 (0.3)	4.7 (0.3)	4.8 (0.3)	4.8 (0.3)	4.9 (0.2)	5.1 (0.2)*
FB						
Adhesive	5.4 (0.3)	4.2 (0.2)*	4.3 (0.2)*	4.3 (0.2)*	4.3 (0.2)*	4.0 (0.3)*
Resin-infiltrated dentin	3.7 (0.3)	3.7 (0.3)	3.8 (0.3)	3.8 (0.3)	3.8 (0.3)	3.8 (0.2)
MB						
Adhesive	5.2 (0.3)	4.3 (0.2)	4.2 (0.3)	4.3 (0.3)	4.0 (0.3)	4.0 (0.2)
Resin-infiltrated dentin	4.1 (0.3)	3.9 (0.3)	3.9 (0.2)	3.8 (0.3)	3.8 (0.2)	3.8 (0.1)
SB						
Adhesive	2.6 (0.2)	2.0 (0.2)*	2.0 (0.3)*	2.1 (0.3)*	1.9 (0.3)*	1.1 (0.2)*
Resin-infiltrated dentin	3.8 (0.3)	3.8 (0.4)	3.2 (0.3)*	3.3 (0.2)*	2.8 (0.3)*	1.9 (0.2*)

n = 5, ( ) = SD

*: Significant differences were found when compared with the 24 h data ($P < 0.05$).

structures might lead to differences in the velocity of sound propagated through a specimen. Significantly higher elastic modulus values were obtained with resin-infiltrated demineralized dentin than with demineralized dentin. When the elastic modulus of adhesive resin that had infiltrated demineralized dentin was compared with that of adhesives, slightly but significantly lower values were found for adhesives used in a self-etching primer system. On the other hand, a higher elastic modulus was obtained with resin-infiltrated dentin than for the adhesive used in an etch and rinse system. This may have been due to solvents remaining inside the cured adhesive and the amounts of the resinous components. Since the adhesive of an etch and rinse system has to dissolve in the water-rich demineralized dentin, the adhesive must have a hydrophilic solvent such as ethanol (16). Thus, differences in formulations might have led to the results we obtained.

Our results indicated that the elastic modulus of the resin made with SB that had infiltrated the dentin was affected by long-term storage in distilled water. Therefore, the hypothesis tested must be partially rejected. The prolonged immersion time in distilled water may have allowed further changes in the resin-infiltrated dentin structure, thereby resulting in a significant decrease of the elastic modulus after 12 months. For specimens made with the self-etching primer system, changes in elastic modulus were found during the first month of storage in water. The elastic modulus of resin-infiltrated demineralized dentin might be determined by the strength and relative volume of collagen fibrils and the adhesive resin employed. Sufficient infiltration of resinous components from the adhesive and the stiffness of collagen fibrils might have determined the results obtained. On the other hand, it is speculated that plasticization of the polymer due to water sorption during long-term storage in water might contribute to the stability of the adhesives used in self-etching primer systems. However, hydrolytic degradation may have taken place in the case of the adhesive used in the etch and rinse system, significantly compromising the structure of the polymer.

The mechanism of water transport and its effects on the mechanical properties of polymers are dependent on several factors (17). Composition and monomer ratio varies according to the specific application required, and such variability will define the chemical stability as well as degradability of resins in a specific environment (18). The hydrophilic nature of different monomers constituting the resin matrix and sensitivity of resin-based materials to the effects of water depend on the degree of polymer cross-linking, the degree of monomer conversion, the presence of fillers, and the volume fraction of intrinsic pores (19).

Analysis of fractured interfaces in long-term studies of resin-dentin bond strength has provided evidence that collagen fibrils may degrade over time (20). Incomplete infiltration of an adhesive into etched dentin reduces the bond strength as well as the durability of the restoration, because acid-etched exposed collagen fibrils unprotected by resin have been shown to be morphologically altered after long-term storage in water (21). In such a case, storage in water would cause partial demineralization of the surface layer, and this would be detected by the surface sensitive method. It is likely that the specimens stored in water in this study also suffered surface demineralization by the water, although this was probably not significant enough to cause an overall change in the specimen made with the self-etching primer system.

In clinical situations, it would be desirable to use adhesive materials that maintain the stability of the resin-tooth interface created by adhesive systems. These kinds of materials have shown stability over time in clinical situations. The results of this study suggest that not all the specimens tested had stability of the elastic modulus. Further studies should therefore be done to clarify suitable methods for achieving long-term stability of the resin-dentin interface by development of both new materials and clinical techniques.

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