SEM-EDS analysis and morphological study of rabbit predentin calcospherites

by

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ABSTRACT

Calcospherites from the lower incisor predentin of rabbits were examined by scanning and transmission electron microscopy, and scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS). In the labial predentin, large globular calcospherites were present at the root apex, decreasing in size toward the incisal pulp horn. In the lingual predentin, small granular calcospherites were present at the root apex. The calcospherites attached the collagen fibers left undigested by NaOCl treatment. The incisal pulp horn of both labial and lingual dentin contained micro-calcospherites. Ultrathin section of globular calcospherites frequently showed von Korff's fibers at the root apex of the labial predentin. The calcospherites consisted of randomly oriented crystals in the labial predentin. The crystals were 30-95 nm long, and 5-8 nm wide. The labial predentin matrix showed a dense population of randomly oriented collagen fibrils. Von Korff's fibers were not found in the lingual predentin. The lingual predentin matrix showed a small population of regularly oriented collagen fibrils. Needle-like crystals, 35 nm to 104 nm long and 5 to 11 nm wide, were observed within and near the collagen fibrils in the lingual predentin. SEM-EDS analysis of calcospherites showed that Ca and P were the major elements, frequently accompanied by S,

and less frequently by Mg. In both labial and lingual predentin, the amount of S in calcospherites reduced from the root apex (initial dentin formation) to the incisal pulp horn (mature dentin formation). S is probably present as sulphated proteoglycans and may have some function in the initial formation of dentin apatite.

INTRODUCTION

In the lower incisor of rabbit, the labial and opposite lingual sides are covered with enamel and cementum, respectively. Coronal (enamel-related) and root (cementum-related) dentin is usually not considered as distinct entitles (STEINFORT *et al.*, 1989). Recently, differences are found between the labial dentin (enamel covered dentin) and the lingual dentin (cementum covered dentin) in incisor dentin (APPLETON, 1993; BEERTSEN *et al.*, 1985; HALS *et al.*, 1988; MISHIMA *et al.*, 1991, 1993; STEINFORT, 1990).

There are few reports on the formation of calcospherites in secondary circumpulpal dentinogenesis. Our purpose now was to investigate the change of calcospherites on dentin formation using scanning and transmission electron microscopy, moreover SEM-EDS.

MATERIALS AND METHODS

Bilateral lower incisors from 14 male Japanese white and New Zealand white rabbits weighing 2.5 to 3.5 kg each were used in the present study. Eight rabbits were decapitated after Nembutal anesthesia. These lower incisors were extracted and immediately fixed in 10 % neutral formaldehyde solution. After the fixation, the incisors of six rabbits were cut transversely into three portions with a torx dental turbine equipped with a diamond disc. They were divided further, with the same turbine, into separate labial and lingual dentin, and treated with NaOCl to expose the mineralization front. The specimens were kept in fresh 10 % NaOCl solution for 30 minutes at room temperature with gentle agitation, and rinsed thoroughly with distilled water. The specimens were dehydrated in a series of ethanol, substituted with isoamyl acetate, and subjected to critical-point drying with carbon dioxide. The pulpal surfaces were gold-coated or carbon-coated in an ion coater and observed with a JEOL JSM-T200 and a HITACHI S-2500Δ scanning electron microscopy (SEM) with an accelerating voltage of 25 kV. Energy dispersive X-ray microanalysis (EDS) was carried out by point-mode with a Kevex system attached to the S-2500Δ. Analytical conditions were: accelerating voltage, 25kV; beam-sample incidence angle, 90°; X-ray emergence angle, 33.6°; X ray-window incidence angle, -1.4°; counting time 100 seconds. X-ray spectra were analyzed using a Kevex Delta computer program and data expressed as atomic percentages for each element detected. The regions of EDS analysis were 9 roughly equally spaced consecutive areas from the root apex to the incisal pulp horn. The incisors of two rabbits were halved parallel to their longitudinal axes using an Isomet low-speed saw (Buehler Co.), after fixation. The cut surface was ground with a whetstone initially and then with diamond paste, after which the specimens were subjected to ultrasonic cleaning for 3 minutes, washed well with distilled water, followed by dehydration and critical-point drying as described above. The polished dentin surface was carbon-coated in the ion coater, and analyzed with the same SEM-EDS system.

For transmission electron microscopy, six rabbits were anesthetized with Nembutal and perfused through the aorta with Tyrode's solution, followed by 2 % glutaraldehyde in 0.05 M cacodylate, pH 7.4. After the perfusion, the incisors were immersed in the same fixative and washed with the same buffer. The root apex of the incisor was sliced transversely into smaller pieces with a razor blade. The other incisal part of the incisor was cut transversely into smaller pieces with the dental turbine. These specimens were postfixed with 1 % osmium tetroxide in 0.05 M cacodylate buffer, and rinsed again in the same buffer. They were dehydrate in a series of ethanol, transferred into propylene oxide, and embedded in epoxy resin (Quetol 812) without decalcification. Thin sections (- 0.1 μm in thickness) were cut with a diamond knife using an ultramicrotome, unstained or stained with 5 % uranyl acetate and 1 % lead citrate, or 1% lead citrate only, and examined with a HITACHI H-8000 and a JEOL JEM-1200EXII transmission electron microscope at 100 kV.

RESULTS

1. Scanning electron microscopy

LABIAL DENTIN: Calcospherites were globular and 7 – 32 μm in diameter at the root apex (fig. 1). The fused calcospherites were observed in anorganic mineralizing front. The size of calcospherites decreases toward the incisal pulp horn. The incisal pulp horn contained micro-calcospherites. Micro-calcospherites ranged from 0.2 μm to 0.9 μm in diameter.

LINGUAL DENTIN: Calcospherites were small and granule in the anorganic mineralizing front of lingual dentin at root apex (fig. 2). The size was 1.3 - 3.3 μm in diameter. The calcospherites attached to the collagen fibers left undigested by NaOCl treatment. The incisal pulp horn contained micro-calcospherites. Micro-calcospherites ranged from 0.2 μm to 1 μm in diameter. At the incisal pulp horn, the shape of size of calcospherites was similar in the labial predentin and lingual predentin.

2. Transmission electron microscopy

LABIAL DENTIN: Globular calcospherites and bundles of collagen fibrils (von Korff's fibers) were observed at the root apex of labial predentin (fig. 3). Obliquely sectioned bundles of von Korff's fibers were scattered in the predentin. The diameter of individual bundles ranged from 1.2 μm to 3 μm. The labial calcospherite consisted of randomly oriented crystals at root apex (fig. 4). The crystals were 30-95 nm long, and 5-8 nm wide.

LINGUAL DENTIN: Calcospherites ranged from 170 nm to 900 nm in diameter at the root apex (fig. 5). Bundles of collagen fibrils were not evident. The matrix showed a small population of regularly oriented collagen fibrils. Secondary branches of odontoblast process were present near the odontoblast layer. The needle-like crystals, 35 nm to 104 nm long and 5 to 11 nm wide, were observed within and near the collagen fibrils in the lingual predentin (fig. 6).

3. SEM-EDS analysis of calcospherites and dentin

SEM-EDS analysis of calcospherites showed that Ca and P were the major elements, frequently accompanied by S, and less frequently by Mg. The Ca/P molar ratio of labial calcospherites was lower than that of lingual calco-

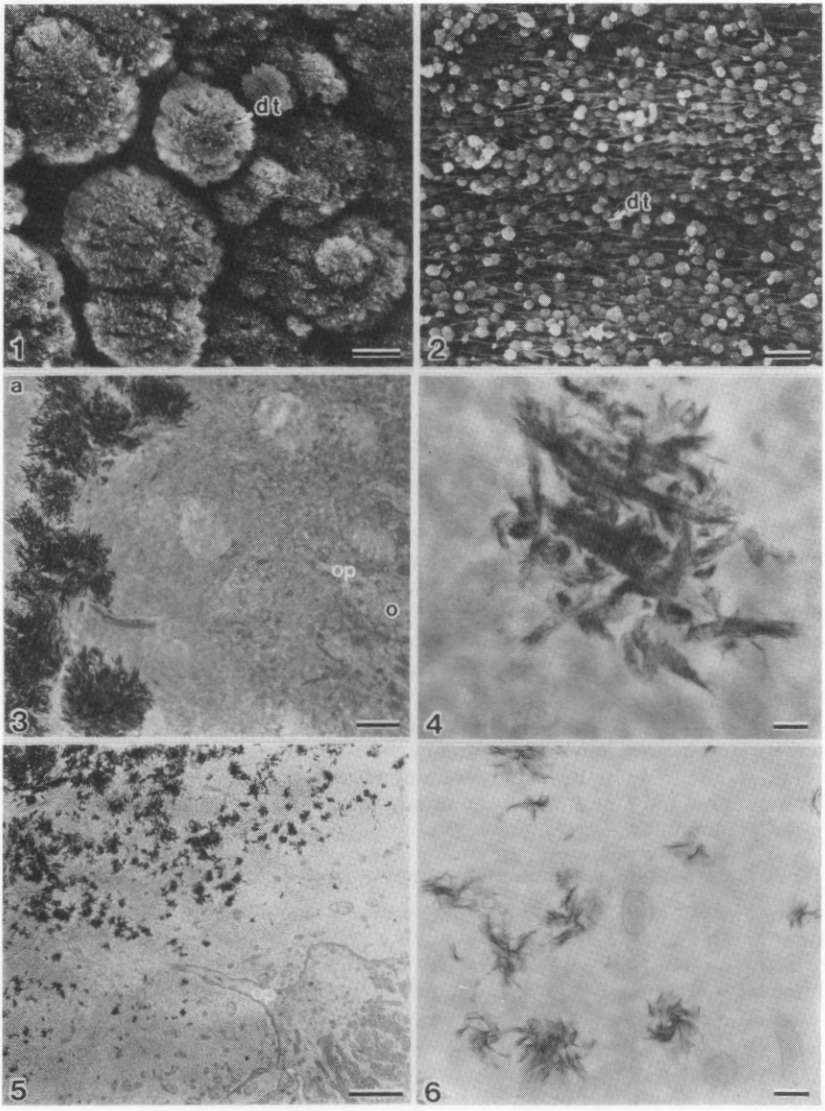

FIGURE 1 – SEM micrograph of anorganic mineralizing front of labial dentin. Root apex. Calcospherites are large and globular. dt: dentinal tubules. Bar = 10 µm.

FIGURE 2 – SEM micrograph of anorganic mineralizing front of lingual dentin. Root apex. Calcospherites are small and granule. dt: dentinal tubules. Bar = 10 µm. La: labial calcospherites, Li: lingual calcospherites.

FIGURE 3 – TEM micrograph of globular calcospherites of labial predentin. Root apex. Ultrathin section with lead citrate staining shows globular calcospherites and bundles of collagen fibrils (von Korff's fibers) at the root apex of labial predentin. op: odontoblast process, o: odontoblast, a: ameloblast. Bar = 2 µm.

FIGURE 4 – TEM micrograph of labial predentin. Root apex. Small labial calcospherite consists of randomly oriented crystals. Stained with lead citrate. Bar = 200 nm.

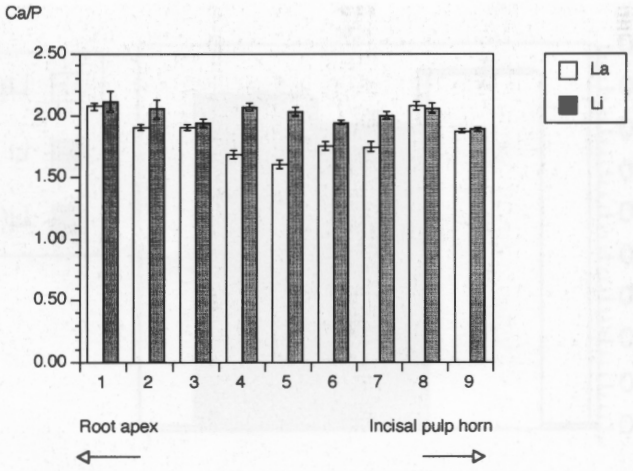

Figure 7 – Ca/P molar ratios by EDS analysis in calcospherites. Numbers (1-9) indicate areas of SEM-EDS analyses referred to in the text. La: labial calcospherites, Li: lingual calcospherites.

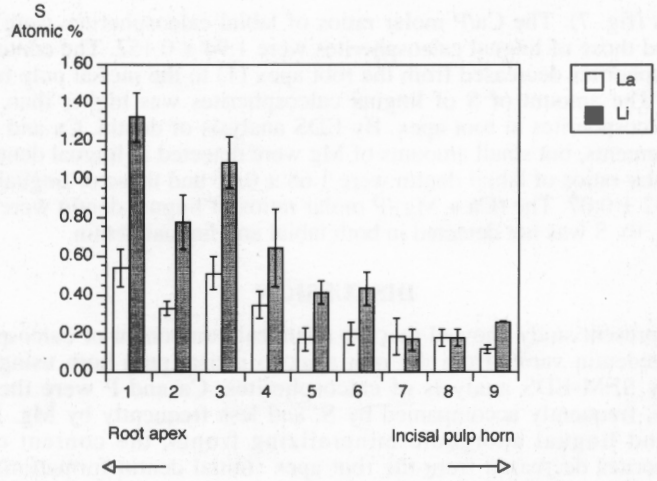

Figure 8 – Atomic percentage of sulphur by EDS analysis in calcospherites. The amount of S in calcospherites decreases from the root apex (1) to the incisal pulp horn (9). La: labial calcospherites, Li: lingual calcospherites.

FIGURE 5 – TEM micrograph of small calcospherites of lingual predentin. Root apex. Bundles of collagen fibrils are not evident, and the matrix shows a small population of regularly oriented collagen fibrils. op: odontoblast process. Stained with uranyl acetate and lead citrate. Bar = 2 µm.

FIGURE 6 – TEM micrograph of lingual predentin. Root apex. The needle-like crystals were observed within and near the collagen fibrils in the lingual predentin. Stained with lead citrate. Bar = 200 nm.

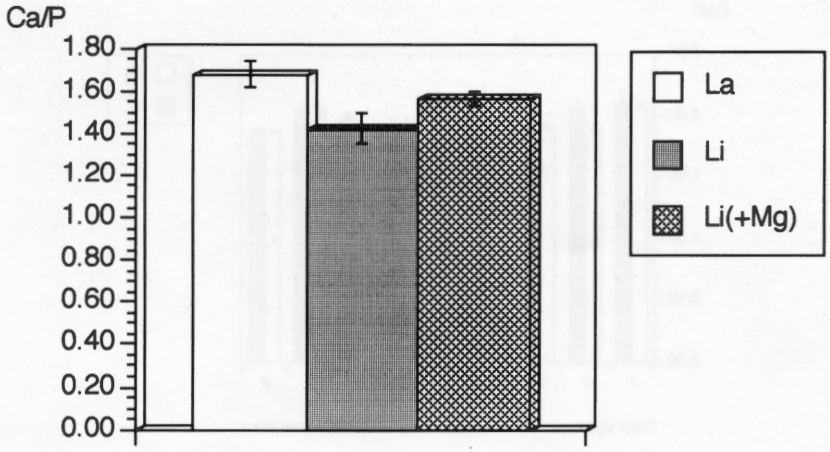

Figure 9 – Ca/P molar ratios by EDS analysis in dentin. By EDS analysis, Ca and P are major elements, but small amounts of Mg are detected in lingual dentin. La: labial calcospherites, Li: lingual calcospherites, Li(+Mg): the molar ratios of lingual dentin represent by (Ca+Mg)/P instead of Ca/P.

spherites (fig. 7). The Ca/P molar ratios of labial calcospherites were 1.89 ± 0.245 and those of lingual calcospherites were 1.94 ± 0.152. The content of S in calcospherites decreased from the root apex (1) to the incisal pulp horn (9) (fig. 8). The amount of S of lingual calcospherites was higher than that of labial calcospherites at root apex. By EDS analysis of dentin, Ca and P were major elements, but small amounts of Mg were detected in lingual dentin. The Ca/P molar ratios of labial dentin were 1.68 ± 0.06 and those of lingual dentin were 1.42 ± 0.07. The (Ca + Mg)/P molar ratios of lingual dentin were 1.56 ± 0.04 (fig. 9). S was not detected in both labial and lingual dentin.

DISCUSSION

The present study showed that the chemical component of calcospherites in the predentin varies from the root apex to incisal pulp horn using SEM-EDS. By SEM-EDS analysis of calcospherites, Ca and P were the major elements, frequently accompanied by S, and less frequently by Mg. In both labial and lingual anorganic mineralizing fronts, the content of S in calcospherites decreased from the root apex (initial dentin formation) to the incisal pulp horn (mature dentin formation). S is probably present as sulphated proteoglycans in the predentin. It has been proposed that predentin is richer in proteoglycans than dentin and some of the predentin proteoglycans have to be degraded enzymatically at the onset of mineralization (STEINFORT, 1990). Our EDS analysis showed that S was not present in calcified dentin. It is considered that S is removed from dentin mineralized matrix in the dentinogenesis. VEIS (1989) proposed that the noncollagenous protein must be able to initiate crystal nucleation in dentin. We think that the sulphated proteoglycans may have some function in the initial formation of dentin apatite. At root apex, the content of S of lingual calcospherites was richer than that of labial calcospherites. In the lingual predentin, small granular

calcospherites were present at the root apex. In contrast, in labial dentin, large globular calcospherites were present at the root apex. It is assumed that the presence of dentin matrix included proteoglycans may be one of the factors controlling the calcospherite morphogenesis. More data are necessary to determine the role of the protein in dentin mineralization.

Calcospherites in predentin are not completely calcified. We assume that the calcospherite apatite may contain many carbonate ions as compared with dentin apatite. Therefore, it is considered that the Ca/P molar ratios of calcospherite are higher than those of dentin. By TEM-EDS analysis, Mg was detected in small amount (MISHIMA *et al.*, 1993). However, SEM-EDS showed that Mg was rarely detected. The result indicates that Mg ions were removed from the apatite crystal structure upon NaOCl treatment (SAKAE *et al.*, 1988).

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