Original

Individual Variations in the Hardness and Elastic Modulus of the Human Cementum

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Abstract: The purpose of this study was to measure the physical properties of enamel and cementum of permanent teeth and to determine the correlation between them. This study was conducted on 50 maxillary premolars that were collected from 26 orthodontic patients (10 males and 21 females, mean age: 19.51 years, range: 12-35 years). The hardness and elastic modulus of the enamel and cementum were measured at the surface of the crown and root at three locations (cervical third: CC, middle third: CM, and apical third: CA). There were individual differences in the hardness and elastic modulus of enamel and cementum. The hardness of the cementum decreased from the cervical to apical regions on the root surfaces in the moderate and soft groups ($p < 0.01$). Individual variations were observed in the hardness and elastic modulus of the human first premolar cementum. A correlation was noted between the hardness and elastic modulus of the enamel and cementum in the CA group (hardness: $r = 0.551$, $p < 0.01$, elastic modulus: $r = 0.552$, $P < 0.01$). These results suggested that the physical properties of cementum may be involved in the occurrence of root resorption caused by orthodontic forces.

Key words: Hardness, Elastic modulus, Cementum, Enamel, Human teeth

Introduction

Orthodontically-induced inflammatory root resorption (OIIRR) is an unwanted side effect of orthodontic treatment. Motokawa et al.¹⁾ reported that teeth undergoing orthodontic treatment may develop some degree of OIIRR. It has been reported that 2%-5% of orthodontic patients experience more than 5 mm of resorption during orthodontic treatment with fixed appliances. It is therefore critical for orthodontists to anticipate this complication and prevent it whenever possible. The etiology of root resorption associated with orthodontic therapy is complex, and heavy force²⁾, lengthy treatment, and abnormal roots all further contribute to resorption³⁾.

Cementum contains 65% inorganic material and 12% water on a wet-weight basis. By volume, inorganic material comprises approximately 45%, organic material 33%, and water 22%. Cementum is less densely mineralized than dentine and enamel and contains no blood vessels and does not undergo physiological remodeling⁴⁻⁶⁾. The chemical composition of cementum may vary

by individual, and morphologically, cementum is classified as both cellular and acellular⁷⁾.

In previous studies, Chutimanutskul et al.⁸⁾ examined the physical properties of the cementum on the buccal and lingual surfaces of the roots at the cervical third, middle third, and apical third. The authors reported a decreasing gradient in the hardness and elastic modulus of cementum in both surface groups, from the cervical to apical thirds. Apical cementum is predominately cellular, less densely mineralized and has lower hardness and elastic modulus values than the more densely mineralized acellular cementum found in the middle and cervical thirds of the root⁷⁾. Therefore, the hardness and elastic modulus of cementum depend on the direction of the structural arrangement and the mineral content of the cementum⁹⁻¹¹⁾. Several studies have reported that the hardness of mineralized tissues were positively correlated with the extent of mineralization¹²⁻¹⁴⁾. Some have suggested that the mineral content of cementum might influence the resistance or susceptibility to root resorption. Therefore, the hardness of cementum may be related to the incidence of OIIRR.

In the present study, we measured the physical properties of enamel and cementum of permanent teeth and examined the correlation between them.

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Figure 1. Location of indentations on maxillary premolar cementum and enamel. Four positions on the buccal root surfaces were used. Scale bar: 5 mm.

Materials and Methods

Sample collection

This study was conducted on 50 maxillary premolars collected from 26 orthodontic patients (10 males and 21 females, mean age: 19.51 years, range: 12-35 years). We obtained informed consent from the patients, and the extracted teeth were used in accordance with a protocol reviewed by the Ethics Committee of Nihon University School of Dentistry at Matsudo (EC 15-001). The selection criteria were:

1. No previous reported or observed dental treatment of the teeth to be extracted,
2. No previous reported or observed trauma treatment of the teeth to be extracted,
3. No previous reported or observed orthodontic treatment of the teeth to be extracted,
4. No past or present signs or symptoms of periodontal disease,
5. No past or present signs or symptoms of bruxism,
6. No significant medical history that would affect the dentition,
7. No physical abnormalities concerning the anatomy of the craniofacial or dentoalveolar complex,
8. Completed apexification.

The teeth were extracted by oral surgeons who were asked to avoid damaging the cervical cementum with the forceps. Immediately after extraction, the teeth were stored in individual containers filled with sterilized deionized water. The extracted teeth were then placed in an ultrasonic bath for 10 minutes to remove the periodontal ligament (PDL) and soft tissue fragments. After the ultrasonic bath, the extracted teeth were gently swabbed with a damp gauze swab until all visible signs of the PDL had been removed. The extracted teeth were then sterilized in accordance with the protocol described in previous publications¹²⁾.

Vickers hardness and elastic modulus tests

We evaluated the Vickers hardness and elastic modulus of the extracted teeth's enamel and cementum and examined the correlation between these values for the enamel and apical third. The extracted teeth's roots were divided into equal thirds (cervical

Table 1. The mean and SD values of the hardness and elastic modulus of enamel and cementum

a) Vickers hardness

	E	CC	CM	CA
Mean	394.46	26.50	20.67	19.00
SD	41.92	7.81	6.35	5.62
Min.	275.99	12.10	11.14	10.69
Max.	480.19	43.85	28.88	30.91

b) Elastic modulus (GPa)

	E	CC	CM	CA
Mean	82.00	11.67	9.13	7.26
SD	16.92	2.00	2.09	1.97
Min.	28.60	5.90	4.30	4.21
Max.	106.25	16.90	14.60	11.00

[CC], middle [CM], and apical [CA]) for the measurements (Fig. 1). The measurement point was the center of the major axis in each region, at the center line on the buccal side. The mean values of three measurements in each part were obtained.

We focused on the CA Vickers hardness in particular, as root resorption following orthodontic treatment often occurs in the apical part. The extracted teeth were classified into soft, moderate, and hard groups according to the CA Vickers hardness as follows: soft group, less than the mean – 1 standard deviation (SD); moderate group, the mean $\pm$ SD; hard group, more than the mean + 1 SD. We compared the hardness of the CC, CM, and CA in each group.

The physical properties of the enamel and cementum were evaluated using a dynamic ultra-micro-hardness tester (DUH-211; Shimadzu Co., Kyoto, Japan) with a load rate of 13.32 mN/s and a holding time of 10 s at a maximum load of 98.07 mN.

Statistical analysis

In the statistical analysis, we compared the mean CC, CM, and CA hardness values of each group using the Steel-Dwass test, with significance set at $P < 0.01$. A box plot analysis was used to determine the hardness and elastic modulus of the enamel and three regions (CC, CM, CA) in the cementum. Correlation coefficients and regression equations were formulated to evaluate any relationship between the physical properties of the enamel and CA.

Results

The mean and SD values of the hardness and elastic modulus of the enamel and cementum are shown in Table 1. A box plot analysis revealed significant individual variations in the mean hardness and elastic modulus of the cementum (respective ranges: 10.69-43.85 and 4.21-16.90 GPa) and enamel (respective ranges: 275.99-480.19 and 28.60-106.25 GPa) (Figs. 2-a, b, 3-a, b). The

Figure 2. The hardness of enamel and cementum.

a: Box plot analysis of the hardness of enamel and cementum (CC, CM and CA).

b: Significant differences were noted among the soft, moderate, and hard group. (* $P < 0.01$ from the Steel-Dwass test).

Figure 3. The elastic modulus of enamel and cementum.

a: Box plot analysis of the elastic modulus of enamel and cementum (CC, CM and CA).

b: Significant differences were noted among the soft, moderate, and hard group. (* $P < 0.01$ from the Steel-Dwass test).

Table 2. Comparison of the hardness and elastic modulus of the CC, CM, and CA in each group

a) Vickers hardness	CC		CM		CA		Steel-Dwass		
	Mean	SD	Mean	SD	Mean	SD	CC vs CM	CC vs CA	CM vs CA
Total	26.50	7.81	20.67	6.35	19.00	5.62	*	*	NS
Soft	24.11	5.62	21.25	9.28	12.84	1.26	NS	*	*
Moderate	25.76	6.36	19.72	5.06	18.09	2.54	*	*	NS
Hard	30.28	10.84	21.86	5.06	26.85	2.37	NS	NS	NS

* $P < 0.01$, NS: not significant.

b) Elastic modulus (GPa)	CC		CM		CA		Steel-Dwass		
	Mean	SD	Mean	SD	Mean	SD	CC vs CM	CC vs CA	CM vs CA
Total	11.67	2.00	9.13	2.09	7.26	1.97	*	*	*
Soft	11.59	1.84	9.27	2.11	5.53	1.09	*	*	*
Moderate	11.12	1.82	8.64	2.08	7.15	1.53	*	*	NS
Hard	12.87	2.13	9.94	1.97	9.41	1.58	*	*	NS

* $P < 0.01$, NS: not significant.

hardness and elastic modulus of the cementum in the CM and CA were lower than in the CC (Table 1-a, b). No significant differences in the values were noted between genders (date not shown).

Regarding the CA hardness of the three groups (soft, moderate, and hard), the hardness decreased from the cervical to apical regions on the root surfaces in the soft and moderate groups ($p < 0.01$), while the differences in the hardness of different regions in the hard group were not significant (Table 2-a). The elastic

modulus decreased from the cervical to apical regions on the root surfaces in all hardness groups ($p < 0.01$) (Table 2-b).

To estimate the physical properties of the cementum based on those of the enamel, we examined the correlation between them and found a positive correlation between the hardness and elastic modulus of the enamel and cementum in the CA (hardness: $r = 0.551$, $p < 0.01$, elastic modulus: $r = 0.552$, $P < 0.01$) (Figs. 4, 5).

Figure 4. Correlation between the hardness of the enamel and cementum.
A correlation was shown between the hardness of the enamel and cementum in the CA group ($r=0.551$, $P<0.01$).

Discussion

Hardness is a major parameter to consider when observing structural changes in the cementum tissue that depend on root resorption caused by orthodontic forces. In this study, the dynamic micro-indentation method was applied to investigate mechanical properties such as the indentation hardness and elastic modulus of the enamel and cementum.

Generally, there are two methods used to measure the indentation hardness of materials. The conventional procedure for testing hardness involves applying a fixed load to a diamond indenter and measuring, with the aid of optical microscopy, the dimensions of the resultant indentation on the surface of the test material after unloading. However, it is difficult to optically measure such dimensions with a high degree of accuracy for indents in micro-scale structures, such as biotissue. The dynamic micro-indentation method therefore has significant advantages over conventional hardness testing. This method is a depth-sensing technique that can accurately characterize the mechanical properties of almost all types of solid materials on a small scale. In other words, this method can continuously record the penetration depth of the cementum tissue during small dynamic loading and unloading at the indentation tip while providing information on the hardness and elastic modulus of the small contact surface.

The present study examined whether or not there was individual variation in the hardness and elastic modulus values of the cementum and enamel of permanent teeth. Regarding the age of the samples in this study, Pinchi et al.¹⁵⁾ reports that the cementum thickens with age. We therefore were limited in the age range of the samples we could use without thickening of the cementum (12-35 years old). Furthermore, there was no significant difference in the values of gender (date not shown).

Figure 5. Correlation between the elastic modulus of enamel and cementum.
A correlation was shown between the elastic modulus of the enamel and cementum in the CA group ($r=0.552$, $P<0.01$).

The results in this study showed that the respective hardness and elastic modulus of the enamel and cementum differed significantly. (Figs. 2-a, b, 3-a, b, Table 1-a,b). Relatively little is known about the individual variation in the physical properties of the enamel and cementum of permanent teeth. In their examination of a small ($n=16$) mixed sample of maxillary and mandibular first premolars, Darendeliler et al.¹⁶⁾ reported large individual variation for both the hardness and elastic modulus. These present and previous findings support the existence of individual differences in the hardness and elastic modulus of the cementum.

Generally, the mineralized surface of the cementum in humans is more resorbed in the apical part after orthodontic tooth movement than in other regions. Therefore, we measured the hardness and elastic modulus in the CA. On dividing the CA hardness into three groups (soft, moderate, and hard), we found that the hardness decreased from the cervical to apical regions on the root surfaces in the soft and moderate groups ($p<0.01$), while the differences in the hardness of different regions in the hard group were not significant (Table 2-a). Furthermore, the elastic modulus decreased from the cervical to apical regions on the root surfaces in all hardness groups ($p<0.01$) (Table 2-b).

Chutimanutskul et al.⁸⁾ reported that the hardness and elastic modulus of the human maxillary first premolar cementum gradually decreased from the cervical to apical regions on the buccal and lingual surface. The cervical two thirds of the root is covered by acellular cementum that consists of mineralized layers. However, the apical cementum is predominately cellular cementum. The hardness and elastic modulus of the apical region are lower than those of the middle and cervical thirds of the root. Rex et al.¹¹⁾ demonstrated that there were large individual variations in the hardness of the cementum of the first premolars based on the calcium (Ca), phosphorus (P), and fluorine (F) concentrations,

with a decreasing gradient in these concentrations from the cervical to the apical third of the root. These previous findings may support the results in the present study. Taken together, these present and previous findings suggest that the low hardness value (soft) may be due to low concentrations of Ca, P, and F and be involved in the outbreak of OIIRR in the apical region. Preparations are currently underway for a new study measuring the proportions of Ca, P, and F in the apical zone in the soft, moderate, and hard groups.

Finally, we observed a positive correlation between the respective hardness and elastic modulus of the enamel and cementum in the CA group (hardness: $r=0.551$, $p<0.01$, elastic modulus: $r=0.552$, $P<0.01$) (Figs. 4, 5). Fong *et al.*¹⁷⁾ showed that enamel-related proteins such as amelogenin were associated with root formation in rats. Tokiyasu *et al.*¹⁸⁾ showed that the enamel matrix derivative activated cementoblasts *in vivo* and *in vitro*. Previous studies have identified amelogenin as an enamel protein associated with enamel formation application that suppresses root resorption by inhibiting odontoclast number *in vitro*¹⁹⁾. Therefore, these factors involved in enamel formation may not only influence the formation of cementum, but also odontoclast activities in root resorption.

Although root abnormalities is a risk factor of OIIRR²⁰⁾, an apparently normal root can resorb severely during orthodontic treatment. This phenomenon may be associated with the hardness and elastic modulus of the cementum. If the cementum hardness and elastic modulus can be estimated based on the enamel value, we may be able to prevent OIIRR. Further studies will be necessary to clarify the relationship between the enamel and cementum with respect to the mechanical properties.

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Conflict of Interest

The authors have declared that no competing interest exists.

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