Effects of the hardness and chemical composition in apical cementum on the occurrence of root resorption

（根尖部セメント質の硬さおよび化学組成が歯根吸収の発生に及ぼす影響）

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Abstract:

Introduction: The purpose of this study was to investigate whether or not individual variation in the hardness and chemical composition of apical cementum affects the degree of root resorption.

Methods: We evaluated the Vickers hardness of 50 extracted teeth's enamel and cementum. In pit formation assay, we measured the resorbed area of apical cementum of 21 extracted teeth *in vitro* using human osteoclast precursor cells (hOCPs). We also investigated the calcium/phosphorous (Ca/P) ratio of 21 extracted teeth using energy dispersive X-ray microanalysis studies (EDSs) to determine the chemical composition of apical cementum.

Results: The results of the study demonstrated that the hardness of cementum decreased from the cervical to apical thirds on the root surfaces. Furthermore, individual variations were observed in the hardness of enamel (range: 275.99 - 480.19) and cementum (range: 10.69 -43.85). It found a positive correlation between the hardness of enamel and apical cementum (r = 0.551, p < 0.01). In pit formation assay, a negative correlation was noted between the hardness and the resorbed area of apical cementum (r = - 0.714, p < 0.01). In the Ca/P ratio a positive correlation was noted between the hardness and the Ca/P ratio of apical cementum (r = 0.741, p < 0.01).

Conclusions: These results suggested that the hardness and Ca/P ratio of cementum may be involved in the occurrence of root resorption.

Key words: Hardness, Cementum, Ca/P ratio, Root resorption, An energy dispersive x-ray microanalysis

Introduction

Orthodontically-induced inflammatory root resorption (OIIRR) is one of the most difficult accidental symptoms to predict in cases of orthodontic tooth movement; this undesirable result can cause the permanent loss of the dental structure of the root apex. In most cases, patients experience a mild degree of root resorption. In exceptional cases, however, excessive root resorption may occur. Lund et al. [1] reported that nearly 7 % of orthodontic patients had one tooth or more with root shortening exceeding 4 mm. The causes of this phenomenon have been reported to include the use of a heavy force [2], the length of treatment [3], the type of root [4] and the patient's genetic predisposition [5]. It is therefore critical for orthodontists to anticipate this phenomenon and to prevent it whenever possible.

Cementum is a non-uniform mineralized connective tissue that surrounds the root dentine and provides the interface through which the root surface is anchored to the collagen Sharpey's fibers of the periodontal ligament [6]. Cementum contains no blood vessels, has no innervation, does not undergo physiologic resorption or remodeling, and is characterized by continuing deposition throughout life [7]. Cementum is less mineralized than dentine or enamel [8, 9], and is classified as both cellular and acellular.

In previous studies, Chutimanutskul et al. [10] examined the hardness and elastic modulus of cementum on the buccal and lingual surfaces of the roots at the cervical third, middle third, and apical third. The authors reported a gradually decreasing in the hardness and elastic modulus of cementum in both surface groups, from the cervical to apical thirds. The apical third of cementum, predominately cellular cementum is less mineralized and lower hardness and elastic modulus values than the middle and cervical thirds, predominately acellular cementum [11]. Mahoney et al. [12] reported that the hardness of enamel and dentin may correlate with calcium mineral content. Thus, the hardness of cementum may correlate with mineral content. Furthermore, Henry et al. [13] reported that the apical third of the root is the most common site of resorption and the incidence decreases as approaching to the crown. Therefore, individual variation in the hardness of cementum may influence an individual resistance to root resorption.

In the present study, we measured the hardness of enamel and cementum and examined the correlation between enamel and apical cementum. In order to examine whether or not individual variation in the hardness of cementum affects the degree of root resorption, we measured the resorbed area of apical cementum using human osteoclast precursor cells (hOCPs) *in vitro*. Furthermore, we performed energy dispersive X-ray microanalysis studies (EDSs) to determine the Ca/P ratio in order to investigate the chemical composition of apical cementum.

Materials and Methods

Sample collection

This study was conducted on 50 maxillary premolars collected from 31 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 distilled 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 until all visible signs of the PDL had been removed. The extracted teeth were then sterilized in accordance with the protocol described in previous publications [14].

Vickers hardness (HV)

We evaluated the Vickers hardness of 50 extracted teeth's enamel and cementum and examined the correlation between these values for enamel and apical cementum. The extracted teeth's roots were divided into equal thirds (cervical cementum [CC], middle cementum [MC], and apical cementum [AC]) for the measurements (Fig. 1). The measurement point in each region was the center line of the major axis on the buccal side. The mean values of three measurements in each part were obtained. The hardness of enamel and cementum were evaluated using a dynamic ultra-micro-hardness tester (DUH-211; Shimadzu Co., Japan) with a load rate of 13.32 mN/s and a holding time of 10 seconds at a maximum load of 98.07 mN.

Pit formation assay

We randomly selected 21 extracted teeth from 50 extracted teeth used for the hardness measurement. The teeth were sectioned buccolingually down the long axis of the tooth through the apex with a diamond blade (Isomet™; Buehler, USA) under irrigation with distilled water. Next, the roots of the apical one-third were sectioned. In all samples, the area of cementum that was in contact with the dish surface was equalized (Fig. 2).

Osteoclasts (hOCPs, 1.0×10^4 cell/well; Takara Bio Inc., Japan) were cultured on the samples in human osteoclast culture medium (Takara Bio Inc., Japan) at 37< for 14 days. Next, the samples were washed three times with PBS. The samples were placed for 30 minutes in 1M NH_4OH and was washed via ultrasonication in order to remove adherent cells and then washed and dried. After drying, the entire surface of each the samples was examined by scanning electron microscope (S-3400N; Hitachi, Japan). The resorption area in apical cementum was determined using an image processing system (Win Roof; Mitani Co., Japan).

Mineral composition analysis

We randomly selected 21 extracted teeth from 50 extracted teeth used for the hardness measurement. The teeth were sectioned buccolingually down the long axis of the tooth through the apex with a diamond blade (Isomet™; Buehler, USA) under irrigation with distilled water. The sections were embedded in epoxy resin (No. 105; Struers Inc., Denmark). The samples were polished on a grinding plate with water-based polycrystal-line diamond suspension (DP-Paste P; Struers Inc., Denmark) from a thickness of 3.0 μm to 0.25 μm. The smooth surface of the polished samples was coated with a conductive carbon layer using a carbon coater (VC-100S; Shinkuu Device, Japan).

The samples were examined by scanning electron microscope (S-3400N; Hitachi, Japan) with an EDX detector operating at 15-kV accelerating voltage and a working distance of 10 mm. The measurement points were compiled for the analysis using the Link ISIS system (Oxford Instruments, UK). The Ca and P concentrations were calculated using the accompanying AZtecEnergy software program (Oxford Instruments, UK).

The Ca and P concentrations were measured on the buccal surface of apical cementum. The measurements were performed at four points on the outer layer of cementum. The mean values of the four measurements were obtained (Fig. 3). The concentration profiles for Ca and P were compared using the Ca/P atomic percent ratio.

Statistical analysis

A box plot analysis was used to determine the hardness of enamel and three regions (CC, MC, AC) in cementum. Correlation coefficients were formulated to evaluate any relationship between the hardness of enamel and AC. Furthermore, correlation coefficients were formulated to evaluate any relationship between the hardness and the Ca/P ratio or the resorption area.

Results

Vickers hardness of enamel and cementum were shown in Table 1. The values of the enamel, CC, MC, and AC were 394.46 ± 41.92, 26.50 ± 7.81, 20.67 ± 6.35, and 19.00 ± 5.62, respectively (Table 1). Vickers hardness of cementum was decreased from the cervical to apical regions on the root surface. There was no significant difference in the values of gender (date not shown). The ranges of individual variations in Vickers hardness of enamel, CC, MC, AC were from 275.99 to 480.19, from 12.10 to 43.85, from 11.14 to 28.88, from 10.69 to 30.91, respectively. (Fig. 4). There was a positive correlation between enamel and AC in the Vickers hardness ($r = 0.551$, $p < 0.01$) (Fig. 5).

In pit formation assay, a scanning electron microscope showed resorption pits formed by osteoclasts on the apical cementum (Fig. 6A). For example, the largest pits was shown in low HV (12.54) area (Soft group). Otherwise, there were small pits in high HV (30.92) area (hard group). In moderate group (HV: 20.16), small pits and their fusions were observed. A negative correlation was noted between the Vickers hardness and the resorbed area of apical cementum ($r = -0.714$, $p < 0.01$) (Fig. 6B).

In the Ca/P ratio a positive correlation was noted between the Vickers hardness and the Ca/P ratio of apical cementum ($r = 0.741$, $p < 0.01$) (Fig. 7).

Discussion

Hardness is a major parameter to consider when observing structural changes in cementum 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 of 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 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.

First, the present study examined whether or not there was individual variation in the hardness of cementum and enamel. 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 (12 - 35 years old) of the samples we could use without thickening of the cementum. There was no significant difference in the values of gender (date not shown). The results of this study demonstrated that the hardness of cementum decreased from the cervical to apical regions on the root surface. Chutimanutskul et al. [10] supported the result of this study. Furthermore, individual variations were observed in the hardness of cementum and enamel. Relatively little is known about the individual variations in the physical properties of enamel and cementum. Darendeliler et al. [16] reported large individual variation for both the hardness and elastic modulus in their examination of a small (n = 16) mixed sample of maxillary and mandibular first premolars. These present and previous findings support the existence of individual variations in the hardness of cementum. We observed a positive correlation between the respective hardness of enamel and apical cementum ($r = 0.551$, $p < 0.01$) (Fig. 5). Fong et al. [17] showed that enamel-related proteins such as amelin were associated with root formation in rats. Tokiyasu et al. [18] showed that the enamel matrix derivative activated cementoblasts *in vivo* and *in vitro*. Therefore, these factors involved in enamel proteins may not only influence the formation of enamel, but also the formation of cementum.

Next, we focused on the hardness of apical cementum in particular, as root resorption following orthodontic treatment often occurs in the apical part. As we focused about the relationship between the hardness of cementum and root resorption, the influence of inflammation in the periodontal ligament, which is a causative factor of root resorption was removed in this study. No structural or histochemical property has been found to distinguish between odontoclasts and osteoclasts [19]. There are several reported procedures for evaluating osteoclastic differentiation [20-23]. Pit formation assay is a traditional method that is commonly used for evaluating bone resorption activity. Pit formation assay, which measure bone resorption using dentin slices or calcium phosphate (CaP)-coated plates, are frequently used to evaluate the function of the osteoclasts [21]. In the present study, in order to clarify the relationship between the hardness of cementum and the resorption degree, we performed pit formation assay using cementum. A correlation was noted between the hardness and the resorbed area of apical cementum (r = -0.714, p < 0.01) (Fig. 6). Reitan et al. [24] reported that the density and hardness of the cementum might influence its resistance to root resorption. Srivicharnkul et al. [25] reported that the orthodontic force did not significantly differ based on the hardness of cementum compared with untreated teeth. Thus, individual variations in the hardness of cementum were considered an important factor of root resorption.

Finally, scanning electron microscopy in combination with an energy dispersive system (SEM-EDS) has become considerably important for odontological studies [26, 27]. Besides being non-destructive, these techniques allow us to obtain the chemical composition from small samples, with detection limits of < 0.5% wt. A positive correlation was noted between the Vickers hardness and the Ca/P ratio of apical cementum (r = 0.741, p < 0.01) (Fig. 7). Alvarez-Perez et al. [6] reported that the range of the Ca/P ratio in apical cementum was 1.3 - 1.6. This previous finding may support the results of the present study. These present and previous findings suggest that the low hardness value (soft cementum) may be due to a low Ca/P ratio.

Taking the above-mentioned results into consideration, individual variation in the hardness and the Ca/P ratio of cementum may influence the resistance or susceptibility to root resorption, and may be involved in the root resorption caused by orthodontic forces. If the hardness of cementum can be estimated based on the value of enamel, it may be possible to know accurately the risk of root resorption for each patient in orthodontic treatment. Therefore, orthodontists may be able to predict root resorption and do orthodontic treatment more safely. Further studies will be necessary to clarify the relationship between enamel and cementum with respect to the mechanical properties.

Acknowledgments

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

	E	CC	MC	AC
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

E: enamel, CC: cervical cementum, MC: middle cementum, AC: apical cementum

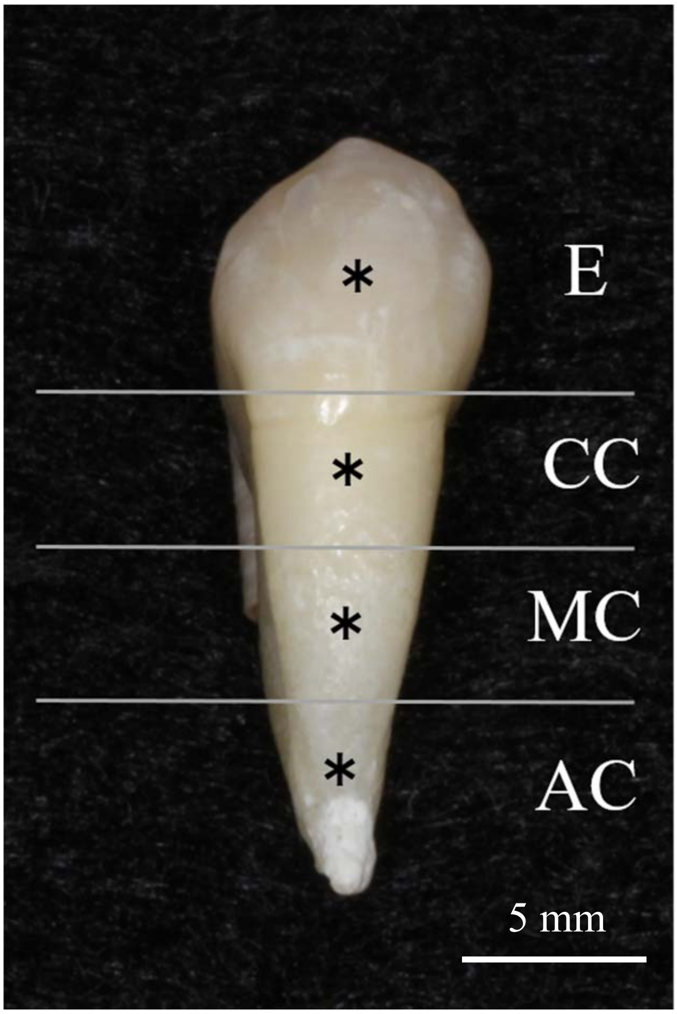


Figure 1. Location of indentations on maxillary premolar cementum and enamel.

The extracted teeth's roots were divided into equal thirds (cervical cementum [CC], middle cementum [MC], and apical cementum [AC]) for the measurements. The measurement point in each region was the center line of the major axis on the buccal side. The mean values in each part were obtained in triplicate. E; enamel. Scale bar: 5 mm.

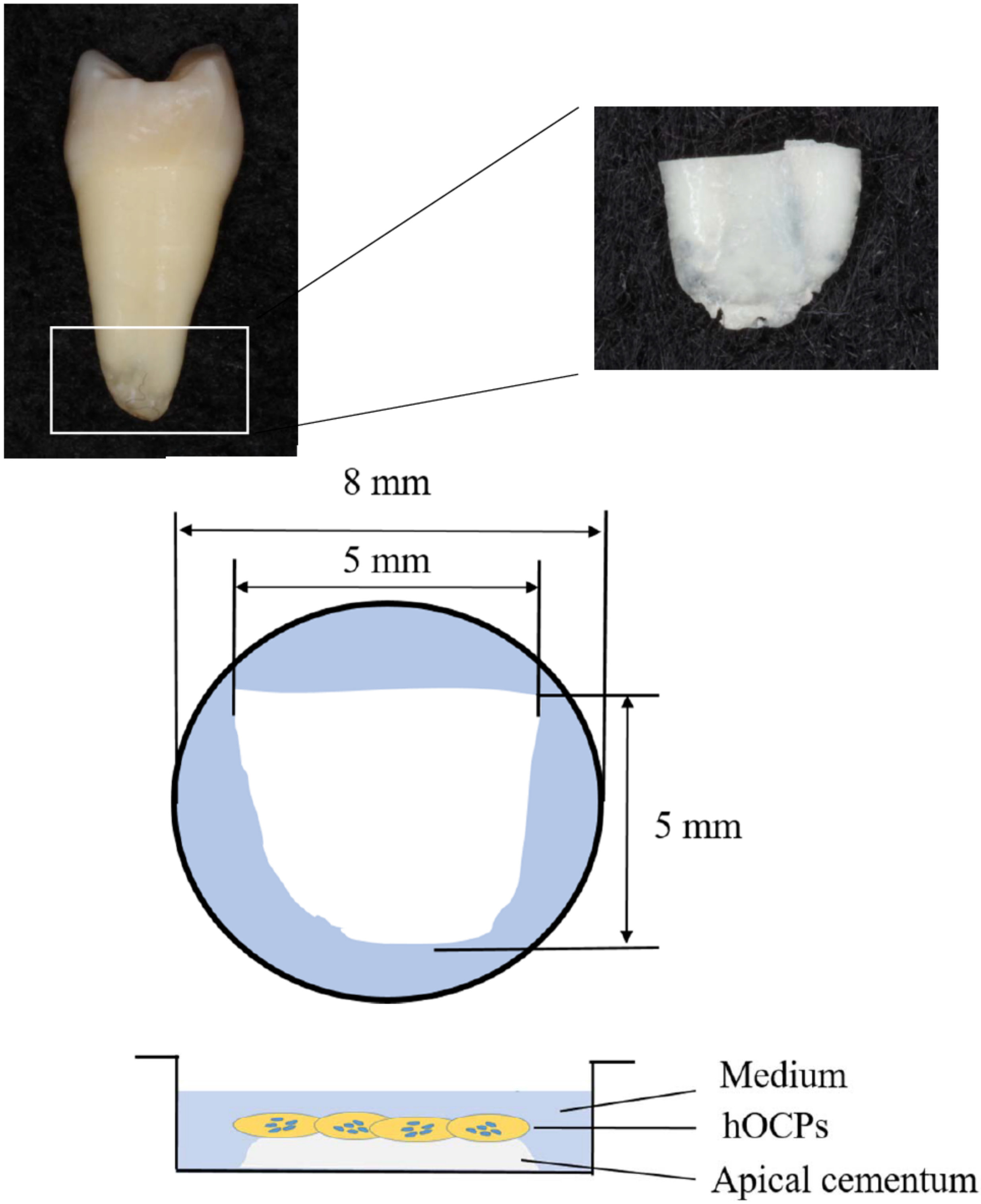


Figure 2. The schema of pit formation assay.

The roots of the apical one-third were sectioned. In all samples, the area of cementum in contact with the dish surface was equalized. Osteoclasts (hOCPs, 1.0×10^4 cell/well) were cultured on the samples in culture medium at 37< for 14 days.

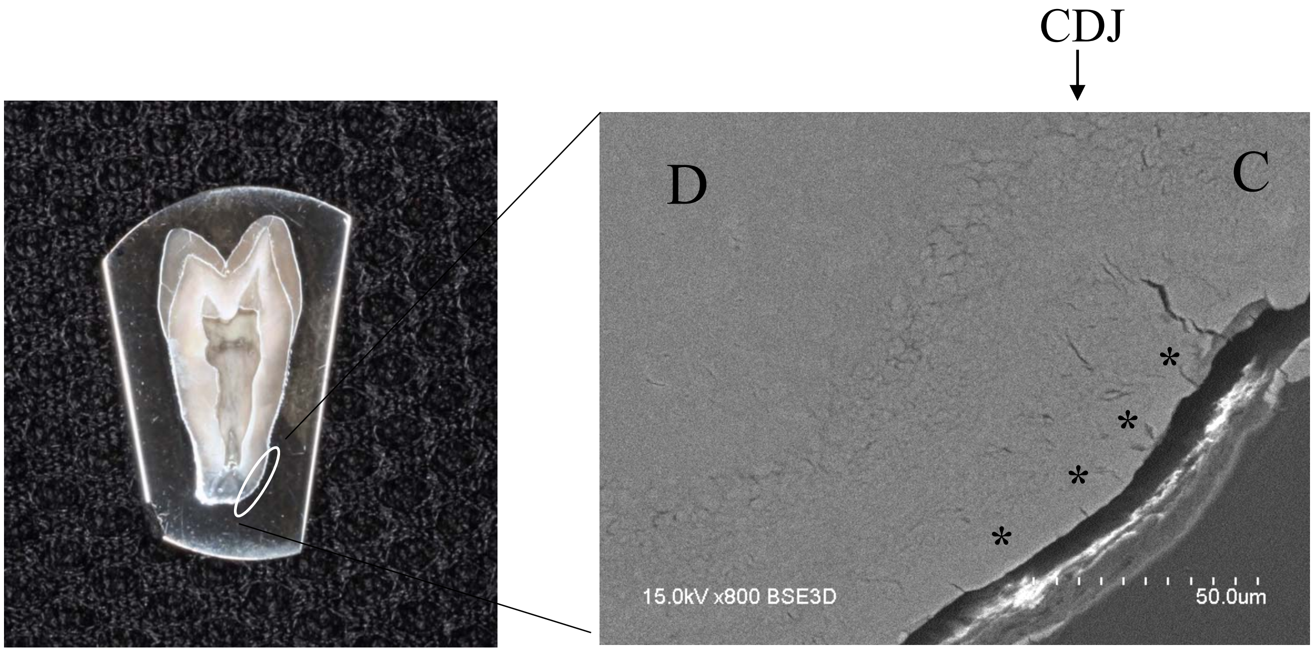


Figure 3. A scanning electron microscope.

The samples were examined by a scanning electron microscope with an EDX detector operating at 15-kV accelerating voltage and a working distance of 10 mm. Dentin (D), cementum (C), and cementodentin junction (CDJ) were observed. The Ca and P concentrations were measured at four points (Asterisk) on the buccal outer layer of apical cementum.

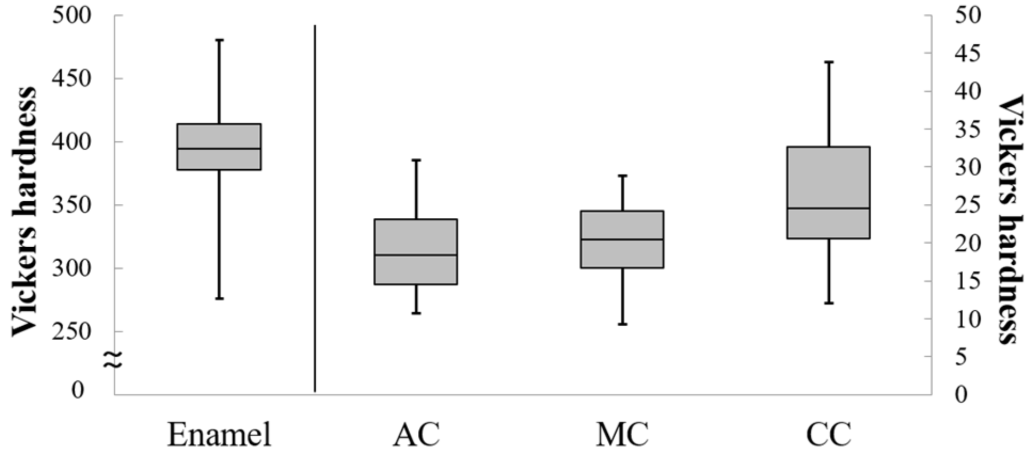


Figure 4. A boxplot analysis of the Vickers hardness.

The Vickers hardness of enamel [E] and cementum (cervical cementum [CC], middle cementum [MC] and apical cementum [AC]) were examined. Each center line in the box showed a median value.

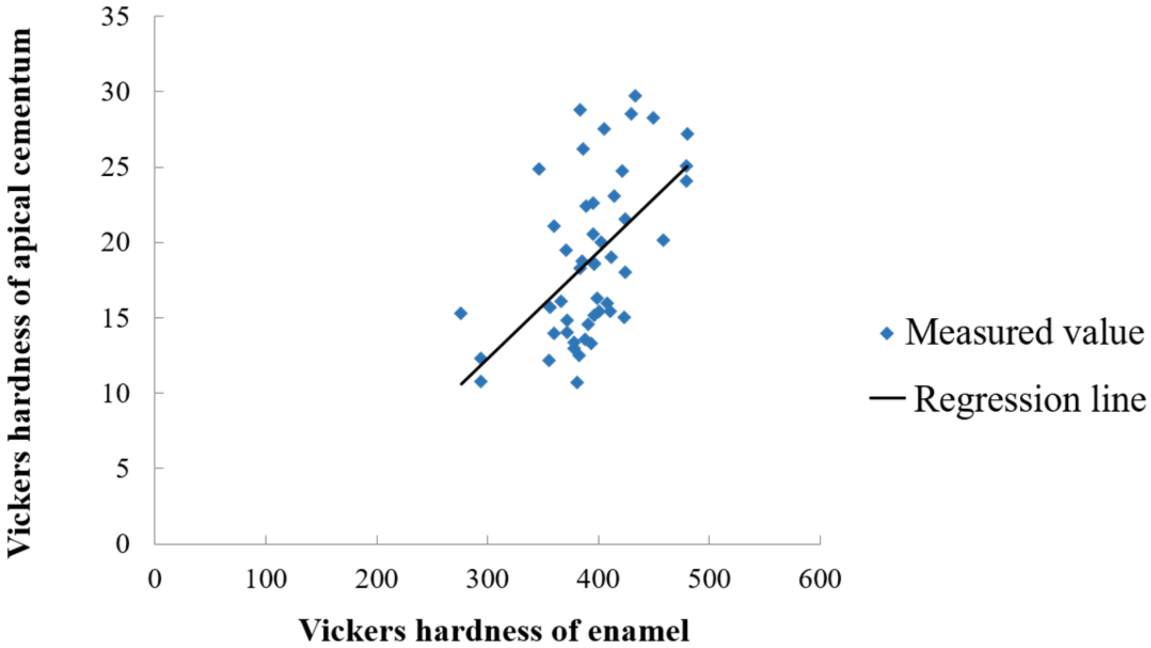
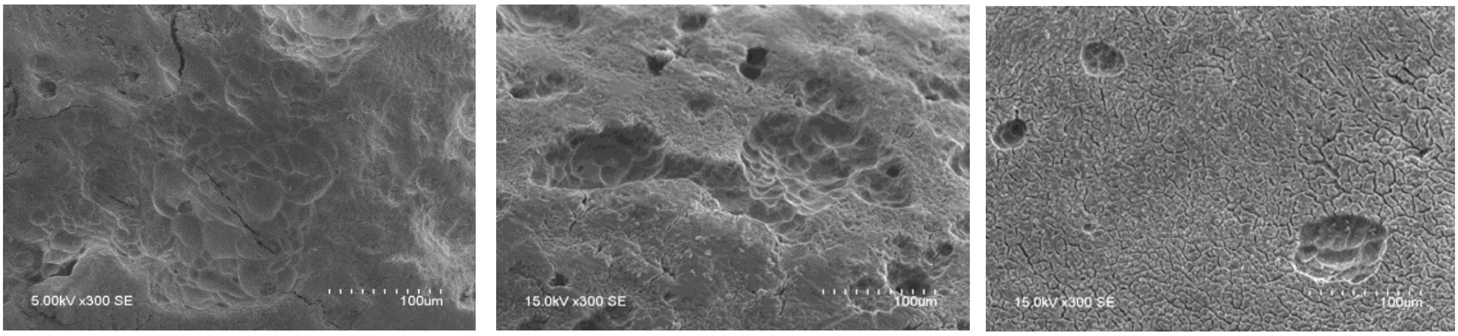


Figure 5. Correlation between the Vickers hardness of enamel and apical cementum.

A positive correlation was shown between the Vickers hardness of enamel and apical cementum ($r = 0.551$, $P < 0.01$).

A



Soft
(HV: 12.54)

Moderate
(HV: 20.16)

Hard
(HV: 30.92)

B

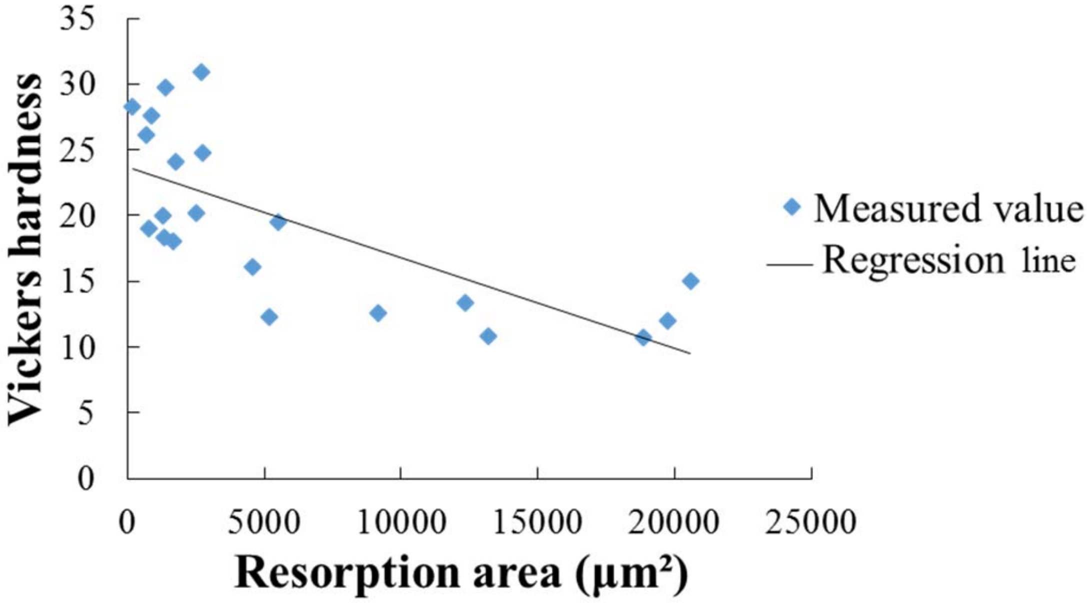


Figure 6. A pit formation assay.

A scanning electron microscope showed resorption pits formed by osteoclasts on the apical cementum (A). The largest pits was shown in low HV (12.54) area (Soft group). Otherwise, there were small pits in high HV (30.92) area (hard group). In moderate group (HV: 20.16), small pits and their fusions were observed. The correlation between the Vickers hardness and the resorbed area of apical cementum (B). A negative correlation was observed between the Vickers hardness and the resorbed area of apical cementum (r = -0.714, $p < 0.01$).

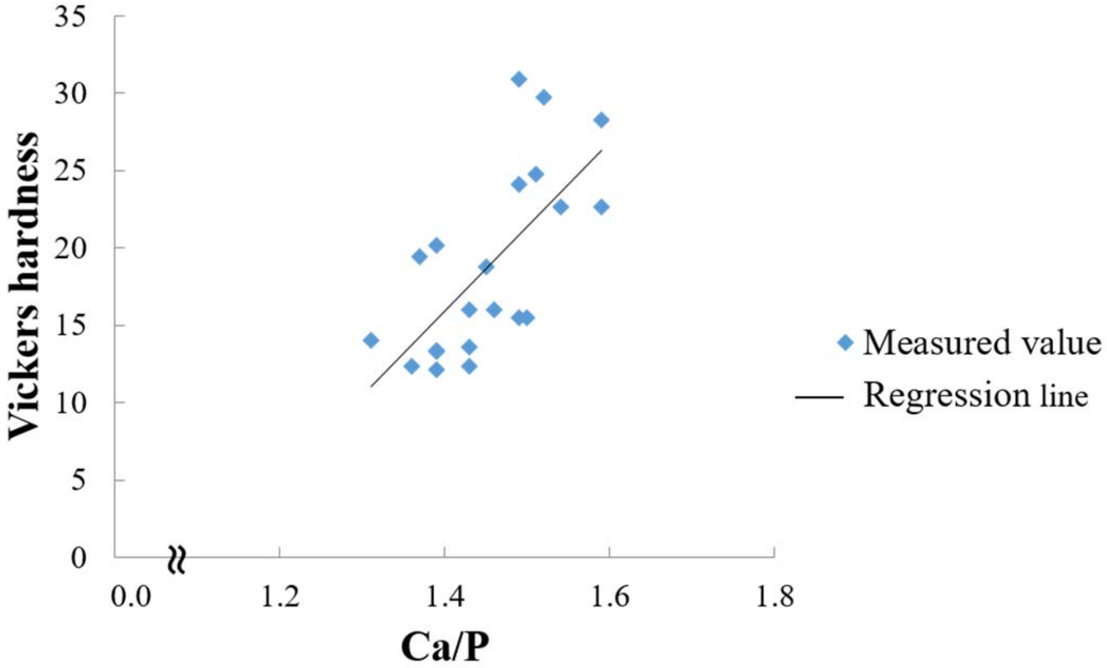


Figure 7. The correlation between the Vickers hardness and Ca/P ratio of apical cementum.

A positive correlation was observed between the Vickers hardness and the Ca/P ratio of apical cementum ($r = 0.741$, $p < 0.01$).