

10-4-1989

Morphology and Chemical Composition of Dental Calculi Mainly Composed of Whitlockite

Toshiro Sakae
Nihon University at Matsudo

Hirotsugu Yamamoto
Nihon University at Matsudo

Hiroyuki Mishima
Nihon University at Matsudo

Takashi Matsumoto
Nihon University at Matsudo

Yukishige Kozawa
Nihon University at Matsudo

Follow this and additional works at: <https://digitalcommons.usu.edu/microscopy>



Part of the [Biology Commons](#)

Recommended Citation

Sakae, Toshiro; Yamamoto, Hirotsugu; Mishima, Hiroyuki; Matsumoto, Takashi; and Kozawa, Yukishige (1989) "Morphology and Chemical Composition of Dental Calculi Mainly Composed of Whitlockite," *Scanning Microscopy*. Vol. 3 : No. 3 , Article 16.

Available at: <https://digitalcommons.usu.edu/microscopy/vol3/iss3/16>

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



MORPHOLOGY AND CHEMICAL COMPOSITION OF
DENTAL CALCULI MAINLY COMPOSED OF WHITLOCKITE

Toshiro Sakae*, Hirotsugu Yamamoto¹, Hiroyuki Mishima,
Takashi Matsumoto², and Yukishige Kozawa

Departments of Anatomy, ¹Pathology, and ²Laboratory of Clinical Pathology,
Nihon University School of Dentistry at Matsudo, Chiba 271, Japan

(Received for publication June 16, 1989, and in revised form October 4, 1989)

Abstract

The morphology and chemical composition of two subgingival calculus samples, which were composed of magnesium-whitlockite with a Mg/Ca molar ratio of approximately 0.1 as shown by X-ray powder diffraction, were analyzed semi-quantitatively using energy-dispersive spectroscopy (EDS) combined with scanning electron microscopy (SEM). The Ca/P molar ratio values for 33 EDS-analyzed materials ranged from 1.24 to 2.03 with an average of 1.49, and the (Mg+Ca)/P molar ratio values ranged from 1.43 to 2.28 with an average of 1.63. The average Mg/Ca molar ratio was 0.10, a value very close to that obtained by X-ray diffraction analysis.

The EDS-analyzed materials were grouped morphologically into three types; Type A materials were typical rhombohedral crystals, Type B were crystals of ill-defined form or small crystallite aggregations and Type C materials had a small granular structure or were amorphous. The values of (Mg+Ca)/P molar ratio for Type A ranged from 1.57 to 2.28 and averaged 1.81, those for Type B ranged from 1.43 to 1.56 and averaged 1.48, and those for Type C ranged from 1.46 to 2.06 and averaged 1.62. Type A crystals had higher molar ratios while Type B crystallites had values similar to that of whitlockite. Type C materials covered the ranges of both Type A and Type B.

These results show that the materials resembling whitlockite morphologically did not have the (Mg+Ca)/P ratio of whitlockite and *vice versa*. This suggests that whitlockite crystals may substitute some ions for PO₄ as well as Mg for Ca to a larger extent than thought previously, and that care is needed in the identification of these materials.

Key Words: Dental calculus, crystal, morphology, magnesium, whitlockite, scanning electron microscopy, energy-dispersive spectroscopy, X-ray diffraction.

*Address for correspondence:

Toshiro Sakae,
Department of Anatomy,
Nihon University School of Dentistry at Matsudo,
Chiba 271, Japan

Telephone no. 0473-68-6111 Ext. 386

Introduction

The characteristic morphology of crystals and thus the morphological features of materials which include them provides important clues to their identification and chemical composition. The morphology of crystalline materials in dental calculi has been studied by various authors [1, 2, 5, 15, 16, 22]. LeGeros *et al.* [19] observed the following morphology in human dental calculi and attributed the features to crystal components on the basis of the Ca/P molar ratio: i) calcified forms of microorganisms including cocci and rods with a Ca/P ratio close to 1.7; carbonated hydroxyapatite, ii) calcophoritic calcifications and dense calcifications with a Ca/P ratio close to 1.7; carbonated hydroxyapatite, iii) aggregated plates or clusters of platelets, and fan-like aggregations with a Ca/P ratio close to 1.33; octacalcium phosphate or OCP, iv) cuboidal forms of varying sizes with a Ca/P ratio close to 1.4; whitlockite.

In these and other reports, whitlockite in dental calculus was shown to consist of cuboidal or rhombohedral crystals, and octacalcium phosphate and brushite as platy forms, but it seems likely that these crystals may occur in other apparent forms, such as platy and blocky rhombohedral forms of whitlockite [24], or less well defined forms.

Whitlockite, the chemical composition of which may be represented as Ca₁₈Mg₂H₂(PO₄)₁₄ for magnesium-whitlockite [8], has a complicated crystal structure and the potential for substitution of many kinds of ions other than beta-tricalcium phosphate, β -Ca₃(PO₄)₂ [3, 4, 6, 7, 14].

The aim of the present study was to demonstrate variations in the morphology and chemical composition of whitlockite in dental calculus and to draw attention to the analysis of crystal components whose shapes are not well defined.

Materials and Methods

Among dental calculi which were collected at the Dental Hospital at Matsudo, two specimens removed from two male patients aged 40 years and 35 years were used in this study after screening by X-ray diffraction analysis had shown that the dental calculi samples were

composed mainly of whitlockite. The calculi were removed from the lower lingual gingival sulcus (ca. 3 mm in depth) of each person. The samples were labeled DC-15 and DC-16, respectively. These samples were stocked in 10% neutral formaldehyde solution for 24 h, rinsed well with distilled water and dried in air. A part of each sample was pulverized using an agate mortar for X-ray diffraction analysis. The powder was not screened by a mesh because of the small amount of samples. Pieces of dried and unpulverized sample were mounted on Al holders with carbon adhesive tape (STR TAPE, Shinto Chemitron Co. Ltd., Tokyo) and coated with carbon using a TB-500 carbon coater (EMSCOPE, Ashford, England) and a carbon cord filament for scanning electron microscopy (SEM) and energy-dispersive spectrum analysis (EDS). The estimated thickness of the carbon coat was about 48 nm.

X-ray diffraction patterns of the powder samples were obtained using an X-ray diffractometer (Rigaku, Tokyo) after mixing with an internal standard material of KCl powder under the following conditions: X-ray tube voltage, 40 kV; tube current, 20 mA; target, Cu; filter, Ni (wavelength, 0.15418 nm); slit system, 1° -0.3 mm- 1° ; scan speed, 1 degree/min. (2 θ); time constant, 2. Unit cell dimensions of whitlockite were calculated by a least squares method.

SEM observation was carried out by the secondary electron imaging technique using the carbon-coated samples with a JEOL T-200 at an accelerating voltage of 25 kV and working distance of 20 mm. EDS analysis was carried out by point-mode analysis with a JED-2000 (JEOL, Tokyo) attached to the T-200 under the following conditions: X-tilt angle, 30° ; take-off angle, 41.18° ; counting time, 100 s. Atomic percentage was calculated using an AUTO-ZAP program on the basis of the total weight of cationic atoms being equal to 100. Point-mode analysis of synthetic monetite using this system showed 43.60 % wt of phosphorus and 56.40 % wt of calcium, or a Ca/P molar ratio of 1.00, in harmony with the ideal chemical composition of monetite, CaHPO_4 . The results of semi-quantitative EDS analysis are represented as molar ratios of Ca/P, (Mg+Ca)/P and Mg/Ca to avoid the effects of any elements not detected in this system.

In accordance with the aim of this study to clarify the relationship between morphology and chemical compositions of materials in dental calculi, the EDS-analyzed materials were divided into three types according to morphology; Type A included typical rhombohedral crystals, Type B included crystals of ill-defined form or small crystallite aggregations, and Type C included materials with a small granular structure or amorphous forms.

Results

X-ray powder diffraction showed that each dental calculus sample was composed solely of whitlockite as crystalline material and did not show peaks of any other crystalline calcium phosphates (Fig. 1). The peak of whitlockite at about 31 degrees (2 θ) had a shoulder toward

higher angles, suggesting the presence of a minor amount of low-crystalline materials, such as poorly crystallized apatite.

The unit cell dimensions of the whitlockites calculated by a least-squares method are listed in Table 1. The values of the a-axis length, 1.0351 nm for DC-15 and 1.0349 nm for DC-16, are close to the a-axis length of magnesium-whitlockite, 1.0350 nm for $\text{Ca}_{18}\text{Mg}_2\text{H}_2(\text{PO}_4)_{14}$ [8], indicating that the whitlockites in the dental calculi contained magnesium ions with a Mg/Ca molar ratio of approximately 0.1 on average (X-ray diffraction usually gives an averaged value of the sample analyzed).

Figures 2a-2c show the Type A crystals having a characteristic rhombohedral form. The crystals shown in Fig. 2 had relatively higher values of Ca/P and (Mg+Ca)/P molar ratios. The values of Ca/P were 1.86, 2.03 and 1.74, and those of (Mg+Ca)/P were 2.01, 2.28 and 1.95, respectively.

There was a change in the morphology of the materials with changing chemical composition. The lower the Ca/P molar ratio the materials showed, the more ambiguous were their form. Figures 3a and 3b show the Type B materials consisting of aggregations of small crystallites or ill-defined forms. These materials had (Mg+Ca)/P ratios close to that of whitlockite, 1.5. Type C material having a small granular structure or amorphous form is shown in Fig. 3c. This material had a (Mg+Ca)/P molar ratio of 1.53.

Table 2 lists the values of Ca/P, (Mg+Ca)/P and Mg/Ca molar ratios for 33 points in the dental calculi; 10 points for Type A rhombohedral crystals, 11 points for Type B ill-defined crystals or small crystallite aggregations and 12 points for Type C granular structures or amorphous materials, following the morphological classification used in this study. The mean Ca/P and (Mg+Ca)/P molar ratios for the 33 points were 1.49 and 1.63, respectively. The mean value for the Mg/Ca molar ratio was 0.10.

Table 3 shows the mean values of Ca/P and (Mg+Ca)/P molar ratios for the three morphologically grouped types. Type A crystals had a mean value of 1.64 for Ca/P and 1.81 for (Mg+Ca)/P, whereas those for Type B were 1.33 and 1.48, and those for Type C were 1.51 and 1.62, respectively.

Figure 4a shows the distribution of the Ca/P molar ratio values for the three types. The Ca/P molar ratio values of Type A crystals ranged from 1.42 to 2.03, those of Type B from 1.24 to 1.42, and those of Type C from 1.28 to 1.90 (Table 2). Figure 4b shows the distribution of the (Mg+Ca)/P molar ratio values for the three types. The (Mg+Ca)/P molar ratio values of Type A crystals ranged from 1.57 to 2.28 and 1.81, those of Type B from 1.43 to 1.56, and those of Type C from 1.46 to 2.06 (Table 2).

Discussion

In this study, the materials in the dental calculi were grouped into three types on the basis of morphological features, namely Type A having typical rhombohedral forms, Type B having aggregations of small crystallites or ill-defined forms and Type C having small granular

Whitlockite Dental Calculi

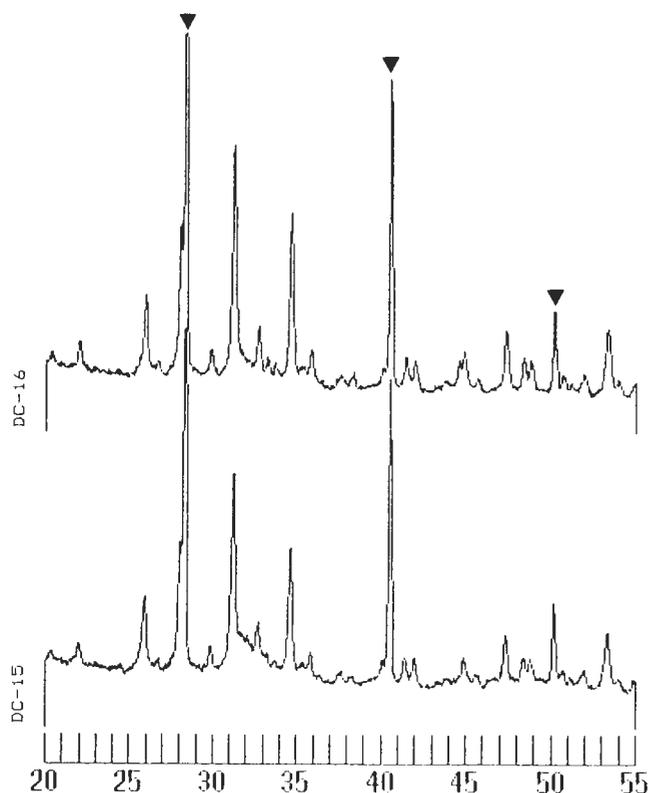


Fig. 1. X-ray diffraction patterns of the dental calculi. Arrow heads indicate the peaks of the internal standard material, KCl. Axis of abscissas represents diffraction angles in 2θ degrees.

structures or amorphous forms. There is a relationship between the morphology and chemical composition of these materials. The distribution of Ca/P molar ratios for the materials indicated that Type A crystals had relatively higher Ca/P values, Type B materials had relatively lower values, and Type C had intermediate values overlapping the ranges of values of the former two.

The Ca/P molar ratios of Type B seemed to resemble at first sight those of brushite or octacalcium phosphate. LeGeros *et al.* [19] classified crystalline components of dental calculus based on semi-quantitative analysis of the Ca/P ratio: close to 1, brushite or DCMP; close to 1.33, octacalcium phosphate or OCP; close to 1.4, whitlockite or β -TCMP; close to 1.7, carbonated hydroxyapatite. However, brushite in sub-gingival calculus is rare [9, 11], and OCP has been observed frequently in supra-gingival calculus [25]. Therefore, it seems better to consider other reasons for the low Ca/P molar ratios of Type B materials in the sub-gingival calculi examined in this study.

Many calcium phosphates can include cations other than calcium in their crystal structures. When magnesium ions were included in the total amount of cations and the molar ratio represented by (Mg+Ca)/P instead of Ca/P, the distributions of the molar ratios of the analyzed materials moved to larger values. Type B materials had the

Table 1. Unit cell dimensions of the whitlockites in the dental calculi.

Sample	a-axis (nm)	c-axis (nm)
DC-15	1.0351(2)	3.724(3)
DC-16	1.0349(2)	3.723(4)
$\text{Ca}_3(\text{PO}_4)_2$ [4]	1.0439	3.7375
$\text{Ca}_{18}\text{Mg}_2\text{H}_2(\text{PO}_4)_{14}$ [8]	1.0350	3.7085
$\text{Ca}_{19}\text{Mg}_2(\text{PO}_4)_{14}$ [12,13]	1.035	3.708

Figures in parentheses are the estimated standard deviations.

Table 2. Ca/P, (Mg+Ca)/P and Mg/Ca molar ratios of the materials.

Analysis No.	Ca/P	(Mg+Ca)/P	Mg/Ca	Type of material*	Sample No.
1	1.24	1.43	0.16	B	DC-16
2	1.26	1.45	0.15	B	DC-16
3	1.27	1.45	0.14	B	DC-16
4	1.28	1.46	0.14	B	DC-16
5	1.28	1.46	0.13	C	DC-16
6	1.30	1.44	0.11	B	DC-15
7	1.33	1.48	0.11	B	DC-16
8	1.33	1.49	0.12	B	DC-16
9	1.34	1.49	0.11	B	DC-16
10	1.36	1.50	0.10	C	DC-16
11	1.38	1.54	0.11	C	DC-16
12	1.41	1.55	0.11	C	DC-15
13	1.41	1.56	0.11	B	DC-16
14	1.42	1.51	0.07	B	DC-16
15	1.42	1.60	0.12	C	DC-15
16	1.42	1.58	0.11	A	DC-15
17	1.44	1.53	0.07	C	DC-16
18	1.44	1.63	0.13	A	DC-15
19	1.46	1.46	0.00	C	DC-16
20	1.47	1.47	0.00	B	DC-16
21	1.48	1.57	0.06	A	DC-16
22	1.49	1.67	0.12	A	DC-15
23	1.57	1.57	0.00	C	DC-16
24	1.58	1.65	0.05	C	DC-16
25	1.60	1.79	0.12	A	DC-15
26	1.62	1.71	0.05	C	DC-15
27	1.63	1.81	0.11	A	DC-15
28	1.68	1.84	0.10	A	DC-15
29	1.69	1.81	0.07	C	DC-15
30	1.74	1.95	0.12	A	DC-15
31	1.86	2.01	0.08	A	DC-15
32	1.90	2.06	0.08	C	DC-15
33	2.03	2.28	0.10	A	DC-15

*: Type A is a crystal showing a typical rhombohedral form; Type B is a crystal of ill-defined form or a crystallite aggregation; Type C is a small granular or amorphous form.

narrowest range of (Mg+Ca)/P molar ratios among the three, and these values were concentrated at around the value of whitlockite, 1.5. On the other hand, Type A crystals having typical rhombohedral forms resembling whitlockite had the widest range of (Mg+Ca)/P values among the three.

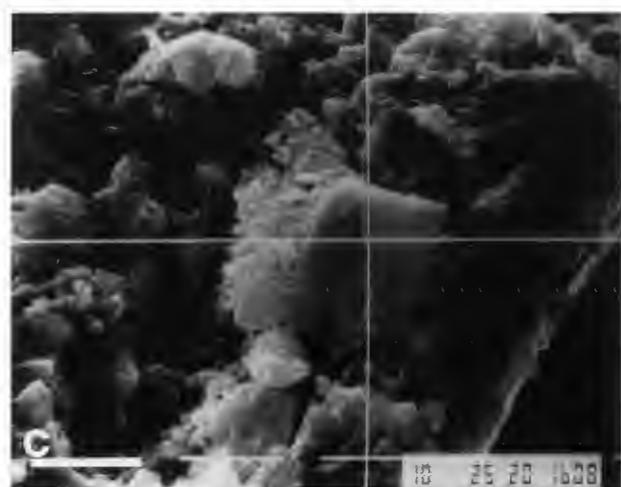
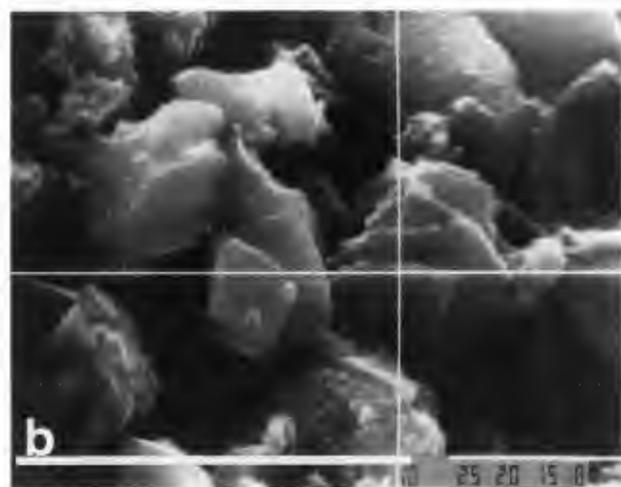


Fig. 2. SEM photographs of the well defined rhombohedral crystals belonging to Type A. (a) typical rhombohedral crystal; analysis No. 31, (b) aggregations of well defined rhombohedral crystals; analysis No. 30, and (c) rhombohedral crystal; analysis No. 21. Bar: 10 μ m.

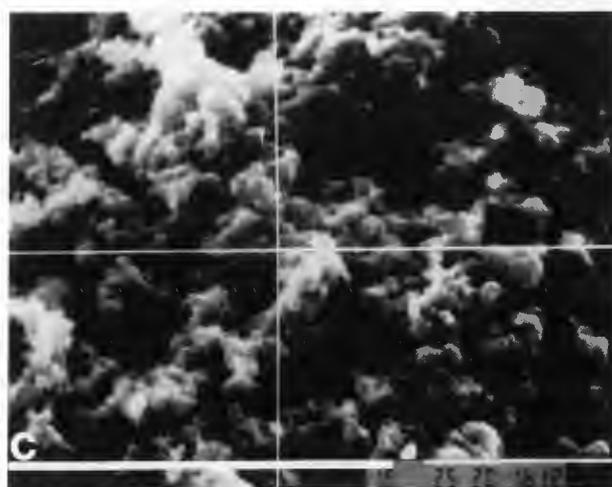
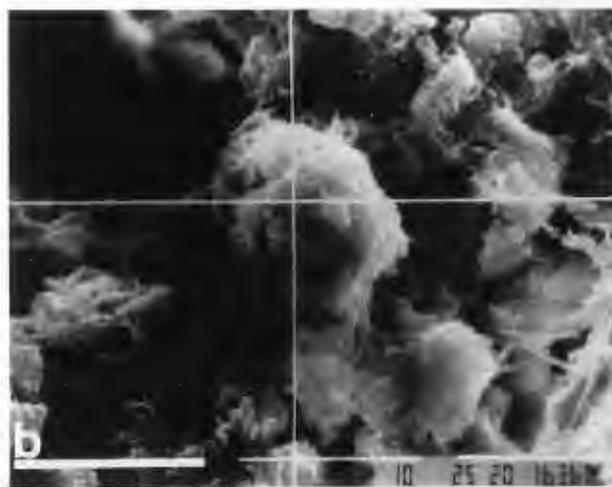
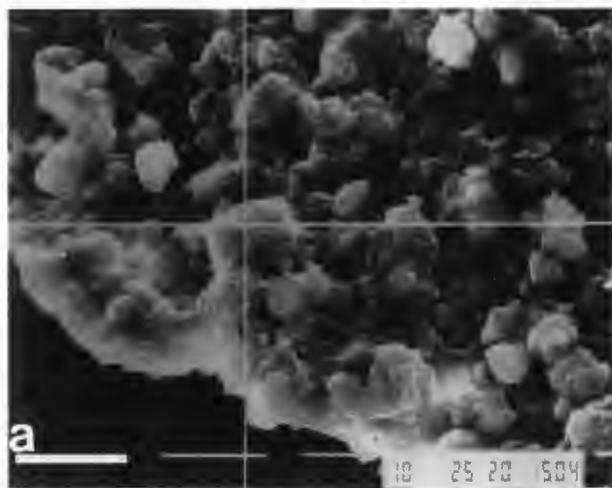


Fig. 3. SEM photographs of the materials belonging to (a, b) Type B and (c) Type C. (a) blocks of ill-defined crystals; analysis No. 6, (b) crystallite aggregation; analysis No. 8, and (c) small granular materials; analysis No. 17. Bar: 10 μ m.

Whitlockite Dental Calculi

These values seemed to suggest that crystals of Type A were apatite rather than whitlockite, despite their apparent morphology.

Although the points for EDS analysis were not selected statistically, it was noted that values for the Mg/Ca molar ratio were very close to those obtained by X-ray diffraction analysis. X-ray diffraction showed that both dental calculus samples were composed of magnesium-whitlockite with a Mg/Ca molar ratio of approximately 0.1. The Mg/Ca molar ratio in biological whitlockites has been reported to be 0.0-0.16 [16, 23, and Table 2 in ref. 17], while Mg can be incorporated in an apatite structure with less than 0.3 % wt, ca. 0.01 Mg/Ca, in a synthetic system [18]. From the results that the Mg/Ca molar ratio for Type C was 0.07 on average and that for Type A was 0.11 on average, and that the X-ray diffraction study did not show a large amount of apatite in the dental calculi, it seemed that the materials in Type C, and also in Type A, were whitlockite rather than apatite.

Keppler [14] cited the work of Franck and Fuldner (1932) in which they showed that the Ca/P molar ratio of whitlockite ranged from 1.5 to 1.9 without any change in structure. It seems that the well defined rhombohedral form of whitlockite in this study showing higher (Mg+Ca)/P molar ratios indicates that some phosphorus ions have been substituted by other ions. These substituting ions are not carbonate, because carbonate ions can inhibit the formation of whitlockite [20, 21] and OCP [10].

In this study it was shown that the crystals showing typical rhomboidal forms did not have a normal Ca/P or (Mg+Ca)/P molar ratio and that materials with a normal Ca/P molar ratio did not show the typical rhomboidal forms. Therefore care is needed in identification, and the variety observed in both the morphology and chemical composition of these materials should be taken into account.

Acknowledgements

The authors thank Dr. Y. Moriwaki and Dr. T. Kani, Asahi University, for their helpful discussions, and the reviewers for their critical comments. Part of the cost of this study was supported by a Scientific Research Foundation Grant from Nihon University (1988-1990).

References

1. Baumhammers A, Conway JC, Saltzberg D, Matta RK (1973). Scanning electron microscopy of supragingival calculus. *J.Periodontol.* **44**:92-95.
2. Bercy P, Frank RM (1980). Microscopie électronique à balayage de la plaque dentaire et du tartre à la surface du cément humain. *J.Biol.Buccale.* **8**:299-313.
3. Calvo C, Gopal R (1975). The crystal structure of whitlockite from the Palermo Quarry. *Amer.Mineral.* **60**:120-133.
4. Dickens B, Schroeder LW, Brown WE (1974). Crystallographic studies of the role of Mg as a stabilizing impurity in β - $\text{Ca}_3(\text{PO}_4)_2$. I. The crystal structure of pure β - $\text{Ca}_3(\text{PO}_4)_2$. *J.Solid State Chem.* **10**:232-248.

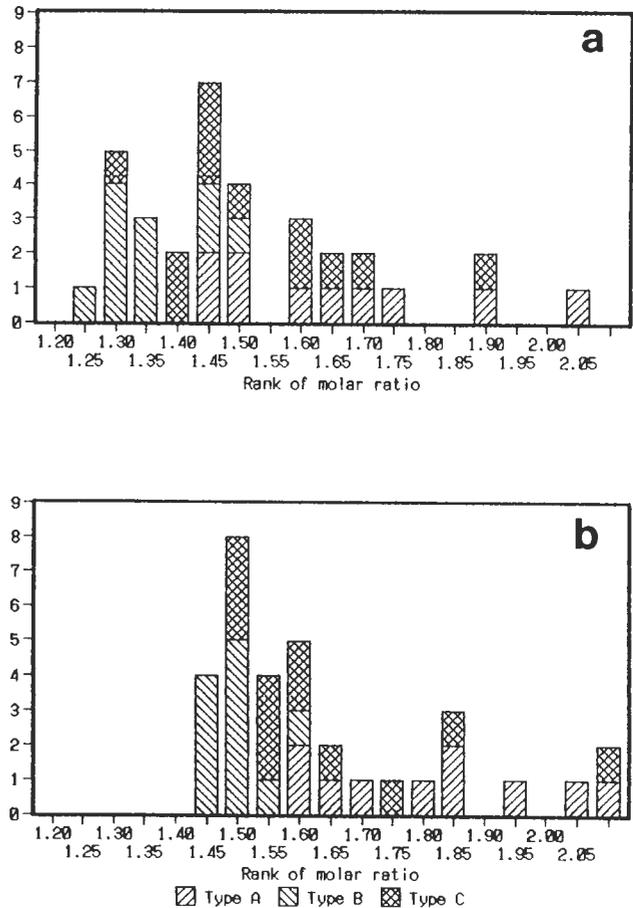


Fig. 4. Distribution of (a) Ca/P molar ratios and (b) (Mg+Ca)/P molar ratios of the 33 materials in the dental calculi. Materials of Type A are the rhombohedral crystals, those of Type B are crystallite aggregations and those of Type C are granules or amorphous materials.

Table 3.
Values of mean molar ratio for the three types.

Type	n	Ca/P	(Mg+Ca)/P	Mg/Ca
A	10	1.64 (0.19)	1.81 (0.21)	0.11 (0.02)
B	11	1.33 (0.07)	1.48 (0.04)	0.11 (0.04)
C	12	1.51 (0.16)	1.62 (0.17)	0.07 (0.05)
Total	33	1.49 (0.19)	1.63 (0.20)	0.10 (0.04)

Figures in parentheses are standard deviations.

5. Friskopp J, Hammarström L (1980). A comparative scanning electron microscopic study of supragingival and subgingival calculus. *J.Periodontol.* **51**:553-562.

6. Frondel C (1941). Whitlockite, a new calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$. *Amer. Mineral.* **26**:145-152.

7. Gopal R, Calvo C (1972). Structural relationship of whitlockite and β $\text{Ca}_3(\text{PO}_4)_2$, *Nature Physical Science*, 237:30-32.

8. Gopal R, Calvo C, Ito J, Sabine WK (1974). Crystal structure of synthetic Mg-whitlockite, $\text{Ca}_{18}\text{Mg}_2\text{H}_2(\text{PO}_4)_{14}$. *Can.J.Chem.* 52:1155-1164.

9. Grøn P, van Campen GJ, Lindstrom I (1967). Human dental calculus. Inorganic chemical and crystallographic composition. *Archs oral Biol.* 12:829-837.

10. Iijima M, Moriwaki Y (1988). Effect of CO_3 , Mg, and F ions on the crystal growth of OCP on cation selective membrane - in relation to enamel formation, 5th Int. Magnesium Symp. Abstr. F-9-11.

11. Jensen AT, Danø M (1954). Crystallography of dental calculus and the precipitation of certain calcium phosphates. *J.Dent.Res.* 33:741-750.

12. Jensen AT, Rowles SL (1957). Lattice constants and magnesium contents of some naturally occurring whitlockites. *Nature*, 179:912-913.

13. Jensen AT, Rowles SL (1957). Magnesium whitlockite, a major constituent of dental calculus. *Acta Odont.Scand.* 16:121-139.

14. Keppler U (1968). Structural investigation of "calciumphosphate" and isotypic structures. *Bull.Soc.Chim.France*, 1968:1774-1777.

15. Kérebél B (1971). Apports du microscope électronique à balayage à l'histologie et à la pathologie dentaires, *Actualités Odonto-Stomatol.* 96:449-472.

16. Knuuttila M, Lappalainen R, Kontturi-Närhi V (1980). Effect of Zn and Mg on the formation of whitlockite in human subgingival calculus. *Scand.J.Dent.Res.* 88:513-516.

17. Kodaka T, Debari K, Higashi S (1988). Magnesium-containing crystals in human dental calculus. *J.Electron.Microsc.* 37:73-80.

18. LeGeros RZ (1984) Incorporation of magnesium in synthetic and in biological apatites. In : *Tooth Enamel IV*, Fearnhead RW, Suga S (Eds.). Elsevier, Amsterdam. pp.32-36.

19. LeGeros RZ, Orly I, LeGeros JP, Gomez C, Kazimiroff J, Tarpley T, Kerebel B (1988). Scanning electron microscopy and electron probe microanalyses of the crystalline components of human and animal dental calculi. *Scanning Microsc.* 2:345-356.

20. LeGeros RZ, Kijkowska R, Abergas T, Orly I (1988). The role of solution Mg/Ca on the in vitro formation of calcium phosphates, 5th Int. Magnesium Symp. Abstr. F-9-1.

21. LeGeros RZ, Daculsi G, Kijkowska R, Kerebel B (1988). The effect of magnesium on the formation of biological and synthetic apatites and whitlockites, 5th Int. Magnesium Symp. Abstr. S-9-8.

22. Lustmann J, Lewin-Epstein J, Shteyer A (1976). Scanning electron microscopy of dental calculus. *Calcif.Tissue Res.* 21:47-55.

23. Molokhia A, Nixon G (1984). Studies on the composition of human dental calculus, *J. Radioanalytical and Nuclear Chem.* 83:273-281.

24. Palache C, Berman H, Frondel C (1951). *Dana's System of Mineralogy*. 7th ed., Vol. II. John Wiley and Sons, New York. pp. 684-686.

25. Sundberg M, Friskopp J (1985). Crystallography of supragingival and subgingival human dental calculus. *Scand.J.Dent.Res.* 93:30-38.

Discussion with Reviewers

T.Kodaka: High voltage of 25 kV used in this methods analyzes wider and deeper area than the low voltage of 10 kV or 15 kV. It is usually said that human mature enamel is analyzed in about 5 - 10 μm diameter under conditions of 15 kV by EDX. The 25 kV data probably contain other crystals in the surrounding area and in the deeper layer.

Authors: Bolon RB (1979; X-ray spectral artifacts encountered in the SEM and TEM. In: *Microbeam Analysis in Biology*, Lechene CP, Warner RR (Eds.). Academic Press, New York. pp.39-52.) calculated the diffusion range of electron as 4.63 μm for Al with an accelerating voltage of 30 kV according to the Reed range equation. Using the same equation, we can calculate the diffusion range of a material having density of 3.0, which is nearly equal to the density of apatite or whitlockite, as to be about 6 μm . The X-ray excitation range is always smaller than the electron diffusion range. Therefore, we estimated that the X-ray signals were come from the analyzed materials, especially in the cases shown in Fig. 2. The case of matrix materials as shown in Fig. 3 had no serious problem, because the analysis data were fundamentally averaged ones.

T.Kodaka: The lower atomic number such as P element is uncompletely detected at the high voltage as compared with Ca element, so that the P concentration is lower than that of the low voltages although the Ca concentration is almost the same. Thus, the Ca/P molar ratio will be higher than that of chemical analysis.

Authors: The EDS data analysis system employed in this study was so well fitted to the experimental conditions including the accelerating voltage of 25 kV that the obtained Ca/P molar ratio of CaHPO_4 was 1.00 as mentioned in the text.