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INVESTIGATION OF TRACE ELEMENT DISTRIBUTION IN PERMANENT ROOT DENTINE BY LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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Abstract : In this study, we report on the effectiveness of using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to analyze the composition of trace elements within root dentinal tissue in permanent teeth. The trace element distribution in different dentine regions and age related changes in their concentration were investigated using the LA-ICP-MS technique, provided semi-quantitative data for 12 trace elements among which Mg, Zn, and Sr ranked as the most abundant. The results demonstrated that most of the observed elements display conformity throughout root dentinal tissue, except for the higher concentrations of Mg in the cervical area and the higher levels of Zn measured in the pulpal layer. Alterations in the levels of dentinal Sr, Cr, Mn, and Se were also observed in relation to the age of dentinal tissue. The findings of this investigation indicate that the combination of EPMA and LA-ICP-MS measurements may prove instrumental to the further research of this topic in the future.

Key words : Trace elements, LA-ICP-MS, EPMA, Root, Dentine

Introduction

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a novel and versatile analytical method, which has been used for trace element determination in inorganic and organic matrices in the last few years. This technique allows remarkably rapid multi element in situ analysis in solids, which therefore makes LA-ICP-MS extremely useful for sample applications where time axis is present, e.g., teeth. Hence, without destruction of the original structure, the analysis is reproducible, and the determination of trace element content of teeth can contribute to the monitoring of environmental exposures, e.g., to trace pre- and postnatal environmental exposures or diseases from trace element distribution of deciduous teeth[1,2)]. Teeth represent inevitably suitable materials for trace element monitoring. Since they are the hardest and most resistant materials of the human body, they might preserve the trace element characteristics for long period of time[3)].

Up to the present, human and animal teeth have been investigated, and predominantly the trace element composition of enamel was demonstrated[4)]. In the current study, the root dentinal tissue was chosen for the following reasons. Dentine forms the bulk of the tooth and because of its anatomical situation; the root dentinal tissue might be preserved even if the crown is strongly destructed or missing.

Only a minimal amount of data is available on trace element distribution in permanent root dentine[5], except some investigation of fluoride concentrations, which seem to accumulate throughout life in dental hard tissues[6]. Because of the lack of such information, we decided to investigate the dentine tissue of permanent teeth.

The aim of the present study was to examine trace element distributions in different regions of the root dentine and to explore possible variation in trace element contents of root dentine in relation to the dentine age.

Materials and Methods

Eleven caries-free teeth between 14 years of age and 77 years of age (mean=43.8 years of age) were studied in the experiment (Table 1). We separated the roots from the crowns using a low-speed diamond cutter (Isomet 11-1180, Buehler). Longitudinal sections were cut from the middle part of the roots in the thickness of approximately 1 mm. Prior to carbon coating, we polished the cut surfaces with a grinding/polishing instrument using Al_2O_3 powder (2,000 and 3,000 mesh) and finally with a 3 μm diamond polisher. The roots were divided into three parts: cervical, middle, and apical thirds. At the vertical mid-line of each region, near-pulpal, inner- and outer- (near-cemental) dentine points were marked under microscopic inspection (10x magnifications).

To determine the quantity and distribution of trace elements among samples using the LA-ICP-MS method, standard reference materials are required. Since the ablation efficiency between the sample (dentine) and the synthetic glass standard is grossly different, the conventional design for LA-ICP-MS measurements is not applicable. Thus, a well-documented approach introduced by Pearce *et al*. (1992)[7] was applied. The EPMA tracings of the major elements were carried out with a JEOL Superprobe 733 analyser (Tokyo, Japan). We determined the accelerating potential as 15 KV, the sample current as 2×10^{-8} and the measurement time as 100 sec. The signal counts were evaluated based on the Bence and Albee method[8], and the samples Ca, P and Mg weight concentrations were calculated. In addition, calcium-phosphorus ratios were determined to assess sample heterogeneity, a

Table 1 Summary table showing the gender, age and types of the teeth used in the study.

Sample number	Gender	Tooth type	Chronological age (years)	Dentine age (years)[a]
1	Female	First premolar	14	9
2	Female	Third molar	24	12
3	Male	Lateral incisor	25	21
4	Male	Third molar	31	19
5	Female	Third molar	35	23
6	Male	First premolar	45	40
7	Male	Lateral incisor	48	44
8	Male	Lateral incisor	57	53
9	Female	Lateral incisor	60	56
10	Female	Canine	70	63
11	Female	Lateral incisor	77	73

[a]Dentine age was calculated as the difference between chronological age and the age at the point of complete crown formation.

Table 2　Macro element composition of permanent root dentine from electron probe microanalysis. Concentrations are expressed in weight percent ; mean (SD[a]).

Section	Cervical			Middle			Apical		
Dentine layer	Outer	Inner	Pulpal	Outer	Inner	Pulpal	Outer	Inner	Pulpal
Calcium*	29.9 (3.7)	30.7 (2.8)	30.9 (2.9)	29.7(4.2)	29.4 (3.4)	30.5 (4.0)	28.9 (2.5)	29.1 (3.7)	30.4 (3.2)
Phosphorus*	13.4 (1.4)	14.5 (1.4)	14.4 (2.0)	13.9 (1.1)	13.5 (1.7)	14.5 (1.9)	13.4 (1.7)	13.4 (2.0)	14.2 (1.0)
Magnesium*	0.92 (0.1)	0.98 (0.1)	1.0 (0.29)	0.86 (0.1)	0.78 (0.2)	0.87 (0.2)	0.7 (0.2)	0.66 (0.2)	0.74 (0.2)

*N＝99 ; [a]standard deviation

factor which has great influence on the ablation characteristics and therefore on the accuracy of LA-ICP-MS analysis.

LA-ICP-MS analysis was performed using a VG Plasma-Quad III (Waltham, MA, USA) with an UV Microprobe laser ablation system. Prior to sample analysis the instrument calibration was achieved by the analysis of an NIST 610 multi-components glass standard (Gaithersburg, MD, USA). Macro and micro elemental data were collected from 99 sampling points (9 sites per tooth). Trace elemental measurements consisted of 12 elements ; Mg, Zn, Sr, Ge, Cr, Cu, Mn, Co, Ga, Ge, Se, Mo.

Statistical analysis were performed using SPSS version 11.0 for Windows (SPSS Inc., Chicago IL). The data were evaluated using the analysis of variance test (ANOVA) to compare elemental distributions among the cervical, middle, and the apical thirds (sections) as well as among the outer, inner, and pulpal root dentine layers. Age-related changes were investigated by Pearson's correlation analysis.

Results

To assess the dentinal Ca, P, and Mg concentrations, the EPMA analysis was performed in different dentinal sections and layers. The average concentrations are given in Table 2. Dentinal density was mapped using the calcium-phosphorus ratios in order to explore variations among the sampling sites. The average Ca/P molar ratio was calculated as 1.68 (±0.2), which remains within the limits of known apatite molar ratios. Table 3 illustrates the results of the factorial ANOVA test for Ca, P, Mg concentrations and for Ca/P and Mg/P ratios in different regions of root dentine. The tests indicated statistically significant differences in the Mg concentrations ($F=16.707$; $P<0.001$) and in Mg/P ratios ($F=24.961$; $P<0.001$) among the different dentine sections. The Tukey's pairwise comparison test revealed that the mean difference in Mg concentration was significantly greater in the cervical section than that of the middle (0.15±0.04) and that of the apical (0.29±0.04) sections.

The LA-ICP-MS measurements were carried out using microscopic control, with the laser beam focused on the dentinal areas corresponding with those analyzed previously by EPMA. Figure 1 shows the typical, round-shaped ablation craters in ca. 80 μm width, surrounded by halo-like effects.

Among the selected trace elements Sr and Zn were the most abundant in dentine, followed by Cr, Ge, Co, Mn and Cu in that order. Trace elements such as Ga, Mo, and Se were detected in less than half the sample points. We also examined the

Table 3　ANOVA results for the distribution of macro elements among different dentine areas. Electron probe microanalysis.

Element (or ratio)	Section	Layer
Calcium	n.s. ($P=0.46$)	n.s. ($P=0.37$)
Phosphorus	n.s. ($P=0.59$)	n.s. ($P=0.17$)
Magnesium	*** ($P<0.01$)	n.s. ($P=0.32$)
Ca/P	n.s. ($P=0.89$)	n.s. ($P=0.77$)
Mg/P	*** ($P<0.01$)	n.s. ($P=0.27$)

***Significant difference ; n.s.＝nonsignificant difference distribution of the trace elements amongst the different dentine areas. The results of the ANOVA-test indicated statistically significant differences between the dentine layers for zinc (Table 4). The near pulpal dentin layer showed prominently higher Zn concentrations than the inner and outer dentine layers (mean differences were 52 ppm±19 ppm and 63 ppm±19 ppm respectively). For Cr, Mn, Cu, Co, and Ge a similar, but not statistically significant tendency, was observed towards the pulpal surface in the apical section (Fig. 2).

To examine age related changes, the macro and micro element concentrations were plotted against the dentine age (Table 5). Pearson's correlation coefficient indicated a positive correlation between the strontium concentration and dentine age. On the other hand, decreasing concentrations of phos-

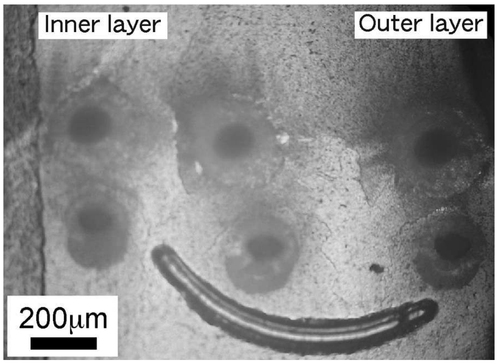

Fig. 1 Laser ablation craters in different dentine layers (scar-pen mark on the right, 10x magnifications). The sampling sites are seen on the cut surface of the root, following the LA-ICP-MS analysis performed repeatedly in the near-pulpal, in the inner and in the outer dentine substances.
Operating parameters: cool gas flow, 13.4 l min^{-1}; auxiliary gas flow, 0.8 l min^{-1}; nebuliser gas, 1.03 l min^{-1}; RF power, 1290 W.
Acquisition parameters: laser ablation rate, 10 Hz; laser energy, 8 mJ, acquisition time, 20s; pre-ablation, 3s; acquisition mode, pulse counting.

Table 4 ANOVA for Zn concentrations in root dentine layers (outer, inner and pulpal).

Source of Variation	df	Sum of Squares	Mean Square	F	P
Dentine Layers	2	0.836	0.418	7.304	0.001
Error	96	5.493	0.057	—	—

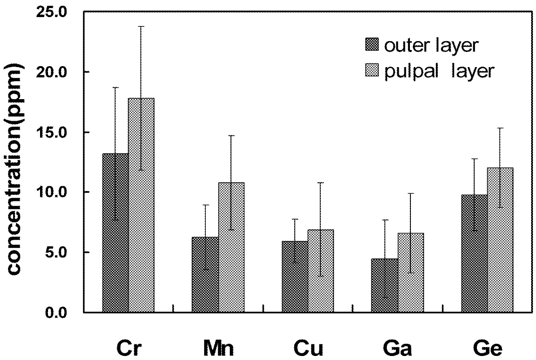

Fig. 2 Some trace elements distribution between the outer and pulpal dentine layer (apical sections).

Table 5a Pearson's correlation table between age and macro elements in root dentine.

	Ca	P	Mg	Ca/P	Mg/P
Correlation coefficient	−0.278	**−0.424**	−0.151	0.177	0.004
Significant probability	0.005	<0.001	0.136	0.079	0.970

Bold numbers indicate significant correlation.

Table 5b Pearson's correlation table between age and trace elements.

	Sr	Zn	Cr	Ge	Co	Mn	Cu	Ga	Mo	Se
Correlation coefficient	**0.314**	−0.263	**−0.321**	−0.167	0.053	**−0.363**	0.140	0.290	0.266	**−0.459**
Significant probability	0.002	0.009	0.001	0.165	0.706	0.001	0.278	0.039	0.149	0.001

Bold numbers indicate significant correlation.

phorus, chromium, manganese and selenium were noted in relation to dentine age.

Discussion

The results of the EPMA analysis demonstrated a homogenous distribution for Ca and P throughout the root dentine tissue. Considering that different sample densities introduce unpredictable variables in the laser ablation process, resulting in variation in the quantification of trace elements; the calcium-phosphorus ratios were used to reveal discrepancies in dentine density from the ablation sites, though in fact, the data showed no major deviations from the average values. The Ca, P, and Ca/P values through dentine reported in the literature previously[9] have confirmed these data.

It is well known that in order to produce absolute quantification with the LA-ICP-MS technique from organic materials, such as bone or teeth, the problem arising from the lack of matrix-matched reference materials must be dealt with. Hence, previous studies surrounding the trace element composition of teeth proposed some alternative methods in order to achieve semi-quantitative results. Lochner *et al*. (1999)[1] analyzed a piece of walrus ivory by ICP-AES and then related the average elemental concentrations to LA-ICP-MS results. In this current study, we adopted a recent advancement in the LA-ICP-MS technique, which has been successfully introduced to the analysis of geological samples. Employing this technique, in an attempt to address the issue of quantification of trace elements in root dentine, electron probe measurement of the concentration of Mg on the same sample sites were used to calculate the element concentrations determined by LA-ICP-MS. The repeatability of EPMA analysis was evaluated on the basis of five repeated measurements of Ca, P (hydroxyapatite) and Mg (MgO) standards and relative standard deviations were under 2%.

Our methodological approach allowed us to assess the element concentrations in different dentinal zones. Among the macro elements, magnesium content from different dentine sections showed statistically significant enrichment in the cervical part of the root. The magnesium/phosphorus ratio also followed an similar pattern in the cervical direction, as has been reported already by Steinfort *et al*. (1991)[9]. Among the micro elements, zinc concentrations elevated significantly towards the pulpal cavity. This data confirms the results of Totdal and Hals (1984)[5], also obtained from measurements on human root dentine. They indicated that alteration in elemental concentrations might correspond to secondary dentine formation, surrounding the pulp chamber. In addition, Brudevold *et al*. (1963)[10] have investigated the Zn content of dentine and highest levels were recorded in the inner layer adjacent to pulp. On this point, it should be also noted that among our samples Cr, Mn, Co, Ge, and Cu displayed an increased presence in the pulpal layer of the root dentine. Thus, changes in the dentinal tubules during aging process result in the calcification of dentinal tubules and the formation of transparent sclerotic dentine, which is found to be more significant in the apical root dentine[11], may also contribute to variation in elemental composition. Furthermore, as trace elements commonly act as cofactors of the enzymes and may bind to proteins, explored differences in protein component between the dentinal zones[12] would also effect the trace element composition.

Among the investigated elements, negative correlation was found between dentine age and phosphorus, chromium, manganese, and selenium. According to an earlier work[13], magnesium content in human cementum and enamel also showed a tendency to decrease with age. Yet contrary to the results of Kato *et al*. (1997), our results did not imply decreasing Mg content in permanent root dentine in relation to age. Interestingly however, strontium did follow an inverse, increasing pattern in relation to dentine age. Therefore, our results suggest that, similar to fluoride, Sr enhancement in dentine may occur in accordance with increasing age. As previous studies have shown, the enrichment of fluoride content in dental tissue clearly depends on actual fluoride uptake and thus the presence of increasing amounts of strontium may be indicative of nutritional and environmental[14] factors.

In summary, the scientific approach used here involved the investigation of the trace element content of root dentine by the LA-ICP-MS method. This technique combined with EPMA analysis yielded semi-quantitative results, which were used to evaluate the dentinal distribution and concentration of trace elements in root dentine in particular in relation to age. Furthermore, the present approach revealed the potential practicability of age estimation based on the alteration of dentinal trace element content. Therefore, a tightly controlled, extensive study is desirable in the future, to further explore if these changes indeed allow accurate age determination for forensic purposes.

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