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HETEROGENEITY OF CRYSTALS ATTACHED TO THE HUMAN ENAMEL AND CEMENTUM SURFACES AFTER CALCULUS REMOVAL *IN VITRO*

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Abstract

Twenty one extracted human teeth with dental calculi on the enamel and cementum surfaces, fixed in 10% neutral formaldehyde, were selected for this study. After ethanol dehydration and air drying, these calculi were removed by tweezers to observe the teeth surfaces under them. The inspection of these surfaces using SEM and EDX revealed hexahedrally based crystals including pseudocuboidal, rhombohedral and variable rugged rocky shapes. These crystals were identified as Mg-containing whitlockite. The pseudocuboidal crystals, measuring about 4.5 µm in maximum length, were widely distributed on the cervical enamel surface previously covered by calculus. On the root surface, however, these areas decreased remakably; the shapes changed from pseudocubes into rhombohedrons and rugged rocky structures, while their sizes were smaller and the Mg content decreased. The difference in frequency and morphological variation of the hexahedrally based crystals might be caused by the different characteristics of enamel and cementum surfaces and the Mg present on these surfaces.

KEY WORDS. human dental calculus, mechanical removal, enamel surface, cementum surface, SEM, SEM-EDX, hexahedrally based crystals, distribution, morphology, Mg-containing whitlockite

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Introduction

Organic components including oral microorganisms and the mineral phase of the calculus-tooth interface have been reported by a number of studies [Zander, 1953; Mandel et al., 1957; Turesky et al., 1961; Schroeder, 1969; Selvig, 1970; Jones, 1972; Canis et al.,1979; Eide et al., 1983; Busscher et al., 1989]. It has been demonstrated that Mg-containing whitlockite is a major constituent of dental calculus [Schroeder and Bambauer, 1966; Jensen and Rowles, 1967; Grøn et al., 1967; Kani et al., 1983], often existed in the innermost calculus layer adjacent to the tooth surface [Kodaka et al., 1988, 1989; Kodaka and Miake, 1991]. In addition, the calculus whitlockite showed various shapes and sizes although they were based on hexahedrons [Kodaka et al., 1988].

According to Everett and Potter [1959], human subgingival calculus was divided into ledge or ling-like formation (59%), finger and fern-like formations (19%), spiny or nodular deposits (62%), and others. In this study the teeth surfaces, after the removal of the dental calculi, were observed by scanning electron microscopy (SEM). The Mg content was detected by energy dispersive electron probe microanalysis (EDX).

Materials and Methods

Twenty-one human teeth with typical forms of dental calculi (ledge-like, finger-like, and spiny) [Everett and Potter, 1959], were selected from extracted teeth. These teeth were fixed in 10% neutral formaldehyde for about one week. They were classified into five types based on the position of the calculus deposits (see Fig. 1: A to E). As shown in the lower right of Figure 1 (F), their positions were divided into enamel surface (ES), the cementum-enamel junction (CEJ) area or the cervical-root surface adjacent to the CEJ (CS-1), and the cervical-root surface away from the CEJ (CS-2). Figure 1 shows that A, B, and C types are ledge-like deposits attached only to the ES (A type, one sample), from the ES to the CS-1 (B type, four samples), and only in the CS-2 (C type, five samples); the D type is finger-like formed deposits attached to the ES, CS-1, and CS-2 (four samples); and E type is spiny deposits attached to the CS-2 only (seven samples).

The teeth regions without calculus were cut off horizontally and vertically with a diamond wheel. The remaining parts were rinsed in running tap-water, dehydrated with ethanol, dried in the air, and coated with carbon in a Hitachi HUS-5GB high vacuum evaporator. After all the samples had been set up under a high vacuum in the evaporator, the calculus could be easily removed from the teeth surfaces with tweezers.

After calculus removal, the non-carbon coated surfaces clearly appeared. These surfaces previously covered by calculus were distinguished macroscopically. The outlines were roughly drawn in Figure 1. The teeth surfaces observed in the SEM came from nine samples in the ES (A, B, and D types), eight samples in the CS-1 (B and D types), and 16 samples in the CS-2 (C, D, and E types). All of them were observed by a Hitachi S-430 SEM after coating with a 10 to 15 nm thick platinum-palladium layer using an Eiko ion sputtering apparatus. The crystals on the teeth surfaces and the teeth surfaces were qualitatively analyzed by a Hitachi X560 SEM fitted with a Kevex 7000Q EDX under conditions of 15 kV accelerating voltage and 1×10^{-10} A specimen irradiation current. In addition, magnesium (Mg) content of larger masses of crystals was quantitatively analyzed by the SEM-EDX under the same conditions. The standard sample for Mg was magnesium oxide and ZAF corrections were used [Moll, 1977].

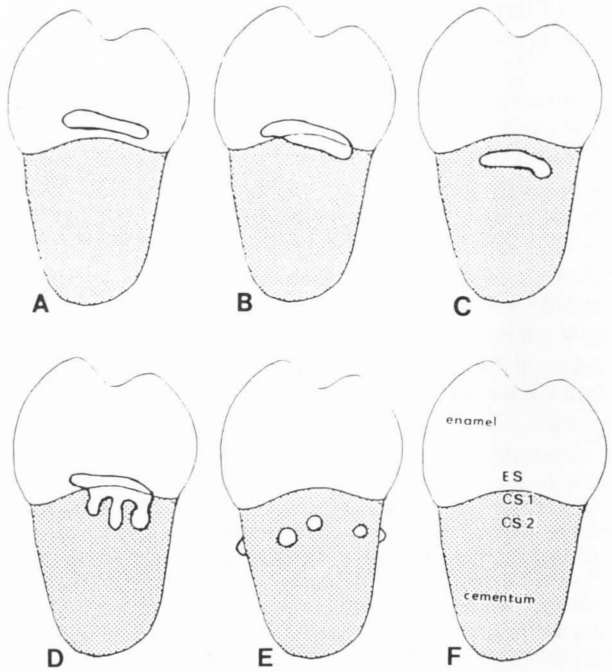
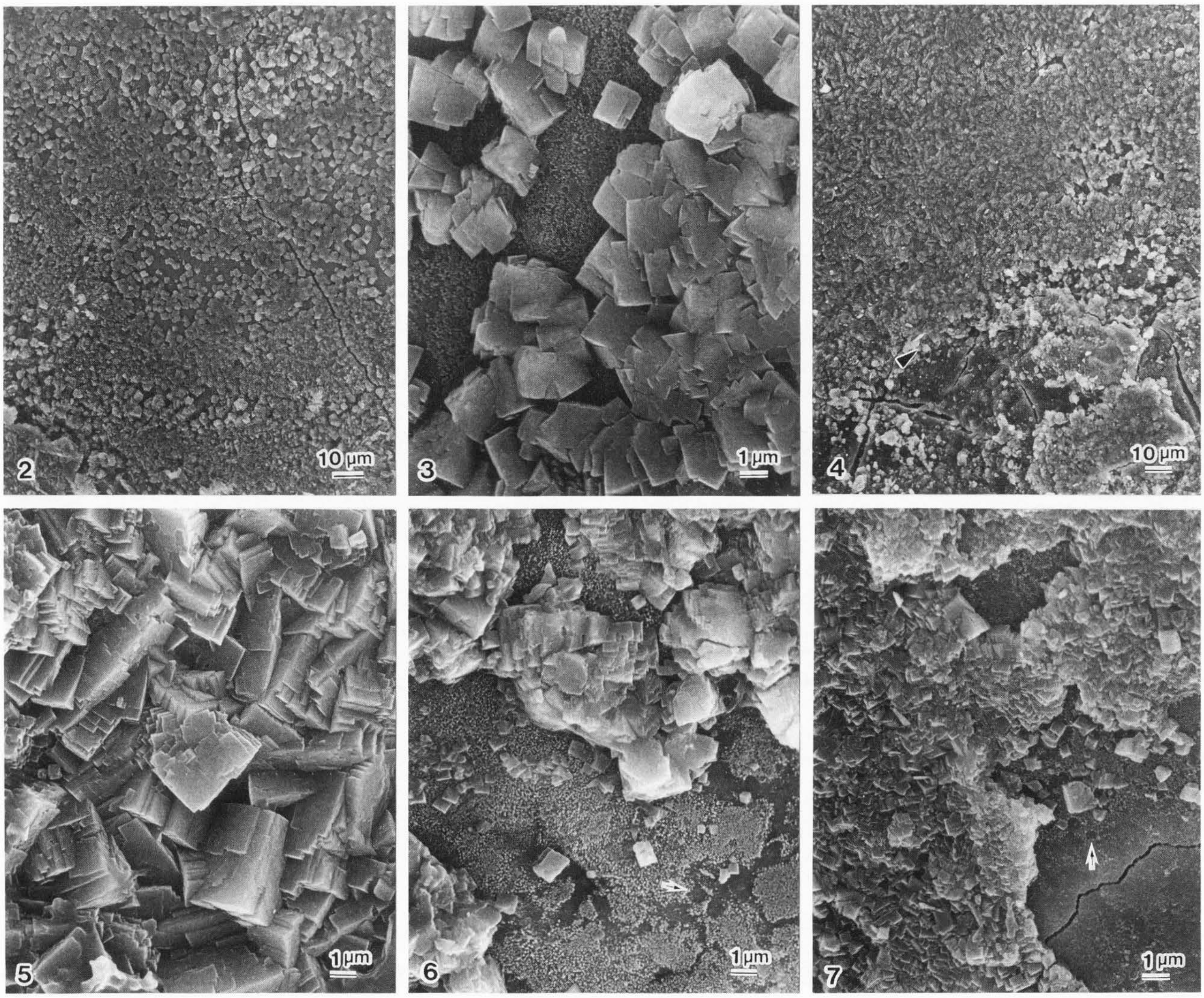


Fig. 1. Classification of teeth with attached dental calculi (A to E types). Lower-right illustration (F) shows the enamel surface (ES), the CEJ area or the cervical-root surface adjacent to the CEJ (CS-1), and the cervical-root surface away from the CEJ (CS-2). A to C types: ledge-like deposits; A type on the ES, B type on the ES and CS-1, and C type on the CS-2. D type: finger-like formed deposits on the ES, CS-1, and CS-2. E type: spiny deposits on the CS-2.

Results

Figures 2 to 19 are the SEM micrographs of the teeth surfaces after mechanical removal of dental calculus. Figures 2 to 7 show the cervical enamel surfaces (ES; A, B, and D types from Fig. 1). Hexahedrally based crystals were always found in the A and B types of ledge-like deposits. The areas with attached crystals covering over 1/3 of the whole surface were observed in three samples of A and B types, which had dental calculus attached. Their frequency was "+3" (Table 1; Figs. 2 and 4). In the two remaining B type samples scattered small areas with crystals were found with "+2" frequency (Table 1). In the D type one sample showed the frequency of "+2", but the remaining three samples showed only a few small areas of crystals. The frequency was "+1" (Table 1).

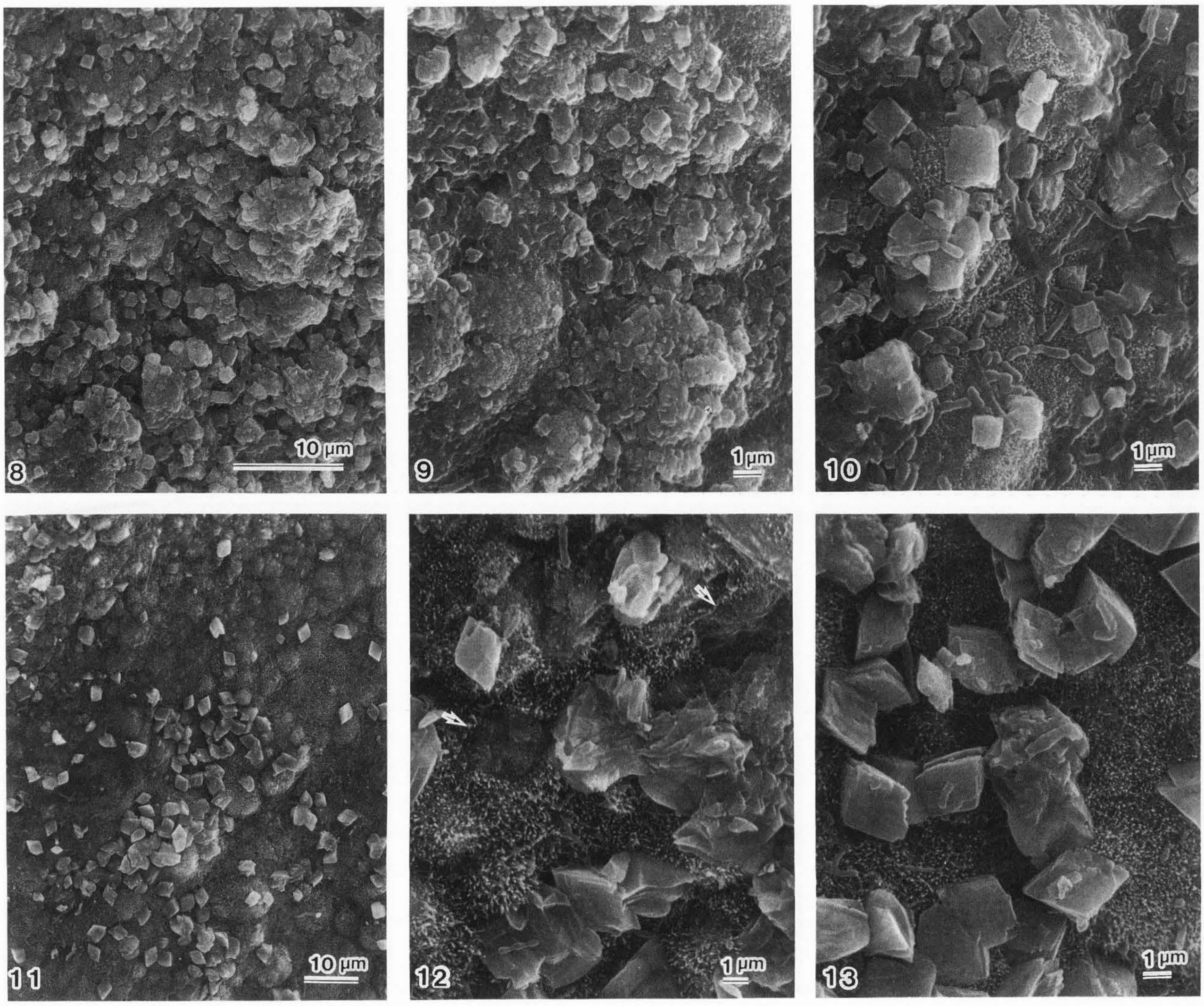
The hexahedrally based crystals on the cervical enamel surface showed a pseudocuboidal shape (Figs. 2, 3, 6, and 7) and groupings of quadranglar blades cubically arranged or variable rugged rocky shapes (Figs. 4 to 6). Although many of them were more or less fused to each other their sizes measured about 0.2 to 4.5 µm in length. The cervical enamel surface except for the attached crystals showed areas of fine granular and smooth surface structures (Figs. 3, 6, and 7). On some areas, the square windows surrounded by the granular structures were observed (Figs. 6 and 7).



Figs. 2 to 7. Hexahedrally based crystals on the enamel surface [ES]. The crystals basically show a pseudocuboidal shape. The maximum size measures about 4.5 µm in length (Fig. 7). Arrowhead in Figure 4: the CEJ. Arrows in Figures 6 and 7: square windows of a smooth surface surrounded by fine granular structures.

The distribution of areas with attached hexahedrally based crystals remarkably decreased on the cervical-root surface (lower of Fig. 4). Figures 8 to 13 show the root surfaces after calculus removal (CS-1; B and D types in Fig. 1). Slight elevations dome-shaped of various sizes formed the cementum surface (Fig. 11). As shown in Table 1, the distribution of these areas had the frequency of "+2" in one sample of the B type group (Figs. 8 to 10), but the remaining three samples and one sample of the D type showed the frequency of "+1" (Figs. 11 to 13). In the remaining three samples of the D type, hexahedrally based crystals were rarely found. Their frequency was "±" (Table 1).

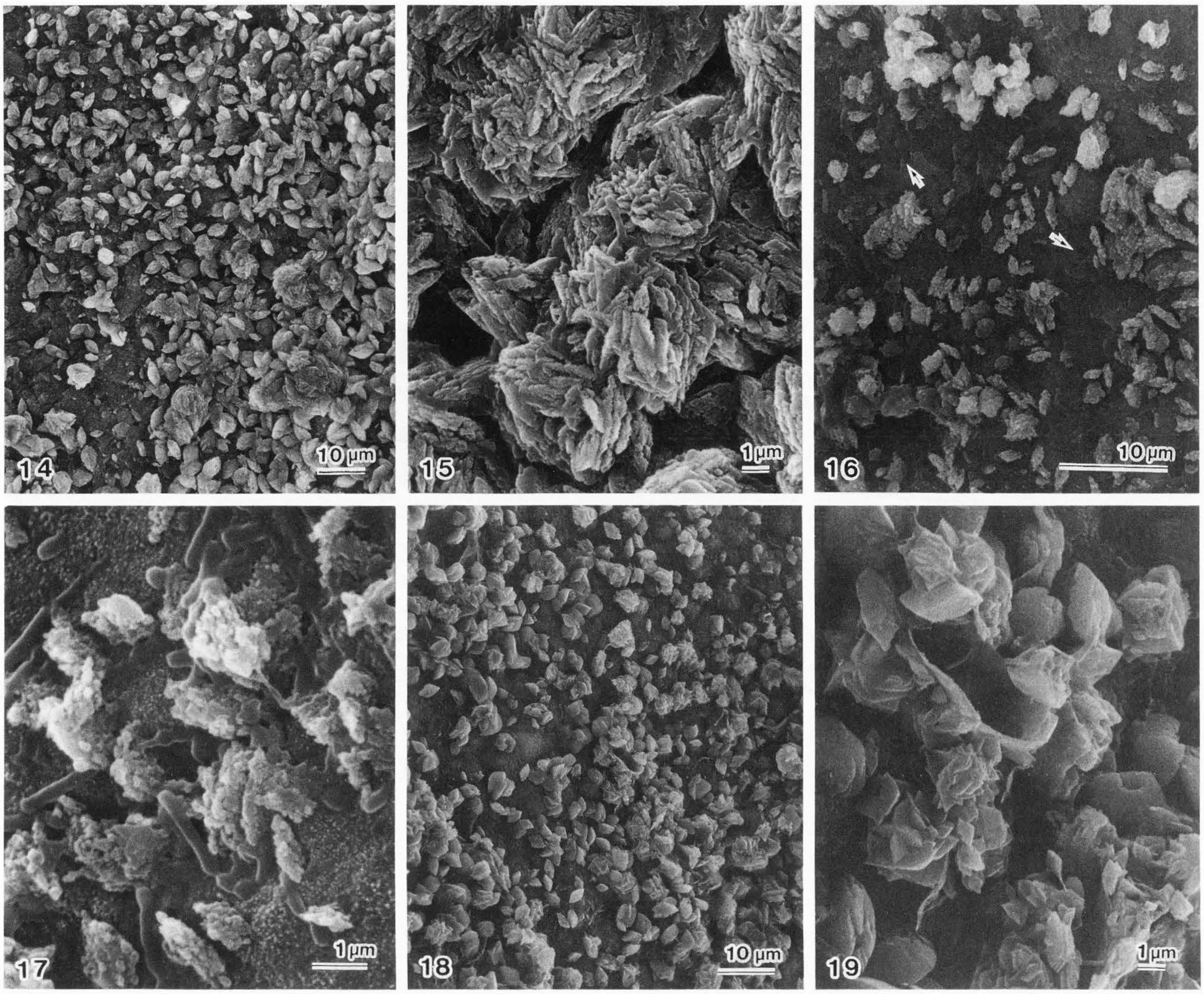
The hexahedrally based crystals on the root surface showed pseudocuboidal (Figs. 8 to 10) and rhombohedral shapes (Figs. 11 to 13). Some rhombohedral crystals fused to each other and formed rugged rocky structures (Figs. 12 and 13). Their sizes measured about 0.2 to 3 µm in length (Fig. 13). The root surface except for the attached crystals was covered with microorganisms (Fig. 10) and fine needle-shaped or granular structures (Figs. 10 to 13), and the square windows surrounded by the needle-shaped structures were observed on some areas (Fig. 13).



Figs. 8 to 13. Hexahedrally based crystals on the CEJ area [CS-1]. The crystals basically show pseudocuboidal and rhombohedral shapes, although some of them fuse to each other and form rugged rocky structures (Figs. 12 and 13). The maximum size measures about 3 µm in length (Fig. 13). Microorganisms (Fig. 10) and fine needle-shaped structures are seen (Figs. 10 to 14). Arrows in Figure. 12: square windows surrounded by granular structures.

Figures 14 to 19 show the cervical-root surface away from the CEJ after calculus removal (CS-2; C, D, and E types in Fig. 1). Dome-shaped elevations forming the cementum surface were considerably flattened (Figs. 16 and 18). As in Table 1, the distribution areas of hexahedrally based crystals showed the frequency of "+2" in two samples of the C type and the E type, and the frequency of "+1" was seen in the remaining nine samples of the C and E types, but all samples in the D type showed the rare frequency of "±".

On the root surface, there were masses of fine rhombohedral crystals (Figs. 14 to 16), aggregations of fine pseudocuboidal crystals (Fig. 17), and rhombohedral crystals with the masses fused to each other (Figs. 14, 18, and 19). These masses and aggregations had variable rugged rocky shapes. The sizes measured about 0.1 to 2.5 µm in length, although some masses were larger (Fig. 15). On the cementum surface except for attached the crystals and microorganisms (Fig. 17), poor granular structures were observed (Figs. 16 and 17). The faint square windows surrounded by the granular structures could be seen on some areas (Fig. 16).



Figs. 14 to 19. Hexahedrally based crystals in the cervical-root surface [CS-2]. Most of the crystals show fine and small rhombohedral shapes and some of them a fine pseudocuboidal shape, although many crystals form variable rugged rocky structures. The maximum size measures about 2.5 µm in length (Fig. 18). Microorganisms and poor granular structures are also seen in Figure 17. Arrows in Figure 16: square windows surrounded by the granular structures.

During the analysis by the SEM-EDX, the Mg element was not detected on the smooth and fine needle-shaped and granular structures on the enamel and cementum surfaces, but always detected from the hexahedrally based crystals. Table 2 displays the Mg concentrations detected on larger masses of the crystals in six samples: A1, B1, B2, C1, D1, and E1 (* in Table 1). The mean Mg content was higher in the crystals on the enamel surface (ES) than those on the cementum surfaces (CS-1, CS-2), but there was no significant difference ($p < 0.05$) between them.

Table 1. Frequency of hexahedrally based crystals attached to the tooth surface after calculus removal.

Type Sample No.	Calculus position ES	CS-1	CS-2
A1	+3*		
B1	+3	+2*	
B2	+3*	+1	
B3	+3	+1	
B4	+2	+1	
C1			+2*
C2			+1
C3			+1
C4			+1
C5			+1
D1	+2	+1*	±
D2	+1	±	±
D3	+1	±	±
D4	+1	±	±
E1			+2*
E2			+1
E3			+1
E4			+1
E5			+1
E6			+1
E7			±
Mean (n)	+2.1 (9)	+0.8 (8)	+0.8 (16)

ES: enamel surface. CS-1: CEJ area. CS-2: cervical-root surface.
+3: wide areas over 1/3. +2: small areas scattered here and there. +1: a few small area. ±: rare.
A to E type: see Fig. 1.
*: quantitatively analyzed by the SEM-EDX (see Table 2).

Table 2. Mg concentration of hexahedrally based crystals. SEM-EDX analysis.

Calculus position	Mg content (weight%)	
ES	0.72±0.49	[0.22 - 1.51]
CS-1	0.50±0.42	[0.08 - 1.13]
CS-2	0.54±0.32	[0.09 - 1.34]

Mean±S.D. (n=10) [Minimum-Maximum]

ES: enamel surface. CS-1: CEJ area. CS-2: cervical-root surface.

Discussion

Hexahedrally based crystals observed in human dental calculus are either in number of whitlockite or brushite crystals [Newesely, 1965; Schroeder and Bambauer, 1966; Schroeder, 1969; Driessens, 1982; Moriwaki et al., 1983]. From the detection of a small amount of Mg (Table 2), the hexahedrally based crystals including pseudocuboidal, rhombohedral, and variable rugged rocky shapes (Figs. 2 to 19), observed in this study, were identified as Mg-containing whitlockite [Jensen and Rowles, 1967; Santos and González-Díaz, 1980; Kodaka et al., 1988, 1989; LeGeros et al., 1988, 1989].

According to our previous studies [Kodaka et al., 1988, 1989; Kodaka and Miake, 1991], the whitlockite crystals of supragingival calculus were frequently seen in the innermost layer and in the intra-spaces of the deposits. However, the crystals were not found in the outer surface exposed to the oral cavity. The crystals covered the outer surface layer exposed to gingival fluid in marginal (supra- and subgingival) ledge-like and subgingival spiny deposits, although the calculus surface facing to the oral cavity in the marginal ledge-like deposits were similar to supragingival calculus. The present SEM results of the observation of teeth surfaces after calculus removal were consistent with the above-mentioned data.

Mg-containing whitlockite crystals are formed by higher Mg sources and a rise of pH of saliva and gingival fluid than other dental crystals [Newesely, 1965; Grøn et al., 1967; Killian and Ennever, 1975; Boyan-Salyers et al., 1978; Knuuttia et al., 1980; Driessens, 1982; Kani et al., 1983; LeGeros et al., 1988, 1989]. According to our previous study [Kodaka and Miake, 1991] (see Table 3), the Mg content was smaller in the inner than in the outer layer of subgingival spiny deposits attached to the root surface, whereas the Mg content increased from the outer to the inner layer of marginal ledge-like deposits attached from the cervical enamel to the root surface. There was no significant difference in Mg content in these inner layers. The SEM-EDX analysis (Table 2) showed no significant difference of Mg content in the hexahedrally based crystals attached to the teeth surfaces, and the Mg element was not detected on the teeth surfaces, only on the crystals.

The frequency of the hexahedrally based crystals (Table 1) and the Mg content on the tooth surface (Table 2) indicate that the supplied Mg was higher on the cervical enamel than on the root surface; and a small increase of pH might be seen on the interface of the calculus and the enamel surface. The frequency of the crystals may be caused by the difference of the supplied Mg content on the teeth surfaces; in addition, the morphological variation as shape and size might be also influenced by the difference of the supplied Mg content and pH range. The Mg source may be derived from saliva, or gingival fluid as well which can penetrate the calculus-tooth interface [Jenkins, 1978; Driessens, 1982]. The enamel mineral with attached deposits might promote the Mg-containing crystals formation as shown in enamel caries [Helmcke, 1955; Newesely and Höhling, 1964; Lester and Boyde, 1968] or arrested enamel caries [Plačková and Vahl, 1967], as well as in a case of arrested dentin caries [Daculsi et al., 1979, 1987; LeGeros, et al., 1989].

Fine needle-shaped and granular structures observed on the teeth surfaces of air-dried samples is not organic matter but mineral (Figs. 3, 6, 7, 10, 12, 13, and 17), and is probably some kind of apatite [Lustmann et al., 1976; Kodaka et al., 1988]. The shape and density of the fine structures were poor and lower on the cementum when compared to the enamel as well as the hexahedrally based crystals, whereas oral microorganisms [Kodaka et al., 1989] tended to be observed only on the cementum surface (Figs. 11 and 18).

The square windows surrounded by the fine needle and granular structures had traces of hexahedrally based crystals (Figs. 6, 7, 12, and 16). These crystals probably were removed with the calculus when the physical force was applied or removed by the vacuum of the evaporator chamber. The square surfaces were covered with a very thin organic membrane, i.e., pellicle as previously reported, if it exists. It is, therefore, suggested that there are smaller amounts of organic substances on the interface between the deposit and the enamel surface (A and B types in Fig. 1) than that of the deep subgingival deposits and the root surface (C and E types in Fig. 1). In other words, the inner calculus surface adjacent to the enamel surface has been fully mineralized as compared with that adjacent to the cementum surface.

The finger-like formed deposits distributed from the enamel to the cementum surface (D type in Fig. 1) were also observed. As shown in Table 1, the ledge-like and spiny deposits (A, B, C, and E types) showed frequent hexahedrally based crystals similar to each other in the enamel and the root surface. The D type of the finger-like formed deposits, however, was somewhat different from the others. They showed less hexahedrally based crystals than the A and B types in the enamel, and also less of the same crystals than the B, C, and E types in the root surface. Though this appearance in the finger-like formed deposits might be caused by their morphological characteristic and formation, it could not be elucidated in this study.

Considering the clinical applications of these findings, the hexahedrally based crystals, Mg-containing whitlockite, may remain when the ledge-like deposits attached to the teeth surfaces are removed with a scaler. The remaining crystals strongly attached to the cervical enamel surface could facilitate plaque attachment, and subsequent mineralization could begin there.

Table 3. Mg concentration of ledge-like deposits (A and B types) attached from the enamel surface (ES) to the CEJ area (CS-1) and spiny deposits (E type) attached to the cervical-root surface (CS-2).

	Mg content (weight%)	
Calculus	Ledge-like deposits (A, B types)	Spiny deposits (E types)
Outer layer*	0.60±0.91	2.40±0.82
Middle layer*	0.96±1.03	2.10±0.86
Inner layer	1.04±1.21	1.27±1.09
	Mean±S.D. ($\underline{n}$=25)	

*: significance level ($\underline{p} < 0.01$)
All data were cited from Kodaka and Miake [1991]

For References and Discussion with Reviewers, see p 720-721.

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Discussion with Reviewers

G. Daculsi: You described some joined crystals as "fused crystals" and in discussion you suggest a fusion process. What evidence do you have for crystal fusion ? What do you think of an epitaxic growing process ?

Authors: The fused whitlockite crystals have been reported with SEM in synthetic calcium phosphates [LeGeros et al., 1988], arrested enamel caries [Lester and Boyde, 1968], in renal calculus [Santos and Gonzallez-Dìaz, 1980], and in dental calculus [Kodaka et al., 1988, 1989; LeGeros et al., 1988, 1989]. Some of these crystals might have been formed by the epitaxial growth on the previously formed or biological aptites. Aoba et al. [1974] reported the epitaxial growth of whitlockite on the enamel apatite *in vitro*. In this study, the higher frequency of whitlockite on the enamel surface than on the cementum surface might be caused by the high purity of the apatite.

M. Alves : How do you know what is supra- and subgingival calculus if the teeth were extracted ?

Authours: The classification of dental calculus attached to human teeth by Everett and Potter [1959] was followed. They classified ledge-like, finger-like, and spiny deposits as subgingival, but we regarded the ledge-like deposits as the transitional type between supra- and subgingival calculus because the calculus is exposed to both the oral cavity and gingival pocket sides [Kodaka and Miake, 1991].

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