PEDIATRIC DENTISTRY/Copyright © 1991 by
The American Academy of Pediatric Dentistry
Volume 13, Number 1

Scanning electron microscopic analysis of dentin in vitamin D-resistant rickets — assessment of mineralization and correlation with clinical findings

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Abstract

In vitamin D-resistant rickets, the basic dental defect is manifested in dentin. Structural investigations of rachitic dentin by light and scanning electron microscopy have revealed the presence of calcospherites separated by interglobular dentin. This occurs when calcospherites do not fuse. This study investigated the morphology and ultrastructure of rachitic dentin with three different clinical and histologic grades of severity to determine if dentin mineralization can be quantified by calcospherite size and degree of calcospherite fusion. The results showed that a correlation existed between calcospherite size, calcospherite fusion, and the degree of dentin mineralization. The present findings indicate that calcospherite size and calcospherite fusion are valid parameters for assessing and quantifying dentin mineralization. In addition, an SEM grading of severity was established based on these two parameters. The SEM grades correlated directly with the clinical and histologic grades, suggesting that the dental clinical manifestations are a reflection of the underlying morphology of the dentin and the extent of abnormal dentin mineralization.

Introduction

Vitamin D-resistant rickets (VDRR) currently is recognized as one of the most common forms of rickets in industrialized nations (Harrison et al. 1966; Stickler and Morgenstern 1989). The condition, inherited as an X-linked dominant trait, is characterized by persistent hypophosphatemia and hyperphosphaturia associated with decreased renal tubular reabsorption of inorganic phosphates (Harrison et al. 1966; Fraser and Scriver 1976).

The most characteristic dental features of VDDR are the multiple "spontaneous" dental abscesses (Pliskin et al. 1975; Vasilakis et al. 1980; Seow 1984; Herbert 1986; Seow and Latham 1986). Seow and Latham (1986) reported that dental clinical findings may be classified into Grades I to III in increasing order of severity based on dental abscess experience and radiographic appearances of the pulp chambers.

The susceptibility to dental abscesses is related to defective dentin mineralization, which may be evident histologically. Structural investigations of rachitic dentin by light and electron microscopy have revealed the widespread presence of calcospherites (Harris and Sullivan 1960; Marks et al. 1965; Tracy et al. 1971; Jones and Boyde 1984; Seow et al. 1989).

In the present investigation, the ultrastructure of rachitic dentin in VDRR was studied by scanning electron microscopy (SEM) to determine if dentin mineralization can be quantified by analyzing the calcospherite size, amount, and distribution of globular and interglobular dentin. In addition, the SEM sections were examined to determine if the SEM findings correlated with the histological and dental clinical gradings of each patient obtained in two earlier studies (Seow and Latham 1986; Seow et al. 1989).

Materials and Methods

Patients

Five patients with an established diagnosis of VDRR contributed both naturally exfoliated and extracted teeth to our study. These same patients were part of a study population who participated in two previous investigations (Seow and Latham 1986; Seow et al. 1989). In the first study (Seow and Latham 1986), the dental clinical grading was established for each patient. Two patients showed evidence of minimal dental manifestations of VDRR (Grade I severity) and another two displayed multiple dental abscesses (Grade III severity). The fifth patient had been diagnosed as having Grade II activity with moderate dental findings. The teeth from each of the five patients were examined subsequently at the histological level in a second study by Seow et al. (1989). In that study, criteria were established to grade the teeth according to their histological appearance, and the authors showed direct correlation of histological grades with the clinical dental grades of the patients.

Preparation of teeth for SEM investigations

The teeth were embedded in a chemical-setting polyacrylic resin (L.R. White — BioRad Sciences, Watford, United Kingdom) and sectioned sagitally in a buccolingual direction into two equal halves using a Leitz Saw Microtome® (Leitz, Wetzlar, Germany). One half of each section was mounted onto an aluminium stub with UV-setting resin. This was polished subsequently using a six-stage aluminium oxide procedure, etched for 30 sec using 1% phosphoric acid, rinsed, dried, and sputter-coated with 50 nm of pure gold. The sections were examined with a Philips 505 scanning electron microscope.

Measurement of calcospherite diameters

A standardized technique was used for viewing the SEM sections, and for measuring individual calcospherites. Each section was tilted at 45° to the cathode ray tube to obtain the best resolution. The coronal dentin was divided into three different levels (outer, middle, and pulpal thirds). Three photographs, each at the fixed magnification of 312x, were taken at the center of each level of dentin for every tooth in the study.

Measurements were obtained from photographs using a millimeter-rule placed across the center of each calcospherite. Two readings of the diameter were taken of each calcospherite, one taken at right angles to the other. The mean of these two readings was recorded. All the calcospherites available in every photograph were measured. The mean number of calcospherites measured ranged from 6–66 for a particular area of dentin scanned.

Mean diameter and standard deviation of the calcospherites were calculated for each tooth, and at each of the three different levels.

Quantifying the degree of calcospherite fusion

The same photographs used for measurement of calcospherite diameters were used for quantifying the degree of calcospherite fusion. The total number of calcospherites in each photograph was counted as were the number of calcospherites that had fused with adjacent ones. The degree of calcospherite fusion was calculated by the following formula:

$$\text{Calcospherite fusion} = \frac{\text{No. of fused calcospherites}}{\text{Total No. of calcospherites}} \times 100$$

Table 1. Calcospherite size at different locations of dentin

Patient	Tooth	Calcospherite Diameter (μm) Mean± SEM Outer 1/3	Middle 1/3	Pulpal 1/3	Tooth Mean ± SEM
N.S.	4	52.0 ± 3.3	62.6 ± 3.3	70.0 ± 5.5	62.6 ±1 .5
N.S.	E	47.9 ± 4.6	57.2 ± 4.9	NM	52.6 ± 3.1
			Overall Patient Mean		57.0 ± 3.0*
K.B.	B	NM	NM	NM	NM
K.B.	C	NM	NM	NM	NM
M.B.	D	NM	NM	NM	NM
M.B.	E	NM	NM	NM	NM
M.G.	B	55.1 ± 6.5	57.4 ± 4.4	70.0 ± 3.2	60.8 ±2 .0
M.G.	C	49.5 ±4 .6	42.3 ± 2.6	36.9 ±3 .2	42.9 ± 2.3
M.G.	E	20.8 ± 1.4	15.1 ± 0.8	6.7 ± 0.2	14.1 ± 0 9
			Overall Patient Mean		57.0 ± 3.0*
S.S.	C	38.5 ± 1.9	30.3 ± 1.6	34.6 ± 2.0	34.4 ± 3.6*

NM Calcospherites not measurable as their fusion was nearly total, and boundaries of individual calcospherites not distinguishable.

* The differences in overall mean calcospherite diameters between patients N.S. and M.G. as well as between N.S. and S.S. are statistically significant ($P < 0.001$), in contrast to the difference between M.G. and S.S. which is not significant, $P > 0.1$.

A calcospherite was considered fused if any part of its circumference contacted at least two adjacent calcospherites.

Statistical Analysis

The Student's *t*-test was used for statistical analysis of the data.

Results

Calcospherite size

Table 1 shows the mean diameter of the calcospherites as measured at various locations of each tooth. From the table it can be seen that there is marked variation in overall mean calcospherite size among the patients studied. The mean calcospherite diameter ranged from 57.0 ± 3.0 µm in patient N.S., and 41.5 ± 1.7 µm in patient M.G., to 34.4 ± 3.6 µm in patient S.S. In contrast, in patients K.B. and M.B., no discrete calcospherites could be discerned since their fusion was nearly complete. The differences in overall mean calcospherite diameters between patient N.S. and M.G. and between N.S. and S.S. were statistically significant, ($P < 0.001$), in contrast to the difference between M.G. and S.S. which is not significant, ($P > 0.1$).

In addition, differences were observed in the diameter of the calcospherites at different locations of the dentin of each tooth. As shown in Table 1, in patient N.S., the calcospherites increased in size, and the dentin appeared better mineralized with advancement toward the pulp. For example, in tooth 4, the mean calcospherite diameter was 52.0 ± 3.3 µm at the outer third of dentin, compared with 62.6 ± 3.3 µm at the middle third, and 70.0 ± 5.5 µm at the pulpal third of dentin. These differences were statistically significant ($P < 0.01$). By contrast, in teeth C and E in patient M.G., the calcospherites decreased in size in a pulpward direction (Table 1). In tooth E, the mean calcospherite diameter was 20.8 ± 1.4 µm at the outer third of dentin, 15.1 ± 0.8 µm at the middle third and 6.7 ± 0.2 µm at the pulpal third. These differences were statistically significant ($P < 0.01$).

Calcospherite fusion

As shown in Table 2, there were significant differences in the mean degree of calcospherite fusion among the patients studied.

In patient N.S., the overall mean fusion of the calcospherites was 65.0 ± 0.5%, compared with 46.0 ± 4.1% in patient M.G., and 43.0 ± 0.3% in patient S.S. While the results of patients M.G. and S.S. were comparable ($P > 0.1$), the differences between N.S. and M.G., as well as between N.S. and S.S., were statistically significant, $P < 0.001$. In addition, the teeth from patients K.B. and M.B. showed near complete fusion, so that individual calcospherites were not discernable.

As in calcospherite size, differences were observed in the degree of fusion of the calcospherites at different

Table 2. Calcospherite fusion at different locations of dentin

Patient	*Tooth*	*Outer 1/3*	*Calcospherite Fusion (% of total) Middle 1/3*	*Pulpal 1/3*	*Mean ± SEM*
N.S.	4	59.0 ± 0.5	75.0 ± 0.4	NM	67.0 ± 4.5
N.S.	E	55.0 ± 0.5	70.0 ± 0.5	NM	63.0 ± 4.5
			Overall Patient Mean		65.0 ± 0.5*
K.B.	B	NM	NM	NM	NM
K.B.	C	NM	NM	NM	NM
M.B.	D	NM	NM	NM	NM
M.B.	E	NM	NM	NM	NM
M.G.	B	51.0 ± 0.3	58.0 ± 0.3	64.0 ± 0.5	58.0 ± 0.4
M.G.	C	49.0 ± 0.4	42.0 ± 0.2	39.0 ± 0.4	43.0 ± 0.3
M.G.	E	43.0 ± 0.4	39.0 ± 0.4	32.0 ± 2.9	38.0± 0.4
			Overall Patient Mean		43.0 ± 0.3*
S.S.	C	47.0 ± 0.4	43.0 ± 0.2	38.0 ± 0.3	43.0 ± 0.3*

NM Calcospherites not measurable as their fusion was nearly total, and boundaries of individual calcospherites not distinguishable.

* The differences in overall mean calcospherite diameters between patients N.S. and M.G. as well as between N.S. and S.S. are statistically significant ($P < 0.001$), in contrast to the difference between M.G. and S.S. which is not significant, $P > 0.1$.

TABLE 3. Three SEM grades of VDRR teeth based on calcospherite size and degree of calcospherite fusion

SEM Grade	*Morphologic Feature*
I	Calcospherite fusion > 75%; dentin relaqtively normal with no discrete calcospherite
II	Calcospherite diameter > 50 µm, and degree of calcospherite fusion between 50 - 75%
III	Calcospherite diameter < 50 µm, and degree of calcospherite fusion <50%

locations of the dentin of each tooth (Table 2). In both teeth from patient N.S. and one tooth from patient M.G. (B), the degree of fusion of the calcospherites increased in a pulpal direction. For example in tooth 4 from patient N.S., the fusion was 59.0 ± 0.5% at the outer third of dentin, compared with 75.0 ± 0.4% in the middle third, and nearly complete (100%) at the pulpal third. These differences were statistically significant ($P < 0.001$). In contrast, in patient S.S., as well as in two teeth (E, C) from patient M.G., the degree of fusion decreased in a pulpal direction.

Establishment of three SEM grades based on calcospherite size and degree of calcospherite fusion

An SEM grading of severity of the dental manifestations of VDRR was established arbitrarily based on the criteria of calcospherite size and degree of calcospherite fusion. This is shown in Table 3.

Correlation of SEM, histologic and clinical gradings

An SEM grade was assigned to each tooth according to the criteria summarized in Table 3. This was performed by one of the authors (ES) who had no prior knowledge of the clinical and histologic gradings ascribed to the patients in two previous studies (Seow and Latham 1986; Seow et al. 1989). Examination of the results from Tables 1 and 2 revealed that patients M.G. and S.S. would be classified as SEM Grade III (Fig 1), patient N.S. as SEM Grade II (Fig 2) and patients K.B. and M.B. as Grade I (Fig 3, see next page for figs).

The SEM gradings then were correlated with the clinical and histological gradings. The results, as shown in Table 4, clearly suggest a consistent correlation of clinical, histologic, and SEM gradings in all the patients studied.

Discussion

Rachitic dentin is characterized by the presence of a large number of calcospherites separated by irregular zones of interglobular dentin. This investigation has shown that the degree of dentin mineralization may be quantified by examination of calcospherite size and degree of fusion, and this in turn may be correlated directly with the clinical manifestations of an affected individual.

In the present study, the quantitation of dentin mineralization is based on current understanding of this process. During normal circumpulpal dentin mineralization, calcospherites are formed from foci of mineral seeding. Local mechanisms promote further mineral deposition around the seeds (Shellis 1983; Couve 1987). The calcospherites thus initiated in predentin grow uniformly by mineral accretion until they contact other calcospherites, so that in completely mineralized nor-

TABLE 4. Correlation of SEM, clinical, and histologic gradings

Patient	*+Calcospherite Diameter (µm) Mean ± SEM*	*+Calcospherite Fusion (% of total) Mean ± SEM*	*SEM Grade*	*Clinical Grade*	*Histologic Grade*
N.S.	57.0 ± 3.0	65 ± 0.5	II	II	II
K.B.	NM	NM	I	I	I
M.B.	NM	NM	I	I	I
M.G.	41.5 ± 1.7	46 ± 4.1	III	III	III
S.S.	34.4 ± 0.6	43 ± 0.3	III	III	III

NM Calcospherites not measurable as their fusion was nearly total, and boundaries of individual calcospherites not distinguishable.

\+ These values were obtained from the overall mean of all available measurements from the teeth of each patient.

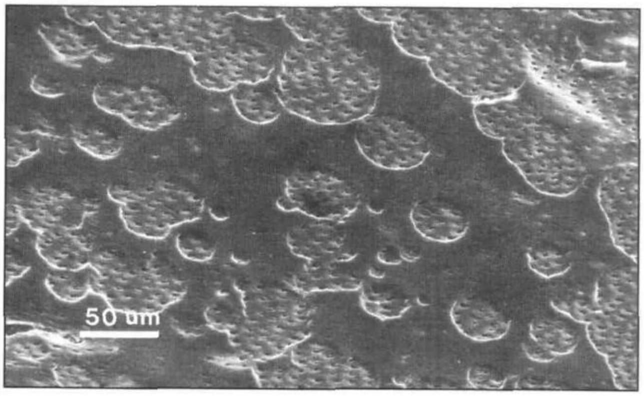

Fig 1. SEM presentation of a tooth with SEM grade III dental manifestations, taken at middle third of dentin. Note that the mean calcospherite diameter is < 50 µm and the degree of calcospherite fusion is < 50%. Mag: original 312 x.

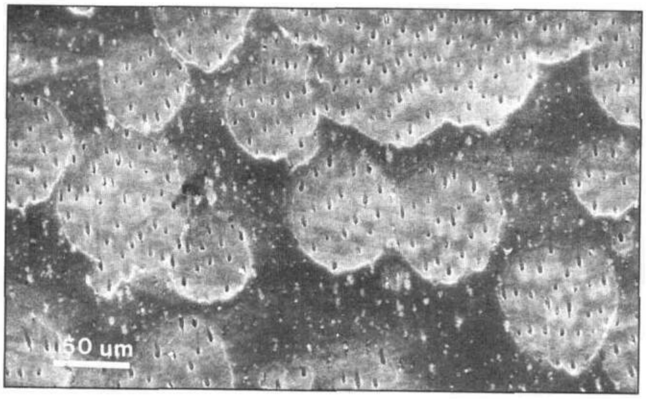

Fig 2. SEM presentation of tooth with SEM grade II dental manifestations, taken at middle third of dentin. Note that the mean calcospherite diameter is > 50 µm and degree of calcospherite fusion is between 50–75%. Mag: original 312 x.

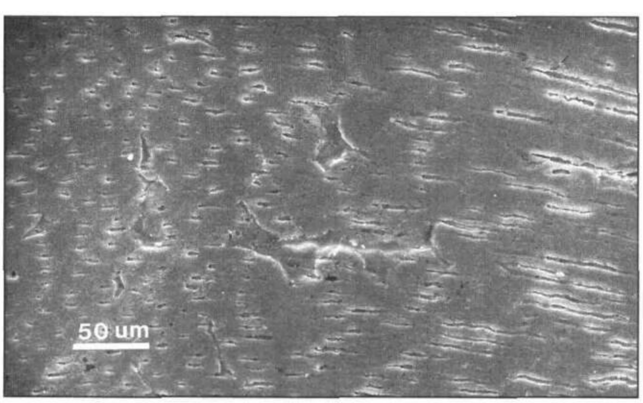

Fig 3. SEM presentation of tooth with SEM grade I dental manifestations, taken at middle third of dentin. Note that the calcospherites have fused to such an extent that the boundaries of individual calcospherites are indistinct. The dentin appears relatively normal and the calcospherite fusion is > 75%. Mag: original 312 x.

mal dentin, the appearance is homogeneous and the outlines of individual calcospherites are indistinguishable (Jones and Boyde 1984). By contrast, incomplete fusion of calcospherites produces areas of interglobular dentin (Shellis 1983; Jones and Boyde 1984; Avery 1986), the calcospherite domains representing areas of normal mineralization and the interglobular spaces consisting largely of unmineralized matrix (Marks et al. 1965; Nikiforuk and Fraser 1979).

It is thus reasonable to assess the state of dentin mineralization by assessing the size and degree of fusion of the calcospherites. Smaller calcospherites would suggest that mineralization has been abrogated at an earlier stage. In addition, the degree of fusion of the calcospherites also may indicate the stage of mineralization; the greater the number of calcospherites fused, the more advanced the mineralization.

The presence of numerous calcospherites in dentin in patients with VDRR suggests that although initiation of mineralization probably is not affected, calcospherite growth and fusion appear to be altered. This derangement in mineralization may result directly from the hypophosphatemia (Nikiforuk and Fraser 1979). However, alterations in the organic matrix that lead to abnormal mineralization also are possible; studies of other inherited defects of dentin such as dentinogenesis imperfecta have indicated abnormalities in the dentin matrix (Sauk et al. 1980; Takagi et al. 1983).

The present study has improved further on the previous histologic evaluation of affected dentin in VDRR by providing an objective method for assessing the degree of mineralization. The SEM grading of dentin manifestations based on this objective method is validated by the consistent correlation of the clinical, light histologic, and SEM grades. The patients with the largest pulp chambers and the highest numbers of dental abscesses (clinical Grade III) show the smallest dentin calcospherites and the lowest degree of calcospherite fusion. Conversely, those with the near-normal pulp sizes and minimal numbers of dental abscesses (clinical Grade I) have the largest calcospherites which may be so well fused that their boundaries are indistinct.

The results of this study further highlight the broad spectrum of dental manifestations of VDRR. Knowledge of the severity of the underlying dental defect is valuable for clinical management of these patients. Routine preventive measures, such as fissure sealants to prevent occlusal caries and topical fluoride therapy may suffice for patients with Grade I severity. However, for patients with Grade II and III severity, more aggressive treatment regimens are required, including prophylactic stainless steel crowns for posterior teeth (Seow 1984; Seow and Latham 1986) and adhesive resins for anterior teeth.

In conclusion, the present study indicates that dentin mineralization in VDRR teeth can be quantified by calcospherite size and degree of calcospherite fusion. These objective criteria may be used in an SEM grading of the severity of dental manifestations of the disease.

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