©*Operative Dentistry, 2004,* **29-2,** 142-149

Laboratory Research

The Nano-Hardness and Elastic Modulus of Carious and Sound Primary Canine Dentin

Y Hosoya • GW Marshall, Jr

Clinical Relevance

The significantly lower nanohardness and elasticity of dentin under the lesion near the pulp and cervical area might have a deleterious effect on resin adhesion.

SUMMARY

This study measured the nanohardness and elastic modulus of carious and sound primary canine dentin and compared the values obtained under the lesion and in sound regions of incisal, center and cervical areas, and outer, middle and inner layers. Six extracted or exfoliated primary canines (three with dentin caries on both proximal surfaces and three that were sound teeth) were mesio-distally sectioned parallel to the long axis of the tooth and polished. The hardness (H), plastic hardness (PH) and Young's modulus (Y) were measured by a nano-indentation tester. Ten indentations at intervals of 10 µm on all regions, areas and layers were made using a load of 1 gf for one second. All indentations were observed using a microscope attached to the tester. All data was statistically analyzed using ANOVA and Scheffe's test at *p*<0.05. For sound teeth, the H, PH and Y values of the inner layer were significantly lower than the outer and middle layers in all areas. The H, PH and Y values of the cervical area were significantly lower than the incisal area in almost all of the outer, middle and inner layers. For carious teeth, the H, PH and Y values of the inner layer were significantly lower than the outer and middle layers in the center area. For the center area, the H, PH and Y values under the lesion were significantly lower than sound teeth in the outer and middle layers. Dentin under the lesion, near the pulp and cervical areas showed significantly lower nanohardness and elasticity.

INTRODUCTION

Improved resin bonding yields strong bonds to enamel with excellent sealing ability (Retief, 1987). However, the resin-dentin seal is much less reliable. Caries modifies the structure and typical carious lesions contain various zones of the affected layer (Fusayama, Okuse & Hosoda, 1966) that have varying mineral levels and properties. Fusayama (1993) compared the structure and characteristics of three layers of dentin of carious teeth (outer caries dentin, inner carious dentin and normal dentin). Outer caries dentin or the discolored layer

---

*Yumiko Hosoya, DDS, PhD, associate professor, Division of Pediatric Dentistry, Department of Developmental and Reconstructive Medicine, Course of Medical and Dental Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Grayson W Marshall, Jr, DDS, MPH, PhD, professor, Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Science, University of California San Francisco, San Francisco, CA, USA

*Reprint request: 1-7-1, Sakamoto, Nagasaki, 852-8588, Japan; e-mail: hosoya@net.nagasaki-u.ac.jp

is infected and unremineralizable and should be removed for carious treatment. Inner carious dentin is classified into turbid, transparent and subtransparent layers. These layers are uninfected and remineralizable and should be preserved for caries treatment. Similar zones have been observed in primary teeth with carious lesions (Hosoya & others, 2000; Hosoya, Ono & Marshall, 2002), but only limited work has been done to define the properties of these altered zones. Although permanent tooth dentin has been studied extensively, the microstructure of dentin in primary teeth has received only limited attention. A better understanding of dentin in primary teeth, especially carious primary dentin, is needed to improve dentin bonding methods and make dental restorations more effective and successful.

The hardness and elasticity of fully mineralized permanent dentin have been reported in numerous studies (Craig, Gehring & Peyton, 1958; Bowen & Rodriguez, 1962; Fusayama & Maeda, 1969; Pashley, Okabe & Parham, 1985; Sano & others, 1994; Kinney & others, 1996; Urabe & others, 2000; Mahoney & others, 2000). Recently, nano-indentation has been used to measure hardness and Young's modulus of dentin on a submicroscopic scale (Urabe & others, 2000; Mahoney & others, 2000). The range in hardness of sound permanent dentin is broad, from 0.2-0.8 GPa (1MPa=10.2 kgf/cm^2, 1GPa=101.93675 kgf/mm^2) (Fusayama & Maeda, 1969; Pashley & others, 1985; Sano & others, 1994; Kinney & others, 1996). Young's modulus of sound permanent dentin ranged from about 10 – 20 GPa (Craig & Peyton, 1958; Bowen & Rodriguez, 1962; Lehmann, 1967; Sano & others, 1994; Kinney & others, 1996; Meredith & others, 1996; Xu & others, 1998; Kinney & others, 1999). Few studies of the hardness of primary dentin have been reported (Johnsen, 1994; Mahoney & others, 2000; Hosoya & others, 2000; Hosoya & others, 2002) and Knoop hardness values for sound primary dentin ranged from 35 to 60 KHN (Johnsen, 1994) depending on location within the tooth.

Fusayama and others (1966) and Ogawa and others (1983) reported the Knoop hardness of carious permanent dentin for discolored, transparent and subtransparent layers and sound dentin and found that all zones, including the transparent zone of the affected layer, were softer than normal dentin. Craig, Gehring and Peyton (1959) also measured the hardness of carious permanent dentin but reported that some zones were harder than normal dentin.

Hosoya and others (2000; 2002) measured the Knoop hardness of carious primary anterior tooth dentin in caries-affected layers including transparent dentin, adjacent sound dentin and dentin regions far from and not related to caries. The results of these reports (Hosoya & others, 2000; Hosoya & others, 2002) showed that primary dentin in areas under lesions and in adjacent regions on the carious side of the teeth had significantly lower hardness than corresponding areas on the sound side. Under the lesion, significantly lower Knoop hardness values were obtained in the region less than 150 µm from the bottom of the cavity and the Ca and P contents at depths less than 100 µm from the bottom of cavity were significantly lower than those at greater distance (Hosoya & others, 2002). Transparent dentin was softer than sound dentin (Hosoya & others, 2000; Hosoya & others, 2002). The reduced mechanical properties of the affected layers of carious dentin suggest that bonding to this weakened structure may be more difficult. This has been reported by several workers for permanent dentin (Nakajima & others, 1995; Nakajima & others, 1999).

The elastic properties of dentin are important for understanding the mechanical properties of calcified tissue and for alterations in the mechanical response due to caries, sclerosis and aging, as well as understanding the effects of adhesive materials to dentin. Mahoney and others (2000) measured the hardness and elastic modulus of sound maxillary primary molar dentin using a nano-indentation tester. However, they used not only sound teeth but also carious teeth as sample teeth and calculated the mixed data. They did not compare the values at different depths or locations of dentin. The elasticity of carious dentin has not been reported for primary carious teeth and only limited studies have been reported for permanent teeth (Marshall & others, 2001).

This study measured the nanohardness and elastic modulus of carious and sound canine dentin and compared the values among areas under the lesions and in sound regions of the incisal, center and cervical areas in the outer, middle and inner layers of each area.

METHODS AND MATERIALS

Sample Teeth

Three sound maxillary primary canines and three mandibular primary canines with caries at the center portion of both proximal surfaces were used for this study. All teeth were extracted or exfoliated by eruption of the succedaneous permanent tooth or extracted as required for orthodontic treatment from Japanese children. The teeth were stored in 4°C physiologic saline solution shortly after extraction or exfoliation. The age of the patients ranged from six years four months to seven years nine months. Informed consent was obtained from the parents and patients in order to collect the teeth. Radiographs were taken to identify the carious areas. The total number of carious lesions was six. In all carious lesions, caries did not extend more than one-fourth the depth of the dentin.

Specimen Preparation

Sound teeth were mesiodistally sectioned parallel to the long axis at the center of the tooth. For carious teeth with caries on both proximal surfaces, the teeth were longitudinally sectioned through the central part of the largest carious lesion. Sectioning was done using a low-speed saw (Buehler Ltd, Lake Bluff, IL, USA) with a circular diamond blade and copious filtered water.

After sectioning, the specimens were polished on wet silicon carbide paper using grit sizes of 600, 800, 1000 and 1200. Final polishing was carried out on felt cloth using 3, 1, 0.1, 0.3 and 0.05 µm-size aluminum oxide suspensions (Baikowski International Co, Charlotte, NC, USA). Optical photomicrographs of the polished specimens were taken with a microscope (Olympus Co, Tokyo, Japan), and the infected, affected and sound portions of the dentin were identified. The sectioned and polished specimens were stored in 4°C distilled water until measurement and were dried at room temperature for 20 minutes prior to the study.

Nano-Indentation Test

Cyanoacrylate (Konishi Co, Tokyo, Japan) was applied onto small areas of enamel of the specimen, and the specimen was then fixed on a flat glass plate to stabilize its surface and orient the surface parallel to the stage of the nano-indentation tester ENT-1100 (Elionix Co, Tokyo, Japan). ENT-1100 is a depth sensing computer-controlled instrument and has a Berkovich indentor, a three-sided pyramid diamond probe. The instrument was enclosed in an isolation chamber with a temperature controller and placed on an ALD anti-vibration isolator in order to minimize influences of environmental conditions such as room temperature, floor-vibration and noise. The specimen was kept in dry conditions during measurement since this machine can control temperature in the chamber at 26°C but not humidity. The loading control system was powered by electromagnetic force and load ranges from 10 mgf to 100 gf. The position of indentation could be programmed and the indents observed with a CCD camera attached to the tester.

Figure 1 shows a load vs displacement curve from the measurement process. Values of hardness (H), plastic hardness (PH) and Young's modulus (Y) were calculated according to the equations (1), (2) and (3), respectively, following the index of Elionix company that was modified from the method reported by Oliver and Pharr (1992):

$$H=3.7926 \times 10^{-2} \times P_{max}/h_{max}^{2} \tag{1}$$

$$PH=3.7926 \times 10^{-2} \times P_{max}/h_{l}^{2} \tag{2}$$

$$Y=1.81092 \times 10^{-3} \times 1/h_{l} \times dp/dh \tag{3}$$

in which P_{max} is the maximum applied load, h_{max} is the maximum penetration depth, h_l is the intercept depth and dP/dh is the contact stiffness from the unloading portion of the load vs displacement curve.

Figure 2 shows the names of the areas and layers of the sample teeth for measurement. For sound teeth, the mesial and distal sides were divided into incisal, center and cervical areas, and for the carious teeth, the mesial and distal sides were divided into center and mesial incisal areas. Distal incisal areas were not measured because the small size of the teeth relative to the lesion size suggested that this area would have been altered by caries. The cervical area of the carious teeth was not measured in this study, because this area could not be reliably measured in the carious teeth due to the presence of caries. Then, each area was divided into outer, middle and inner layers. For regions under the lesion, the term "under the lesion" was used instead of the term "outer layer."

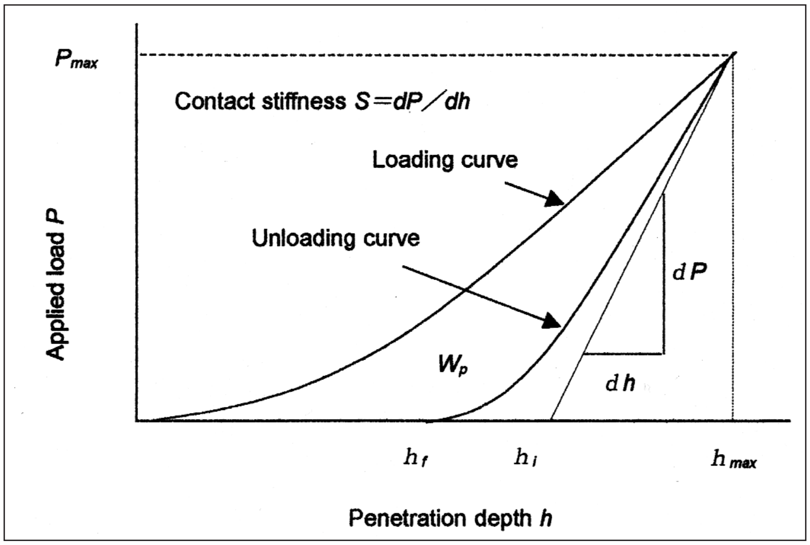

Figure 1. *Load vs displacement curve in the measurement process.*

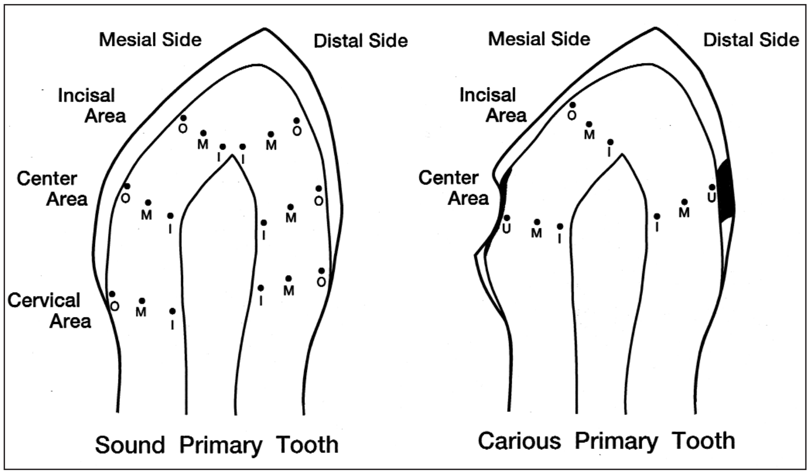

Figure 2. *Names of the areas and layers of the sample teeth in measurement (O: outer layer, M: middle layer, I: inner layer, U: under the lesion layer).*

Ten indentations at intervals of 10 µm in each of the subdivided regions were made perpendicular to the outline of the dentinoenamel junction or DEJ using a load of 1 gf for one second. The positions of the indentations were as follows. The first point of the outer layer was made at 10 µm beneath the DEJ. The first point of the middle layer was made at the center from the DEJ to the pulp chamber wall. The last point of the inner layer was made at the most inner measuring point in primary dentin close to the pulp chamber wall. Indentations were observed using a microscope with a CCD camera attached to the tester under 700x magnification. Some of the indentations were also observed using a scanning electron microscope (SEM) S-3500 (Hitachi Ltd, Tokyo, Japan). For regions under the lesion, measurement was started just internal to the infected and destroyed dentin layer, and the shape of the indentation at the first point was verified by examining the indents with the CCD camera and SEM. Irregular or unclear shaped indentations were removed from the data. Therefore, all of the selected indentations were marked on the intertubular dentin.

All data was statistically analyzed using ANOVA followed by Scheffe's multiple comparison testing and correlation coefficients at $p<0.05$.

RESULTS

First, the means and standard deviations of the values of 10 indentations on each layer were calculated and compared to different areas and layers on the same sample. Tooth to tooth differences existed but regional differences were similar on all samples. Then, the values for all samples were mixed and statistically compared among different areas and layers in this study.

Table 1 shows the hardness, plastic hardness and Young's modulus of sound primary canine dentin. In general, related to the incisal, center and cervical areas, the hardness, plastic hardness and Young's modulus decreased from DEJ to pulp and the values of the inner layer were significantly lower than the outer and middle layers. For cervical areas, the hardness and plastic hardness of the outer layer were significantly higher than the middle and inner layers. Comparing values in the same layer among the different areas, the hardness, plastic hardness and Young's modulus were reduced from incisal to cervical areas and, in general, the values of the cervical area were significantly lower than those of the incisal and center areas.

Table 2 shows the hardness, plastic hardness and Young's modulus of carious primary canine dentin. For the center area or under the decayed area, all values of the inner layer were significantly lower than the outer and middle layers. In the incisal area, which was a sound region of the carious teeth, the values of the outer layer were significantly higher than the middle and inner layers.

Table 3 compares the hardness, plastic hardness and Young's modulus of carious primary canine dentin and sound primary canine dentin. The center area had values under the lesion that were significantly lower than the sound teeth in the outer and middle layers.

Two- or three-way ANOVA and Scheffe's tests indicated that the depth of dentin and caries decay had the most significant influence on the values, and the area of dentin also had a second significant influence on the values.

Figures 3 and 4 show the average hardness and Young's modulus, respectively, for sound and carious primary canine dentin on both the mesial and distal sides of the teeth. The trends for both were similar.

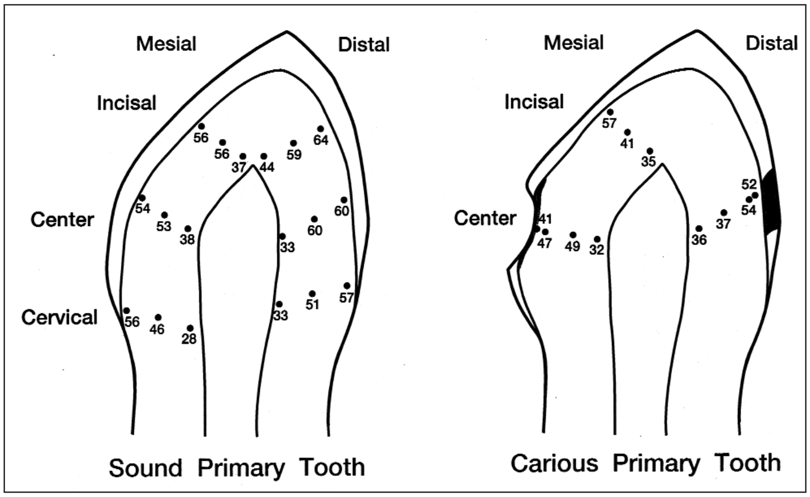

Figure 3. *Average hardness values of sound and carious primary canine dentin.*

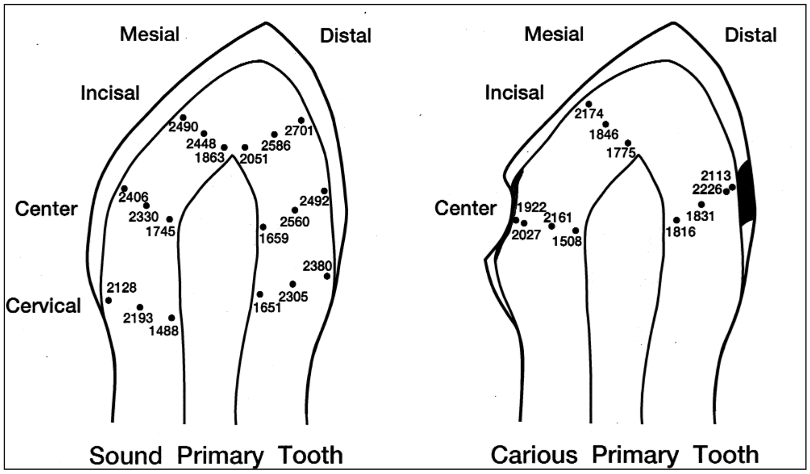

Figure 4. *Average Young's modulus of sound and carious primary canine dentin.*

Table 1: *Hardness, Plastic Hardness and Young's Modulus of Sound Primary Canine Dentin (unit: kgf/mm^2)*

Area	Layer	Hardness Mean (SD)	Plastic Hardness Mean (SD)	Young's Modulus Mean (SD)	Number of Measurements
Incisal	Outer	60.0 (8.0)	71.5 (12.6)	2597 (367)	59
	Middle	57.3 (13.9)	74.3 (19.7)	2517 (423)	60
	Inner	40.6 (10.7)	50.6 (14.8)	1957 (364)	60
Center	Outer	57.0 (11.3)	71.6 (15.8)	2447 (393)	60
	Middle	56.8 (12.6)	71.6 (18.0)	2445 (379)	58
	Inner	35.4 (10.4)	44.3 (14.5)	1702 (347)	60
Cervical	Outer	56.8 (6.7)	76.5 (11.7)	2254 (341)	60
	Middle	48.7 (12.4)	60.8 (18.8)	2249 (312)	60
	Inner	30.6 (9.4)	36.6 (13.4)	1569 (263)	60

Vertical line: no significant difference at $p<0.05$

Table 2: *Hardness, Plastic Hardness and Young's Modulus of Carious Primary Dentin (unit: kgf/mm^2)*

Area	Layer	Hardness Mean (SD)	Plastic Hardness Mean (SD)	Young's Modulus Mean (SD)	Number of Measurements
Center	Under Lesion	46.9 (19.5)	57.5 (25.0)	2023(618)	59
	Middle	42.9 (17.7)	53.5 (23.5)	1996 (700)	60
	Inner	33.6 (12.0)	41.0 (15.8)	1662 (498)	60
Incisal	Outer	57.1 (15.1)	77.2 (20.7)	2174 (593)	29
	Middle	40.7 (21.0)	52.0 (30.2)	1846 (596)	30
	Inner	34.8 (11.2)	42.0 (14.7)	1775 (429)	30

Vertical line: no significant difference at $p<0.05$

Table 3: *Hardness, Plastic Hardness, and Young's Modulus of Carious and Sound Primay Dentin (unit: kgf/mm^2)*

Layer	Tooth	Area	Hardness Mean (SD)	Plastic Hardness Mean (SD)	Young's Modulus Mean (SD)	Number of Measurements
Under Lesion	Carious	Center	46.9 (19.5)	57.5 (25.0)	2023 (618)	59
Outer	Carious	Incisal	57.1 (15.1)	77.2 (20.7)	2174 (593)	29
Outer	Sound	Center	57.0 (11.3)	71.6 (15.8)	2447 (393)	60
Middle	Carious	Center	42.9 (17.7)	53.5 (23.5)	1996 (700)	60
Middle	Carious	Incisal	40.7 (21.0)	52.0 (30.2)	1846 (596)	30
Middle	Sound	Center	56.8 (12.6)	71.6 (18.0)	2445 (379)	58
Inner	Carious	Center	33.6 (12.0)	41.0 (15.8)	1662 (498)	60
Inner	Carious	Incisal	34.8 (11.2)	42.0 (14.7)	1776 (429)	30
Inner	Sound	Center	35.4 (10.4)	44.3 (14.5)	1702 (347)	60

Vertical line: no significant difference at $p<0.05$

Under the lesion, not only the average values but also the values at the first measuring point located in the discolored layer were plotted on the figures. Values under the lesion and in the middle layers under the decayed areas or center area of the carious teeth were significantly lower than either the outer or middle layers at the center areas in the sound teeth.

Hardness and Young's modulus showed significant correlations (corr = 0.932, $p<0.0001$).

DISCUSSION

The nano-indentation technique has several advantages for determining hardness over conventional microhardness methods such as Vickers and Knoop hardness. This technique has the ability to produce small indentations under small loads and can measure both the hardness and elastic modulus of materials. Previous reports have suggested wide variations in the basic mechanical properties of dentin. Some of this variation may result from using techniques such as microhardness, which yield averaged values that include contributions from the tubules, peritubular dentin and intertubular dentin. Since the quality of each dentin component might vary with location, this could contribute to the wide range of values. However, work by Kinney and others (1996) indicated that much of the variation in permanent teeth could result from differences in intertubular dentin rather than peritubular dentin. In this work, we used a nanoindentation technique that allowed measurement of the intertubular dentin alone.

Sound Teeth

In this study, the hardness values of sound primary canine dentin ranged from 30 to 60 kgf/mm^2 and plastic hardness ranged from 37 to 77 kgf/mm^2 (Table 1, Figure 3). These values were lower than that for primary molars (0.92 GPa or 94 kgf/mm^2) as reported by Mahoney and others (2000) but were in agreement with previous studies of sound permanent dentin (Fusayama & Maeda, 1969; Pashley & others, 1985; Sano & others, 1994; Kinney & others, 1996). Hardness is calculated by maximum penetration depth that includes both plastic and elastic deformation of dentin. Plastic hardness is calculated based only on the plastic deformation of dentin and corresponds to Vicker's hardness. In this study, variations in hardness and plastic hardness were almost the same for all layers and areas. Young's modulus of dentin for sound primary canines ranged from about 1600 to 2600 kgf/mm^2 (Table 1, Figure 4). These values were in agreement with a previous study of primary molar dentin (19.89 GPa or 2029 kgf/mm2) (Mahoney & others, 2000) and sound permanent dentin (Craig & Peyton, 1958; Bowen & Rodriguez, 1962; Lehmann, 1967; Sano & others, 1994; Kinney & others, 1996; Meredith & others, 1996; Xu & others, 1998; Kinney & others, 1999).

No report using a nano-indentation technique has compared the hardness and elasticity among the depths and areas of primary dentin. Meredith and others (1996) and Hosoya and others (2000; 2002) reported that the hardness of dentin decreased with distance from the dentinoenamel junction, while Pashley and others (1985) reported a highly significant inverse correlation in permanent dentin between dentin microhardness and tubule numerical density that increases with depth. However, Kinney and others (1996) reported that most of the decrease in dentin hardness upon approaching the pulp chamber could be attributed to changes in the hardness of intertubular dentin, not just the increase in the number of tubules.

In this study, for all areas, hardness, plastic hardness and Young's modulus of the inner layer were significantly lower than that of the outer and middle layers (Table 1). These results for the hardness and plastic hardness agreed with previous studies (Craig & Peyton, 1958; Bowen & Rodriguez, 1962; Lehmann, 1967; Sano & others, 1994; Kinney & others, 1996; Meredith & others, 1996; Kinney & others, 1999), but the result of the Young's modulus was in disagreement with suggestions by Kinney and others (1999). They utilized atomic-force microscope (AFM) based nanoindentation measurements and suggested that Young's modulus was dominated by the properties of intertubular dentin and could show a slight increase from outer to inner dentin. However, the current study showed significant correlations among the values of hardness and Young's modulus, which were in agreement with the previous report for primary molars (Mahoney & others, 2000).

In this study, there was no significant difference in hardness, plastic hardness and Young's modulus between the outer and middle layers of all areas, except for the hardness and plastic hardness of the cervical area. Since the outer layer of this study was positioned 10-110 µm beneath the DEJ, the finding that there was no significant hardness difference between the outer and middle layers might be due to the influence of the mantle dentin.

Carious Teeth

Craig and others (1959) reported that permanent dentin surrounding a caries lesion had a hardness of 10 KHN greater than sound dentin; the hardness at the center of the lesion was significantly lower and transparent dentin was 10 KHN harder than the adjacent area. Fusayama and others (1966) and Ogawa and others (1983) reported that Knoop hardness for the discolored layer of carious permanent dentin ranged from 20 to 27 KHN, the transparent layer from 27 to 48 KHN, the subtransparent layer from 48 to 68 KHN, and sound dentin varied from 21 to 68 KHN. In our previous study (Hosoya & others, 2002), the average Knoop hardness values of primary canine dentin under the lesion ranged from 31 to 43 KHN in the outer layer, 35 to 44 KHN in the middle layer and 30 to 43 KHN in the inner layer, respectively. However, values less than 150 µm from the bottom of cavity were about 29 KHN and were lower than the region 250 µm away from the cavity. The hardness of many layers and areas of the carious side of carious primary canines, even in apparently unaffected dentin, were significantly lower than the sound side of carious primary canines, suggesting that they were affected by caries. In our previous study on carious primary anterior teeth (Hosoya & others, 2000), the transparent layer had significantly higher hardness than the more superficial decayed layer, but it was not significantly different from adjacent areas or areas to the side of the lesion. In another previous study (Hosoya & others, 2002), there was no significant difference in hardness between transparent dentin and non-transparent dentin in the same areas and layers. Marshall and others (2001) reported that the hardness and elastic modulus of intertubular dentin decreased slightly or was unchanged in the transparent dentin of permanent teeth.

In the incisal area that was sound in carious teeth, the hardness, plastic hardness and Young's modulus of the outer layer were significantly higher than the middle and inner layers. However, in the center area that was under the decayed area, there was no significant difference in hardness, plastic hardness and Young's modulus under the lesion (outer) and middle layers (Table 2). All mechanical values under the lesion were significantly lower than the corresponding regions of sound teeth (Table 3), although caries did not extend more than one-fourth the depth of the dentin in the teeth used for this study. Therefore, the results of this study indicate that not only in the obviously affected layer but also in the areas adjacent to the lesion not obviously affected by caries, the dentin was softer and had a lower elastic modulus than sound dentin (Figures 3 and 4). In this study, the lesions were small and only one linear measurement was done under the carious area. Therefore, the values of the transparent dentin could not be statistically analyzed. However, no significant difference was obtained between the values of the first measuring point under the lesion, which was positioned 10 µm from the bottom of cavity and the discolored dentin and the average values under the lesion, which included all measurements 10-110 µm from the bottom of cavity and included not only discolored dentin but also transparent dentin (Figures 3 and 4). This further suggests that transparent dentin may not be sclerotic as noted by other studies (Fusayama & others, 1966; Ogawa and others, 1983; Hosoya and others, 2000; Marshall and others, 2001).

The results of this study showed that hardness and elastic modulus of dentin differed with intratooth location and decreased with distance from the DEJ in primary canine dentin (Tables 1-3, Figures 3 and 4). Hardness and elastic modulus of dentin might also differ with tooth type and environmental factors during the time of tooth formation and mineralization. In this study, all the sound teeth were maxillary primary canines but all the carious teeth were mandibular primary canines. In addition, the position of the carious lesion was almost at the center area of the proximal surfaces but was not the same among the specimens. The depth from the DEJ to the first measuring point under the lesion differed among the carious teeth and differed between the sound and carious teeth. These differences, due to varying lesion shapes, caused considerable variation among the specimens but, in general, both dentin under the lesion and apparently normal dentin adjacent to the lesion had reduced hardness and elastic modulus compared to sound dentin. None of the areas associated with carious teeth had increased values, thus, there was no evidence of sclerotic dentin in these lesions.

Although the use of nano-indentation permits smaller areas than microhardness to be measured, both the microhardness and nano-indentation equipment used for this study were carried out on dried tissues. When demineralized dentin is dried, it collapses (Marshall & others, 1998), and indentations on the collapsed layer could be influenced by the underlying mineralized tissue. Improved methods such as an AFM-based indentation, which allows submicroscopic indentations of hydrated tissues, may be needed to improve accuracy of the values of various altered layers of carious dentin (Balooch & others, 1998; Marshall and others, 2001).

This study and our previous work (Hosoya & others, 2000; Hosoya & others, 2002) indicate that lower hardness and elastic modulus values were obtained under the caries region and also in the region adjacent to the caries that was apparently not affected by the lesion. This could have a deleterious effect on resin adhesion to these regions. After etching, the hardness might be lower or the depth of demineralization might be increased so that bonding to these areas might require specific etching treatments that have not yet been defined. Previous reports (Nor & others, 1997; Olmez & others, 1998; Hosoya & others, 2001) suggested that primary dentin is more susceptible to acid or some chemical conditioning treatments and, therefore, shorter application times for the dentin conditioner in primary dentin may be appropriate. Further study is required to understand the precise mechanism of adhesion between carious dentin and resinous materials and whether different treatments are needed to optimize bonding for primary and permanent dentin.

CONCLUSIONS

1. Hardness and elastic modulus of sound primary canine dentin were consistent with prior reported results for permanent dentin.
2. Hardness and elastic modulus for primary canine teeth with carious lesions showed markedly lower mechanical properties than sound primary dentin. No areas with increased mechanical properties were observed, thus, there was no evidence of sclerotic dentin associated with primary canine caries.
3. Apparently unaffected dentin in areas adjacent to carious lesions had reduced mechanical properties compared to primary dentin in sound teeth.

Acknowledgements

The authors acknowledge Mr Hideo Suzuki and Mr Takahiko Uematsu (Elionix Co) for their technical assistance in the operation of the nanoindentation tester. This study was partially supported by the Japanese Ministry of Education, Science, Sport and Culture grant 11672053 and 14571955.

(Received 30 January 2003)

References

Balooch M, Wu-Magidi IC, Lundkvist AS, Marshall SJ, Marshall GW, Seikhaus WJ & Kinney JH (1998) Viscoelastic properties of demineralized human dentin in water with AFM-based indentation *Journal of Biomedical Materials Research* **40** 539-544.

Bowen RL & Rodriguez MM (1962) Tensile and modulus of elasticity of tooth structure and several restorative materials *Journal of the American Dental Association* **64** 378-387.

Craig RG & Peyton FA (1958) Elastic and mechanical properties of human dentin *Journal of Dental Research* **37** 710-718.

Craig RG, Gehring PE & Peyton FA (1959) Relation of structure to the microhardness of human dentin *Journal of Dental Research* **38** 624-630.

Fusayama T, Okuse K & Hosoda H (1966) Relationship between hardness, discoloration, and microbial invasion in caries dentin *Journal of Dental Research* **45** 1033-1046.

Fusayama T & Maeda T (1969) Effect of pulpectomy on dentin hardness *Journal of Dental Research* **48** 452-460.

Fusayama T (1993) New concepts in the pathology and treatment of dental caries A simple pain-free adhesive restorative system by minimal reduction and total etching Ishiyaku Euro America Inc, St Louis, MO p 1-16.

Hosoya Y, Marshall SJ, Watanabe LG & Marshall GW (2000) Microhardness of carious deciduous dentin *Operative Dentistry* **25(1)** 81-89.

Hosoya Y, Kawashita Y, Marshall GW Jr & Goto G (2001) Influence of Carisolv™ for resin adhesion to sound human primary dentin and young permanent dentin *Journal Dentistry* **29** 163-171.

Hosoya Y, Ono T & Marshall GW (2002) Microhardness of carious primary canine dentin *Pediatric Dentistry Journal* **12** 91-98.

Johnsen DV (1994) Comparison of Primary and Permanent Teeth Oral Development and Histology 2nd ed JK Avery, New York: Thiema Medical Publishers p 287.

Kinney JH, Balooch M, Marshall SJ, Marshall GW Jr & Weihs TP (1996) Hardness and Young's modulus of human peritubuler and intertubular dentin *Archives of Oral Biology* **4** 9-13.

Kinney JH, Balooch M, Marshall GW & Marshall SJ (1999) A micromechanics model of the elastic properties of human dentin *Archives of Oral Biology* **44** 813-822.

Lehmann M (1967) Tensile strength of human dentin *Journal of Dental Research* **46** 197-201.

Mahoney E, Holt A, Swain M & Kilpatrik N (2000) The hardness and modulus of elasticity of primary molar teeth: An ultra-micro-indentation study *Journal of Dentistry* **28** 589-594.

Marshall GW, Wu-Magidi IC, Watanabe LG, Inai N, Balooch M & Kinney SJ (1998) Effect of citric acid concentration on dentin demineralization, dehydration and rehydration: An AFM study *Journal of Biomedical Materials Research* **42** 500-507.

Marshall GW, Habelitz S, Gallagher R, Balooch M & Marshall SJ (2001) Nanomechanical properties of hydrated caries human dentin *Journal Dental Research* **80** 1768-1771.

Meredith N, Sherriff M, Stechell DJ & Swanson SA (1996) Measurement of the microhardness and Young's modulus of human enamel and dentine using an indentation technique *Archives of Oral Biology* **41** 539-545.

Nakajima M, Sano H, Burrow MF, Tagami J, Yoshiyama E, Ebisu S, Ciucchi B, Russell CM & Pashley DH (1995) Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives *Journal of Dental Research* **74** 1679-1688.

Nakajima M, Ogata M, Okuda M, Tagami J, Sano H & Pashley DH (1999) Bonding to caries-affected dentin using self-etching primers *American Journal of Dentistry* **12** 309-314.

Nor JE, Feigel RJ, Dennison JB & Edward CA (1997) Dentin bonding: SEM comparison of the dentin surface in primary and permanent teeth *Pediatric Dentistry* **19** 246-252.

Ogawa K, Yamashita Y, Ichijo T & Fusayama T (1983) The ultrastructure and hardness of the transparent layer of human carious dentin *Journal of Dental Research* **62** 7-10.

Olmez A, Oztas N, Basak F & Erdal S (1998) Comparison of the resin-dentin interface in primary and permanent teeth *Journal of Clinical Pediatric Dentistry* **22** 293-298.

Oliver WC, Pharr GW (1992) An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments *Journal of Materials Research* **7** 1564-1583.

Pashley DH, Okabe A & Parham P (1985) The relationship between dentin microhardness and tubule density *Endo Dental Traumatology* **1** 176-179.

Retief DH (1987) Are adhesive techniques sufficient to prevent microleakage? *Operative Dentistry* **12** 140-145.

Sano H, Ciucchi B, Matthews WG & Pashley DH (1994) Tensile properties of mineralized and demineralized human and bovine dentin *Journal of Dental Research* **73** 1205-1211.

Urabe I, Nakajima M, Sano H & Tgami J (2000) Physical properties of the dentin-enamel junction region *American Journal of Dentistry* **13** 129-135.