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著者名(英)	XU,Shiting/KITAJO,Hiroyuki/KAWASHIMA,Isao/YAMANE,Yuro/MURATA,Masaru/SHIBATA,Toshiyuki/ARISUE,Makoto/ENDO,Kazuhiko/SHI,Sizhen/OHNO,Hiroki
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〔ORIGINAL〕

X-ray scanning analytical microscopic and scanning electron microscopic studies of an unusual case of dens invaginatus

Shiting XU[*,***], Hiroyuki KITAJO[**], Isao KAWASHIMA[***], Yuro YAMANE[***], Masaru MURATA[**], Toshiyuki SHIBATA[**], Makoto ARISUE[**], Kazuhiko ENDO[***], Sizhen SHI[*] and Hiroki OHNO[***]

* Department of Pediatric Dentistry, School of Dentistry, Tongji University, China
** Department of Oral Maxillofacial Surgery, School of Dentistry, Health Sciences University of Hokkaido, Japan
*** Department of Dental Materials Science, School of Dentistry, Health Sciences University of Hokkaido, Japan

*(Chief : Prof. Sizhen SHI)
**(Chief : Prof. Makoto ARISUE)
***(Chief : Prof. Hiroki OHNO)

Abstract

A mass was extirpated from the region of the maxillary right third molar of a 20 year-old patient. It was proved to be an extreme and unerupted case of dens invaginatus. The clinical and histopathological features have been presented in a previous paper. Ameloblasts and odontoblasts were noted along the surface of enamel or dentin. In the present study, X-ray scanning analytical microscopy (XSAM), a newly developed technique, together with scanning electron microscopy (SEM) was applied for the study of this malformed tooth. SEM study showed a strange arrangement of well-formed enamel and dentin in normal structures. The distribution of calcium(Ca) and phosphorus(P) was clearly observed by the XSAM method. It revealed that the bulk of dentin was composed of two kinds of dentin quite different in mineral content while their SEM appearances were similar. The part of the dentin near the dentinoenamel junction had much higher Ca and P concentrations than the outer parts. In conclusion, the disagreement between the microscopic findings and the XSAM findings suggests the importance of applying various methods in studies to obtain a comprehensive understanding of such malformation. In addition, the present investigation also points out the complexity of the disturbance ranging within the term dens invaginatus.

Key words : X-ray scanning analytical microscopy, SEM, Dens Invaginatus.

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Introduction

The term, dens invaginatus applies to a group of developmental defects in which part of the enamel organ invaginates into the dental papilla during odotogenesis, resulting in the formation of an enamel-lined cavity which communicates with the surface[1]. It is thought to be relatively common with the incidence varying from 0.25% to 10% due to the different criteria used[2,3]. Tooth crowns as well as roots may exhibit variation in size and form[4]. In extreme cases the invagination may extend into the apex and the tooth is grossly deformed[5-7].

The etiology of such malformation is still unknown and different classifications are proposed[8,9]. As only a few reports on structural studies of dens invaginatus have been published there is no consensus on that point. The structure of enamel or dentin of dens invaginatus has been reported to be normal or hypomineralized by different authors[10-12]. As far as we know, only two chemical analysis studies have been reported[11,12].

The purpose of the present study was to correlate the structural findings with elemental distribution by applying scanning electron microscopy (SEM) and the elemental mapping image method of X-ray scanning analytical microscopy (XSAM). XASM is a newly developed elemental analysis technique that has just been applied to dentistry science in recent years[13]. The sample surface under scanning is irradiated by an X-ray microbeam from the X-ray guide tube and the transmitted X-ray and fluorescent X-ray are detected to obtain the elemental data for the surface. It can be used to measure the concentrations of the components both in dental hard tissues and soft issues.

THE CASE

A 20-year-old woman was referred to the Department of Oral and Maxillofacial Surgery, School of Dentistry, Health Sciences University of Hokkaido, for examination and treatment of a mass in the region of her maxillary right third molar. The woman's medial history was unremarkable. There was no history of trauma or local damage. No signs were evident with the mass.

Extraoral examination revealed no facial asymmetry or abnormalities. Intraorally, there was a mass located in the buccal maxillary alveolar bone about 10 mm distal to the cervical line of the maxillary right second molar. The mass size was estimated at about 12mm in diameter. It was also noticed that the irremovable mass was as rigid as bone, with a normal mucosal covering, accompanied by compressive pain.

A panoramic radiograph showed a tooth-like heterogeneous radiopaque mass located in the distal aspect of the maxillary right second molar. The sharply defined mass was about 18mm in its distal-mesial dimension, surrounded by a peripheral radiolucenct margin.

CT examination demonstrated that the CT value of the irregular radiopaque structure was similar to that of enamel and dentin. It also indicated that some parts of the mass were covered by soft tissue. The maxillary right second tooth was shifted mesially due to the pressure of the mass.

The preoperative clinical diagnosis was complex odontma. It was therefore suggested that the lesion should be removed. The extirpation of the mass was done under local anesthesia. A mucoperiostal flap distal to the maxillary right second molar was formed to expose the buccal aspect of the alveolar bone. There a bone defect was found. It was about 3mm in diameter. Since there was some soft tissue attached to the mass, it was easy to separate the mass from the alveolar bone and take it out. The bone cavity was smooth-walled and there was no connection with the maxillary sinus.

Grossly, the mass was a malformed tooth, 14×13×13mm in size (Fig. 1). It was shell-like in shape with crooked roots that encircled the crown.

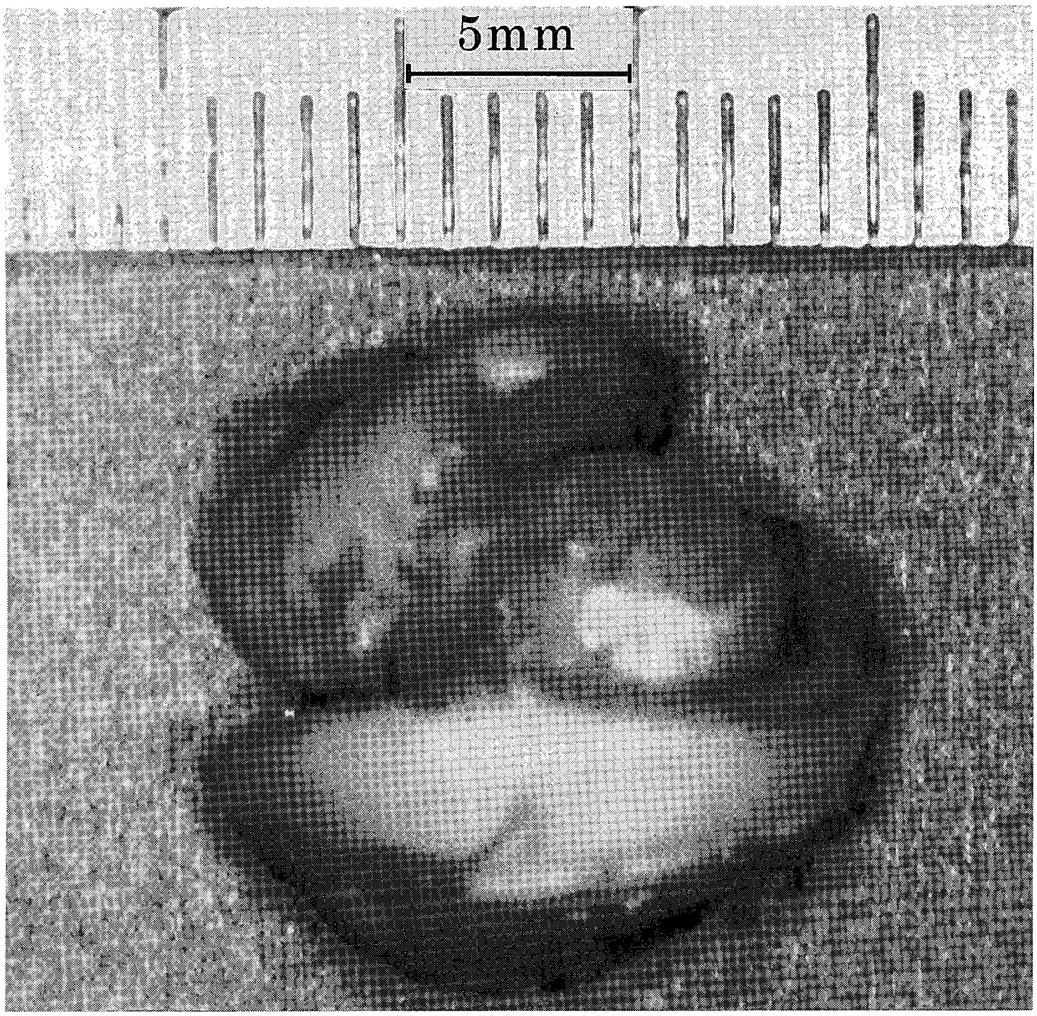

Fig. 1 Gross appearance of the unusual case of dens invaginatus. Note the abnormality of the crown and root.

Materials and methods

After extirpation, the tooth was at once sent to the laboratory for histogical investigation. It was embedded in epoxy resin without decalcification and cut longitudinally to a thickness of 100 um by means of diamond saw with a water coolant. After being double stained by basic fushin and methylene blue, the section was examined by light microscopy.

A section of the same thickness from an extracted normal third molar from one patient was prepared in the same way and served as a control for XSAM analysis.

The XSAM analysis procedures

A schematic diagram of X-ray scanning analytical microscopy (XGT-2000W, Horiba Ltd, Tokyo, Japan) is shown in Fig. 2. As one of the advantages of the method, no special pre-treatment was needed for the sample. The incident X-ray was generated from the Rh anode at 50kV and 1mA. The XGT diameter was set at 100μmϕ, the same as the X-ray beam size. Two kinds of detectors were used to receive the transmitted and fluorescent X-ray signals. To get an accurate integrated image, the scanning area was set at 14.3×14.3mm^2, while the scanning time was set at about 300 sec/scan and repeated 66 times. Thus, the transmission image and elemental mapping image were obtained. The same procedures were done for the control section.

The SEM observation procedures

To get more accurate data by SEM examination after light microscopic observation, the surface of the specimen was once again polished to remove the thin superficial resin film and washed carefully and extensively with distilled water. After being fixed on aluminium stubs, the sample was sputter-coated with a gold-palladium film. Images were obtained using a scanning electron microscope (X-650 Hitachi, Tokyo, Japan) at an accelerating voltage of 25kV.

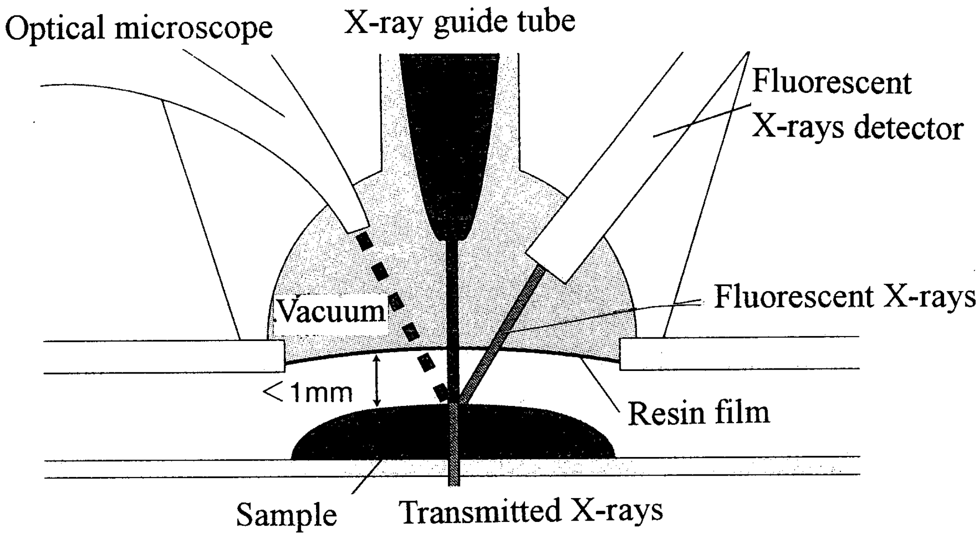

Fig. 2 Schematic diagram of X-ray scanning analytical microscope (XSAM).

Results

Light microscopy findings

Microscopically, the mass was mainly composed of enamel and dentin with a small amount of cementum, while the arrangement was quite different from the usual. The enamel was totally invaginated into the crown and surrounded by the dentin (Fig. 3). Adjacent to the surface of the invaginated enamel were some columnar cells that were recognized as ameloblasts. Enamel matrix accompanied this occasionally. Columnar odontoblasts were also noted along the surface of the dentin[14]. Dentinal tubules were arranged in a uniform and regular pattern to the enamel-dentin junction. A thin layer of cementum was also present on the surface of the root-part dentin. Surprisingly, there was no cavity for the pulp tissue. The soft tissue around the mass was recognized as pulp tissue. On the basis of the microscopic findings, the mass was diagnosed as dens invaginatus of the maxillary right third molar rather than complex odontoma.

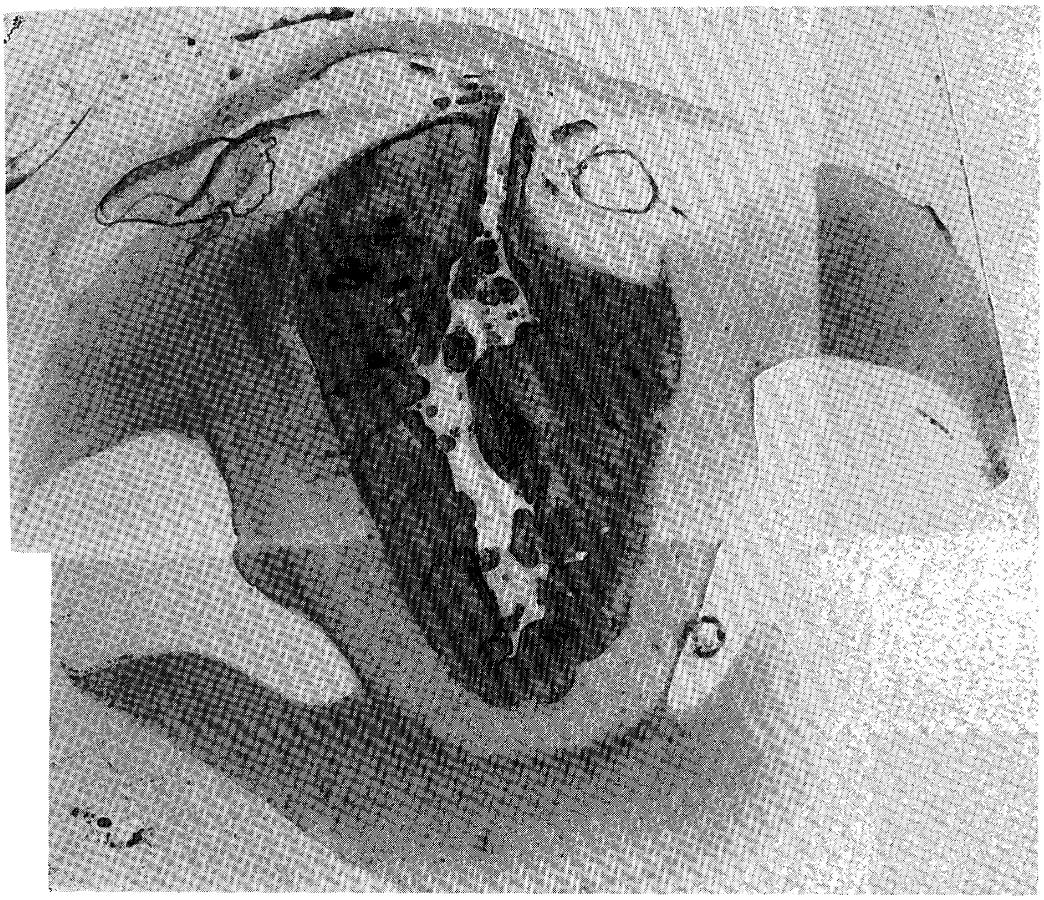

Fig. 3 Histological image of a section from the abnormal tooth. (basic fushin-methylene blue, original magnification, ×12.5)

SEM findings

The overall SEM view of the section revealed abnormality in the arrangement of dental hard tissues (Fig. 4). But it did not show haphazard organization like a complex or compound odontoma. The enamel was totally invaginated and surrounded by the dentin. The strange shape of the root could also be noticed. The enamel was very thick, which was quite different from the dens invaginated cases in other reports[2-8]. A cavity lined with enamel was formed

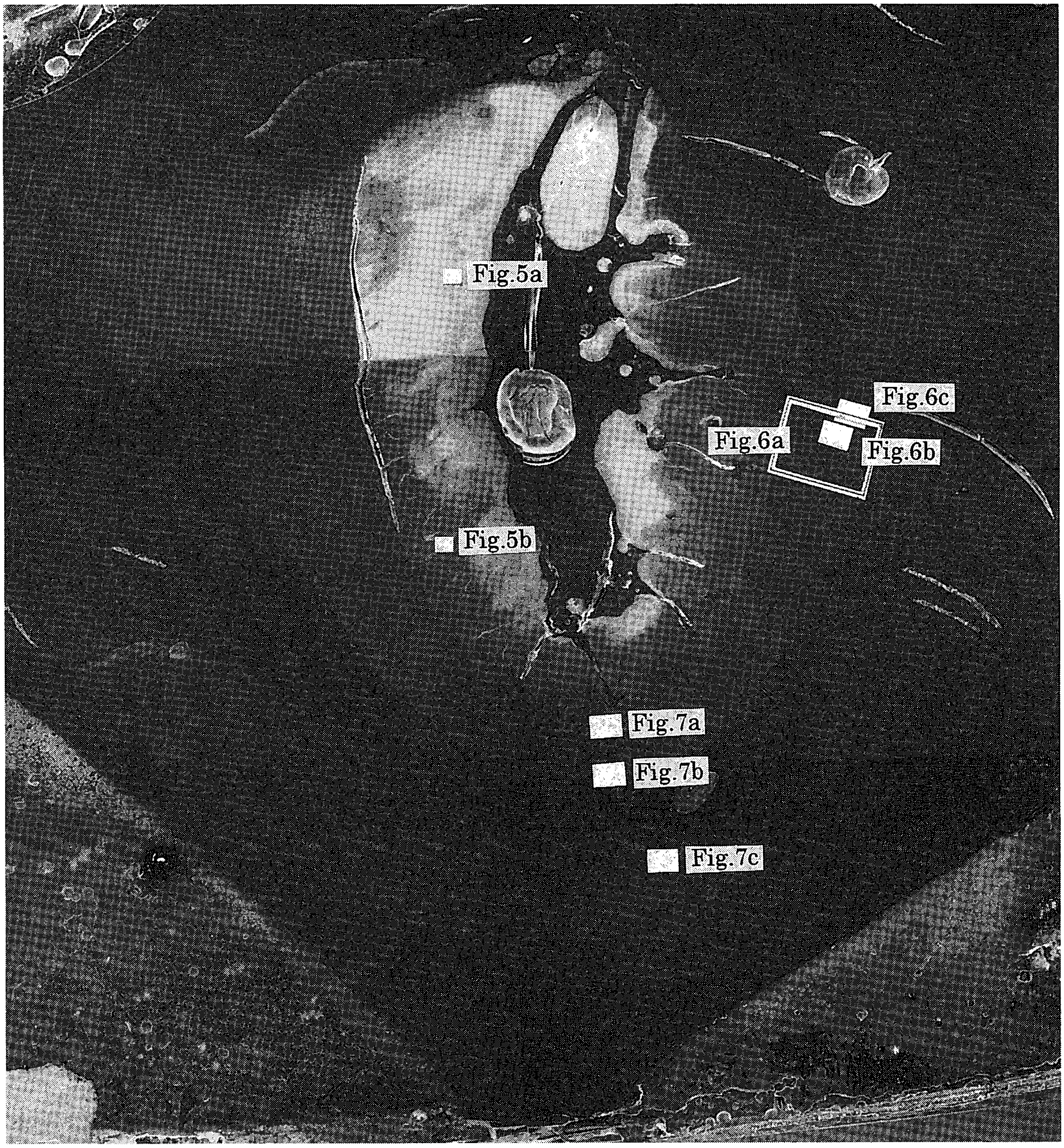

Fig. 4 Abnormal arrangement of the tooth structure. Locations of the following figures are illustrated. (SEM, original magnification, ×27)

in the tooth with an opening at the top. Some enamel nodules of various sizes could be observed in the cavity. Normal enamel structures were presented in the bulk of the enamel (Fig. 5a, 5b). Different orientation of enamel prisms which indicated the Hunter-Schregen bands, were observed. An overall view of the dentin is shown in Fig. 6a, which clearly demonstrated the curvature of the dentin tubules. The dentin tubules extended in an S-shaped curve as is normal but became irregular and twisted when they came to the mineralization front, which can be seen more clearly in Fig. 6b and 6c. In other parts normal and similar dentin structures were found (Fig. 7a, 7b, 7c).

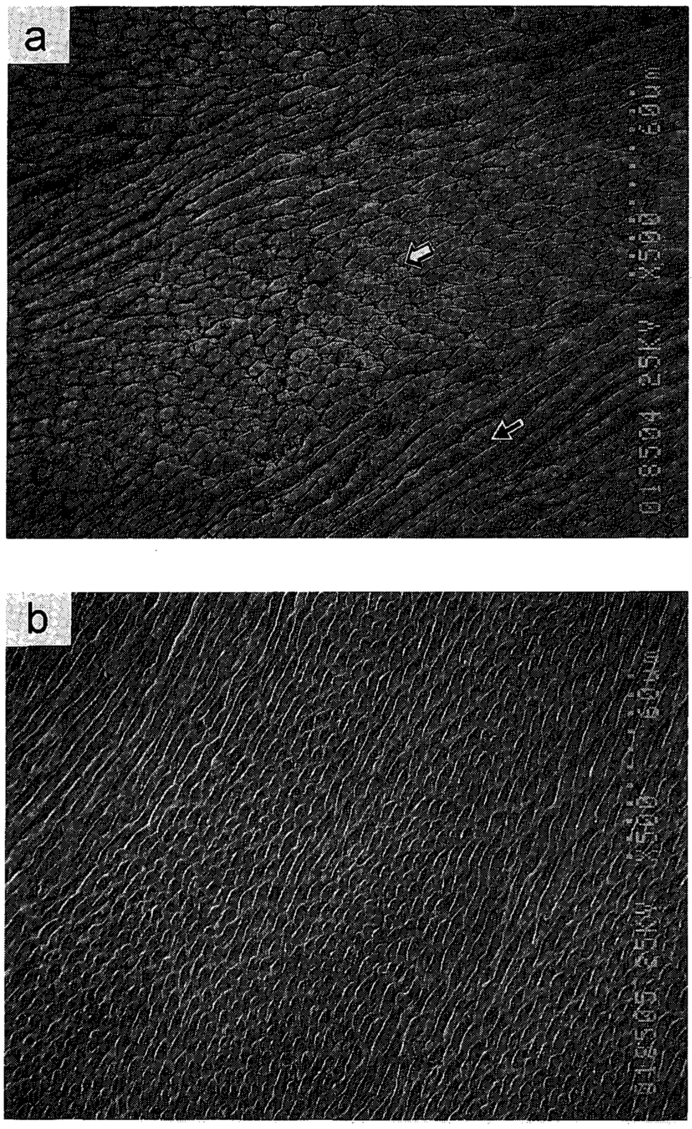

Fig. 5 (a, b). Invaginated enamel with a regular prism pattern. Near-longitudinal rod orientation (black arrow) and near-cross-sectional rod orientation (white arrow). (SEM, original magnification, ×500)

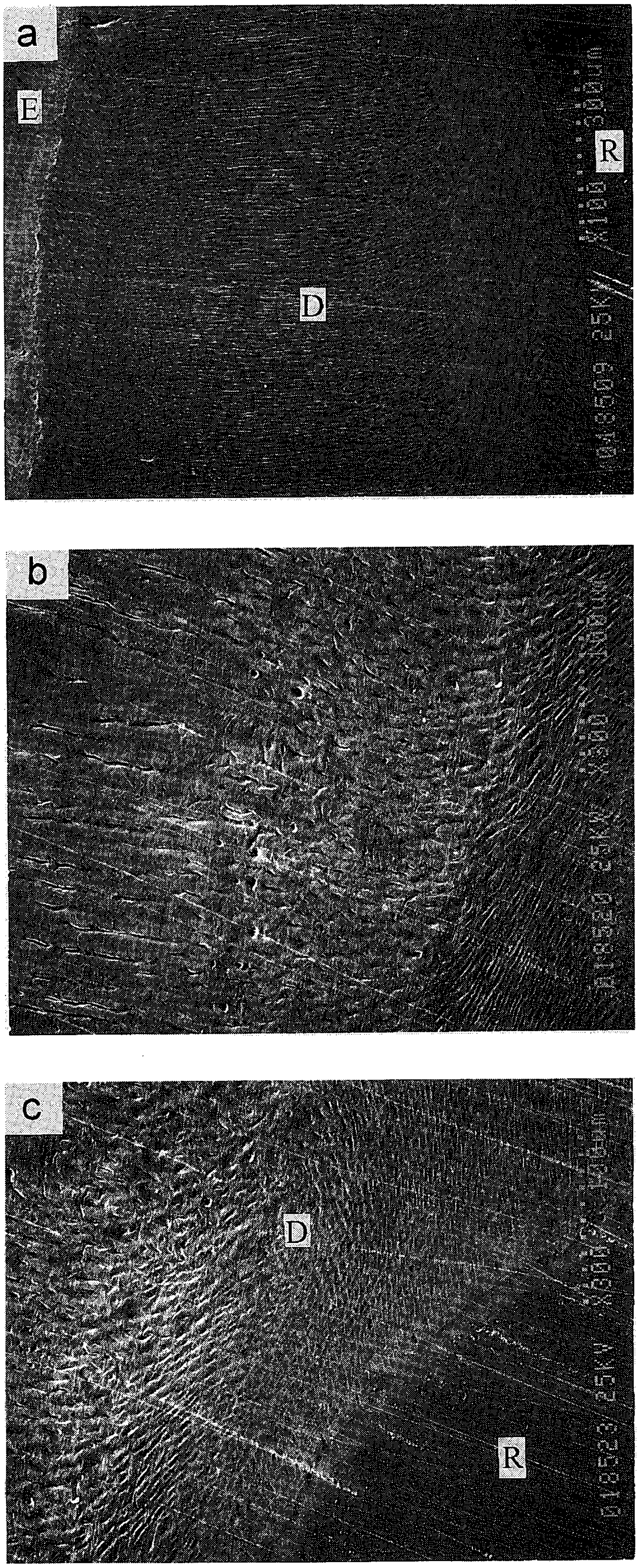

Fig. 6 (a). Overall view of dentin showing the S-shaped curvature of dentin tubules. E, enamel; D, dentin; R, resin. (SEM, original magnification, ×100). (b, c). Dentin tubules became irregular and twisted when they came to the mineralization front. D, dentin; R, resin. (SEM, original magnification, ×300)

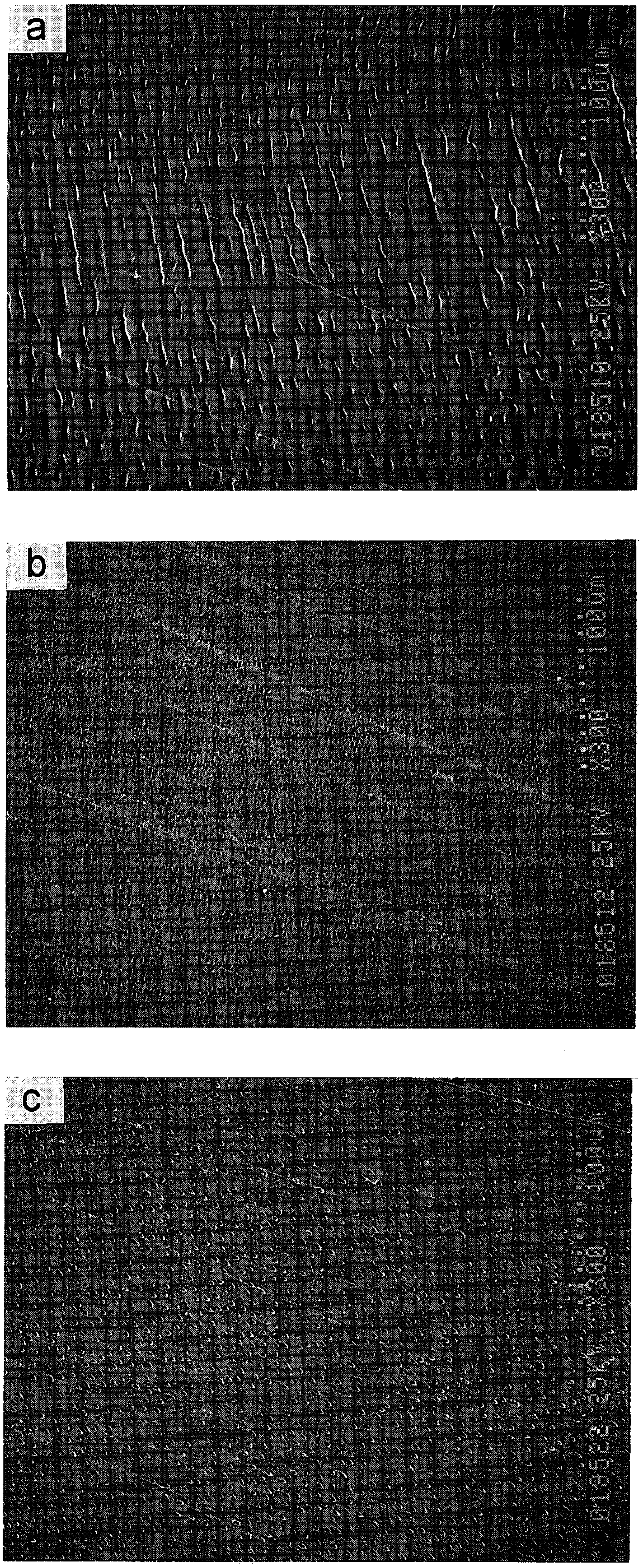

Fig. 7 (a, b, c). Dentin in consecutive parts showing similar and normal appearances. (SEM, original magnification, ×300)

XSAM findings

A transmitted image of whole the section was obtained by XSAM (Fig. 8a), which showed the density difference, other words, the mineral content difference between parts of the section. Elemental mapping images of two primary elements, calcium (Ca) and phosphorus (P) of the specimen were also obtained (Fig. 8b, 8c). The distribution of Ca and P throughout the line can

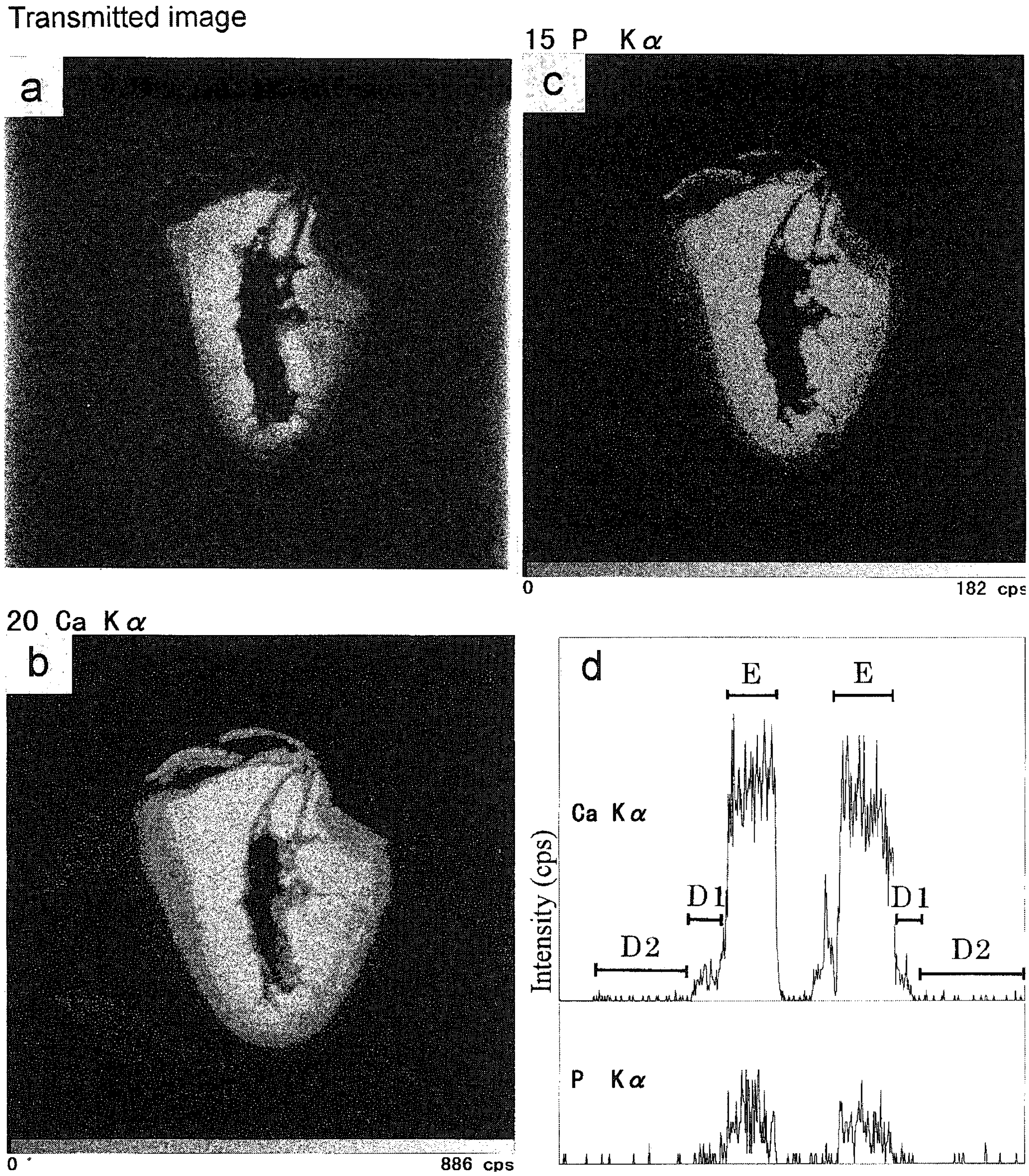

Fig. 8 (a). Transmitted image of the section by XSAM, from which mineral content distribution is known. Line of elemental analysis of Ca and P is also illustrated. (b, c). Elemental mapping images of the tooth section for calcium and phosphorus. Note the difference of mineral content in the dentin part. (d). Elemental line analysis of Ca and P of the dens invaginatus (Fig. 8a). Difference in mineral content in outer part of dentin (D1), inner part of dentin (D2) and enamel (E) can be clearly seen.

be clearly recognized, showing the two elements were widely distributed throughout the tissue. The distribution characteristics of Ca were almost the same as for P. High concentrations of Ca and P were noticed in the enamel part, while in dentin relatively low concentrations were found. It was surprising to note that there was a clear concentration difference in dentin itself. Accordingly, the dentin could be divided into two parts as shown in Fig. 9. The element line analysis of Ca and P showed the relationship between the element concentrations and the different parts examined (Fig. 8d). The increasing trend was clearly demonstrated. The concentrations of Ca and P were quite low in the outer part of dentin (D2), a little high at the inner part of dentin (D1) and at last reached their peaks in enamel (E). Although according to the XSAM analysis the mineral concentration difference in dentin were quite obvious, the structural appearances seemed to be similar and normal (Fig. 7a, 7b, 7c).

The same analysis was also done for the control tooth (Fig. 10a, 10b). The same concentration difference was observed between the enamel and dentin. Unlike the abnormal tooth section, no obvious concentration difference could be detected in dentin. The diagram of the line analysis of the control also indicated this.

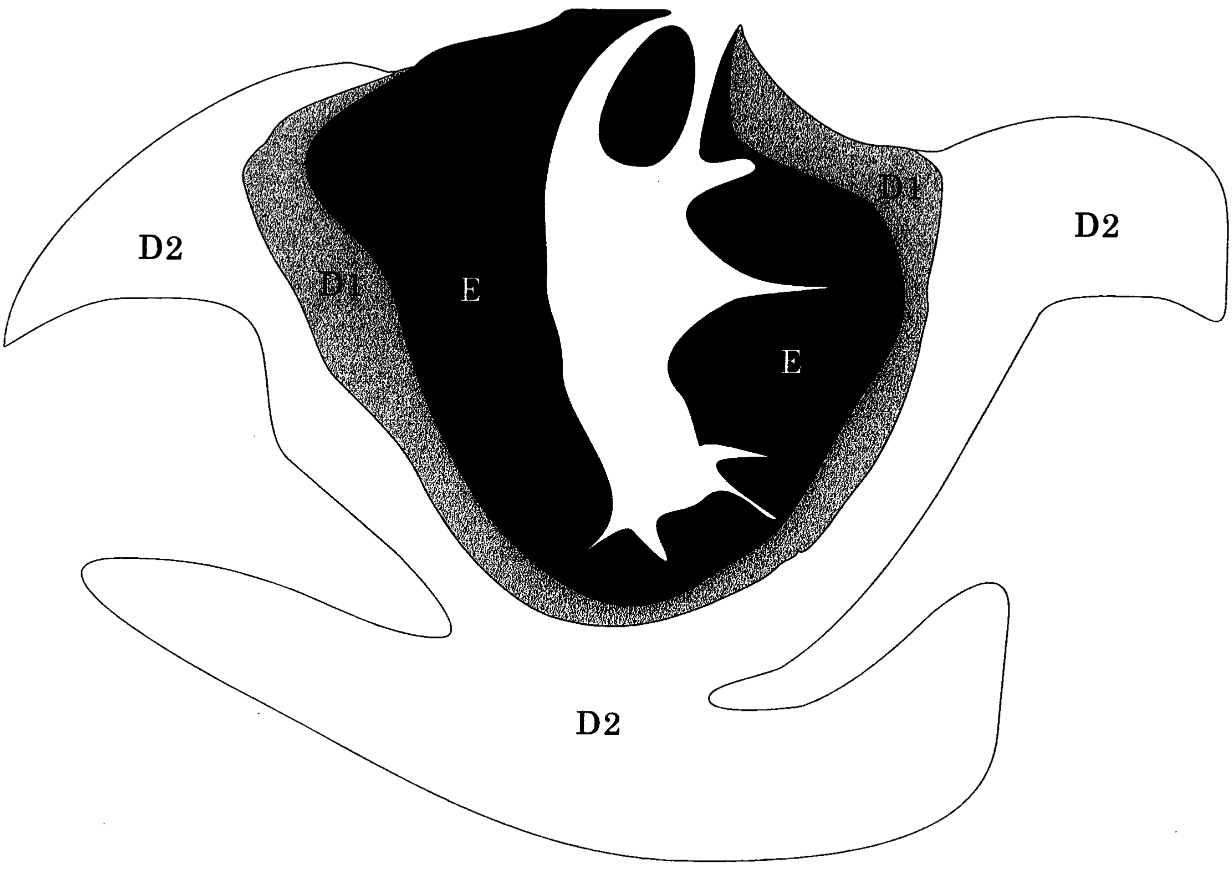

Fig. 9 Schematic presentation of the tooth section image in Fig. 5 showing the difference in element concentrations. E, enamel; D1, dentin with high concentrations of Ca and P; D2, dentin with low concentrations of Ca and P.

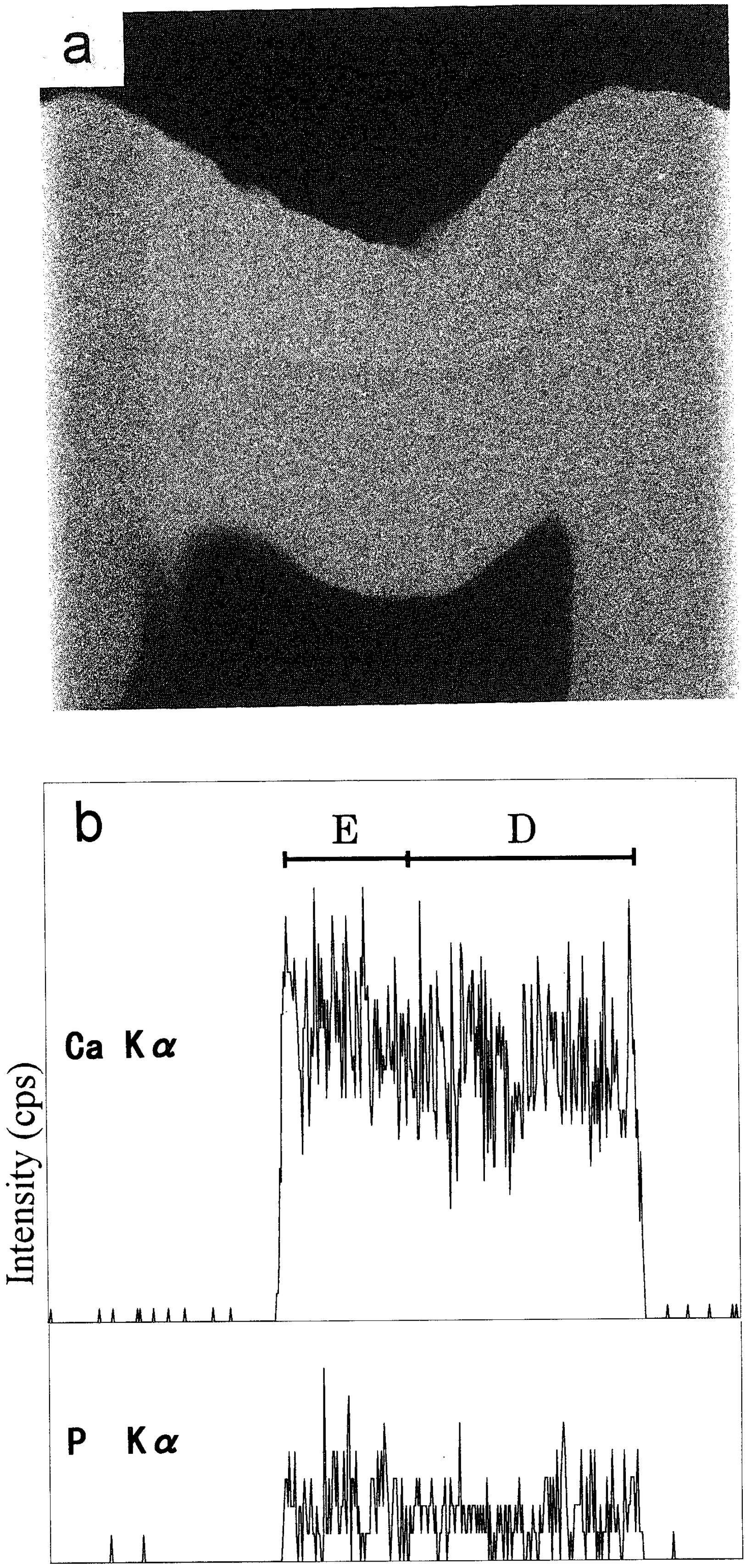

Fig. 10 (a). Transmitted image of the normal tooth served as a control. (b). Elemental line analysis of Ca and P of the normal tooth that served as a control showing almost the same elemental concentrations in the dentin part.

Discussion

This present tooth was an unusual case of dens invaginatus, although it had invaginated enamel and a deformed crown and root, the distinguishing features of that malformation. It was an unerrupted one in the molar region, which is believed rare according to the literature[5]. This case also had one feature quite uncommon in dens invaginatus that has only been reported in a few papers[15,16]: enamel nodules scattered over the inner enamel surface of the invagination. In addition, pulp tissue was found attaching the tooth without a true pulp cavity. This case can not be included in any commonly accepted classifications, for example, Oehlers'[8].

In the past decades, many theories have been proposed to explain the etiology of this malformation[8,15,17]. Now it is well known that the crown pattern is determined during the bell stage of tooth development[17]. Both the growing dental papilla and the stellate reticulum cells exert pressure on the dental epithelium, which therefore is in a state of equilibrium. It is presumed that during the process of mototic division of internal dental epithelium, the pressure from the stellate reticulum side prevailed over that from the dental papilla side due to different proliferation rate. That resulted in an unusual shape of the dental epithelium. Former internal epithelium then covered the convex surface while the external dental epithelium lined the concavity side. In this way the crown pattern of our case was determined and the deposition of enamel and dentin followed, which led to this unusual case of dens invaginatus.

The light microscopic examination showed the existence of a few ameloblasts and odontoblasts near the surfaces of the enamel and dentin[14], which should indicates that the tooth still had some limited growth potential. This has only been reported in a few papers on dens invaginatus before[16]. In some way, it could be related to complex odontoma, which may be composed of dentin, enamel, enamel matrix and pulp-like tissue in its period of active growth[1]. However, complex odontomas are characterized by haphazard distribution of all dental tissues, unlike our case.

The SEM observation revealed a normal structure in the bulk of both dentin and enamel, which differs from previous reports[10,19]. Beynon reported hypomineralized enamel at the base of the invagination while the dentin showed an irregular structure[19]. However, there are some other studies agreeing with ours, in that they did not observe irregularity in the dentin[10,16]. Disagreement between the various microscopic reports may result from the wide range of morphology in this abnormality.

Studies of the elemental composition of dental hard tissues are very useful in the understanding of the inorganic composition of the tooth. Many methods are now available to do this work, such as electron probe microanalysis (EPMA), proton probe microanalysis (PPMA), and X-ray photoelectron spectroscopy (XPS)[20].

The method applied in present study was first introduced in medicine in 1997[21], and has some advantages of its own. XSAM makes it possible to analyze the specimen in air without pre

-treament even if it contains water, quite suitable for the analysis of biological specimen. It also makes it practical to examine parafin-embedded specimens, which is an advantage over other methods.

In this study, XSAM provided us with an overall element distribution image for calcium(Ca) and phosphorus(P) of the sample. It revealed that the bulk of dentin was composed of two kinds of dentin quite different in mineral content, which has not been reported before[5]. The part of the dentin near the dentinoenamel junction had much higher Ca and P concentrations than the outer parts, while in the control tooth almost uniform concentrations of Ca and P was found through the bulk of dentin (Fig. 10).

On the other hand, the SEM observation (Fig. 7a, 7b, 7c) showed similar dentin tubule structures while their mineral concentrations could be high or very low. This phenomenon was not found in the normal tooth that served as a control. It may due to the different development stage of dentin. Such interesting findings may deepen our understanding of the process behind disturbed mineralization.

According to this finding, it is reasonable to suppose that the inner parts of dentin, near the dentinoenamel junction, may have better crystallinity than the outer part. Further crystal structure studies are needed to prove this presumption.

It has been reported that invaginated enamel has more Ca and P than outer enamel[11]. In our case, there was only invaginated enamel and no outer enamel. Thus, no comparative study could be done.

In conclusion, the disagreement between the microscopic findings and the XSAM findings may suggest the importance of applying various methods in studies to obtain a comprehensive understanding of such malformation. In addition, the present investigation also points out the complexity of the disturbance ranging within the term of dens invaginatus.

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