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BACILLUS-SHAPED DEPOSITS COMPOSED OF HEXAHEDRALLY BASED CRYSTALS IN HUMAN DENTAL CALCULUS

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Abstract

In human supra- and subgingival calculus, bacillus-shaped deposits showing various rocky-pile forms composed of hexahedrally based crystals were observed by scanning electron microscopy. The crystal size measured approximately 0.1 - 1.5 μm. The electron probe microanalysis always detected calcium, phosphorous, and magnesium. Their molar ratios resembled those of magnesium-containing whitlockite and moreover the crystals also gave the electron diffraction pattern of whitlockite. The bacillus-shaped deposits happened to coexist with the intracellular calcifying microorganisms, furthermore, oral microorganisms partially replaced by the hexahedrally based crystals were found. The crystal deposits were never seen in the surface layers of calculus exposed to the oral cavity, but occurred in the innermost layers and intra-spaces of supragingival and ledge-type subgingival calculus and in the outer layers of deep subgingival calculus.

KEY WORDS: dental calculus, scanning electron microscope, bacillus-shaped deposits, oral microorganisms, hexahedral crystals, electron probe microanalysis, magnesium content, molar ratios, electron diffraction, whitlockite.

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Introduction

Since it was found that Bacterionema matruchotii, an oral microorganism, calcified by the deposition of hydroxyapatite under suitable medium conditions [Ennever, 1960], many studies on the intracellular calcification of oral microorganisms have been reported. The inorganic crystals were needle-shaped hydroxyapatite in Streptcoccus salivarius [Rizzo et al., 1962] and in B. matruchotii [Takazoe et al., 1963; Ennever and Creamer, 1967; Sidaway, 1980]. The needle-shaped hydroxyapatite was also found in Actinomyces viscosus, Veillonella aclalescens, Eikenella corrodens, and Haemophilus parainfluenzae [Sidaway, 1980]; and many other species of microorganisms isolated from human dental calculus calcified themselves [Rizzo et al., 1963; Sidaway, 1978, 1979].

However, it was found that the intracellular calcifying microorganisms of B. matruchotii contained magnesium-containing whitlockite as well as hydroxyapatite when the calcifying medium contained calcium and magnesium [Killian and Ennever, 1975; Boyan-Salyers et al., 1978].

It is widely accepted that whitlockite is one of main inorganic constituents in human dental calculus [Jensen and Rowles, 1957; Rowles, 1964; Schroeder and Bambauer, 1966], and is most abundant in subgingival calculus [Jensen and Danø, 1954; Grøn et al., 1967; Kani et al., 1983; Sundberg and Friskopp, 1985], although Ruzicka [1984] reported that characteristic diffraction rings of whitlockite were never observed when intra- and extracellular calcification of subgingival calculus was examined by electron diffraction.

Rock whitlockite, which randomly distributed magnesium in the lattice, takes a hexagonal system [Frondel, 1941] and shows rhombohedral crystals in outline [Frondel, 1943; Keppler, 1965; Calvo and Gopal, 1975]. Synthetic crystals containing magnesium showed a rhombohedral [Newesely, 1965] or cuboidal form [LeGeros et al., 1988]. Biological magnesium-containing whitlockite showed pseudocubic formations or groupings of quadrangular blades cubically arranged in renal calculus [Santos and González-Díaz, 1980] and cuboidal crystals in dental calculus [LeGeros et al., 1988].

In our previous study of human supra- and subgingival (marginal) calculus using scanning electron microscopy and electron probe microanalysis [Kodaka et al., 1988], various hexahedrally based crystals were identified as magnesium-containing whitlockite that conformed in shape with their magnesium content and molar ratios of calcium, phosphorous, and magnesium [Jensen and Rowles, 1957; Keppler, 1965; Calvo and Gopal, 1975]; moreover, bacillus-shaped calcified deposits, a characteristic form in some aggregations of the hexahedrally based crystals, were sometimes found in the innermost calculus layers and in the intra-spaces of calculus deposits. These calcified deposits also took the form of large or small masses and even board-like aggregations. In the present study relationships between the bacillus-shaped deposits and oral microorganisms in human supra- and subgingival calculus have been investigated by electron microscopy.

Materials and Methods

Fifty human lower incisors with attached supragingival and/or subgingival calculus were extracted because of periodontal disease. Subgingival calculus used in this study was ledge-type deposits along the gingival margins and spiny deposits attached to the root surfaces in deep gingival pockets [Everett and Potter, 1959]. They were fixed in 10 % formaldehyde at pH 6.5 for about one month. This was followed by washing under running water for 30 minutes and by drying in the air for one day. In white supragingival calculus connected with a small amount of subgingival deposits generally coloured in gray-green, the small deposits were previously removed and excluded. Each piece of calculus was removed from the tooth and fractured into several pieces. These pieces were used for observations in scanning electron and transmission electron microscopes (SEM and TEM).

Half of the calculus pieces were treated with about 8 % sodium hypochlorite (NaOCl) for about one hour in order to remove unmineralized microorganisms and other organic debris [Holma et al., 1970], and subsequently rinsed in running water for one hour to remove NaOCl-remnants which might crystallize in dried specimens. The pieces were dehydrated with alcohol and dried in the air. The remaining pieces were untreated. All were coated with carbon. The structures of bacillus-shaped deposits were observed and at the same time the elements were determined qualitatively by the Hitachi X-560 SEM fitted with a Kevex 7000Q energy dispersive detection system (SEM-EDX).

Each aggregation of microorganisms, observed in natural and fractured surfaces of supragingival calculus untreated with NaOCl, was analysed quantitatively at 10 points by the SEM-EDX. One of the aggregations of bacillus-shaped deposits untreated with NaOCl clearly showing its structure was also quantitatively analysed. The microprobe conditions were 15 kV accelerating voltage and 1×10^{-10} A specimen current. The standard samples were native fluorapatite, magnesium oxide (MgO), and cadmium sulphide (CdS). All the specimens, both treated and untreated with NaOCl, were coated with a 7 - 10 nm thick layer of platinum. They were photographed in a Hitachi S-430 SEM or a Hitachi S-700 field emission type SEM (FE-SEM).

The pieces of deep subgingival calculus of spiny deposits without NaOCl treatment were observed in the SEM after coating with carbon and then the undecalcified specimens containing aggregations of bacillus-shaped deposits were used for TEM studies. Unstained and carbon coated ultra-thin sections measuring about 50 - 60 nm thick were prepared. The diffraction patterns of bacillus-shaped deposits were investigated in a Hitachi H-600 TEM. Identificaton of the lattice planes was carried out using the ASTM card-index. By the Hitachi H-600 TEM fitted with a Kevex 7000 EDX (TEM-EDX) the calcification values were quantitatively measured under the conditions of 100 kV accelerating voltage and 1×10^{-10} A specimen current. The standard samples were native fluorapatite and magnesium oxide.

Results

Hexahedrally based crystals in supragingival and subgingival (marginal) ledge-type calculus of human permanent teeth were frequently observed at the interface between calculus and teeth, and in the intra-spaces of calculus deposits including extra-, intracellular calcifying deposits, and elongated

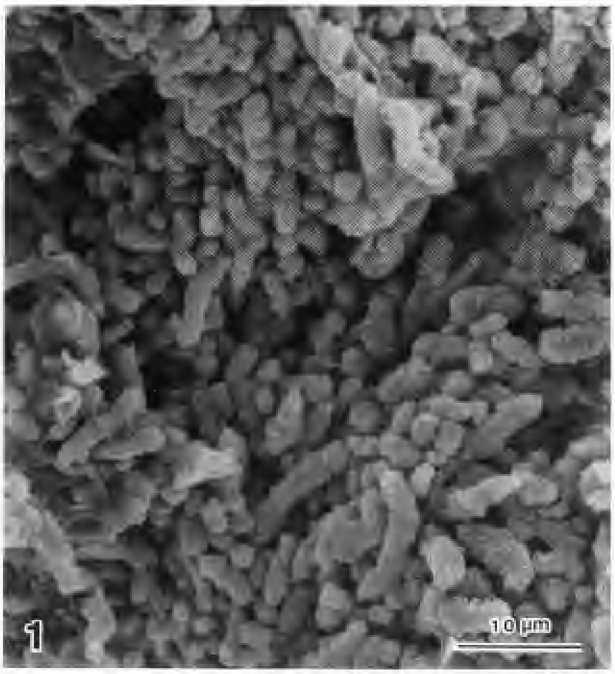


Fig. 1. Low magnified SEM image of bacillus-shaped deposits in an inner surface layer of marginal ledge-type calculus. Untreated with NaOCl.

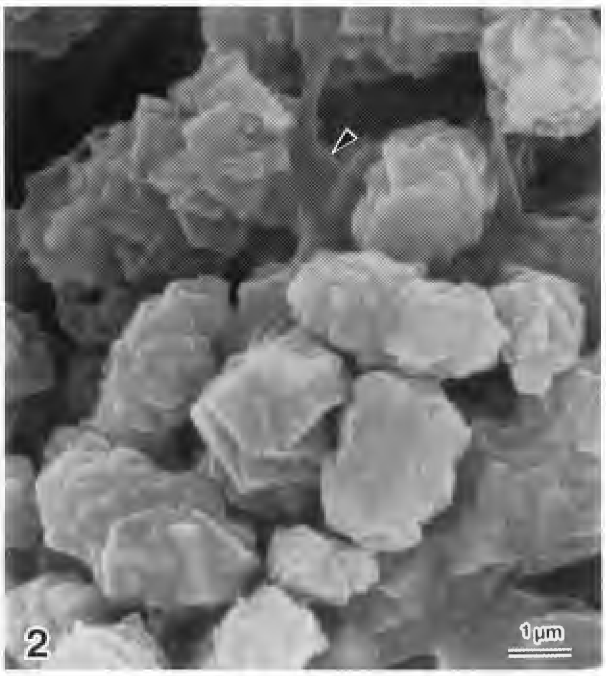


Fig. 2. Part as shown in Figure 1. Rocky-pile form composed of hexahedrally based crystals with traces of amorphous substance (arrowhead). Untreated with NaOCl.

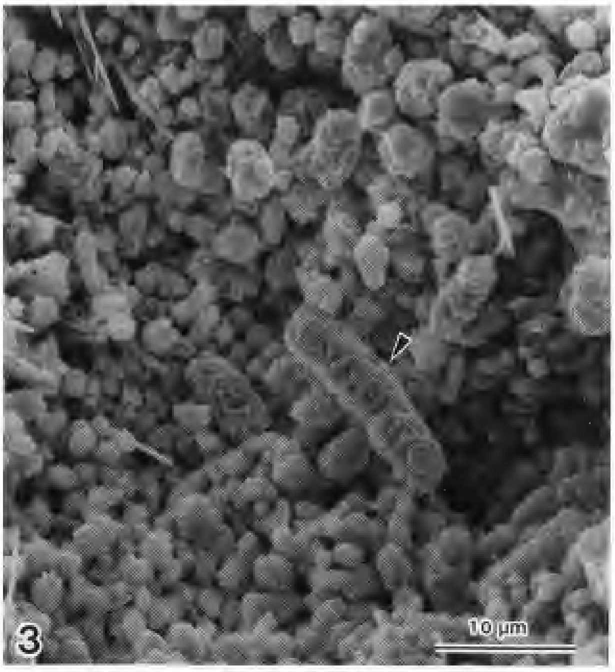


Fig. 3. Bacillus-shaped deposits clearly showing a rocky-pile form in an inner surface layer of supragingival calculus. Untreated with NaOCl and analysed by the SEM-EDX (arrowhead; Table 1).

ribbon-like crystals. In these spaces bacillus-shaped deposits were sometimes seen under a low magnified SEM (Fig. 1). At the higher magnification of a part shown in Figure 1 the bacillus-shaped deposits were observed as a rocky-pile form composed of hexahedrally based crystals which thronged and fused with each other, although traces of amorphous substance were often attached to the surfaces (Fig. 2).

In some specimens, the bacillus-shaped deposits more clearly illustrated a rocky-pile form (Fig. 3) as well as the NaOCl-treated deposits (Fig. 4). The specimen of Figure 4 was one untreated piece out of several pieces obtained from the same calculus already observed in Figures 1 and 2. These crystal sizes measured approximately 0.1 - 1.5 µm in length. The rocky-pile shaped deposits showed various shapes and sizes in different sites although they basically took rod-like and filamentous outlines. Although the bacillus-shaped deposits were observed in the innermost layers and intra-spaces of supragingival (Figs. 3, 9, and 10) and marginal ledge-type calculus (Figs. 1, 2, 4, and 11 - 13), they were not found in the outermost layers exposed to the oral cavity.

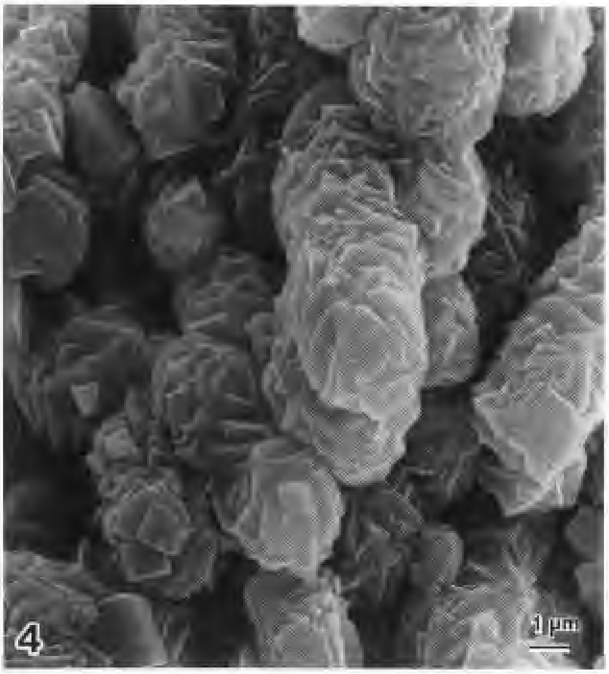


Fig. 4. Bacillus-shaped deposits showing a rocky-pile form treated with NaOCl in one piece obtained from the calculus of Figures 1 and 2.

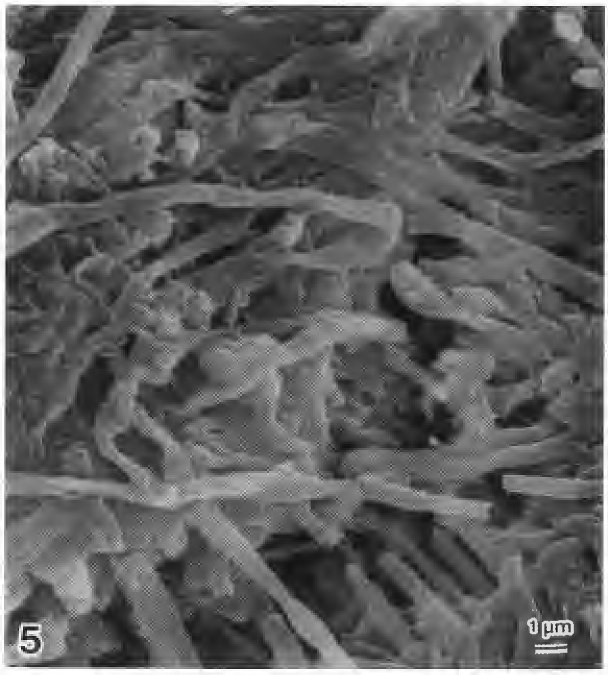


Fig. 5. Natural surface containing stunted and teared filamentous microorganisms in supragingival calculus. Untreated with NaOCl and analysed by the SEM-EDX.

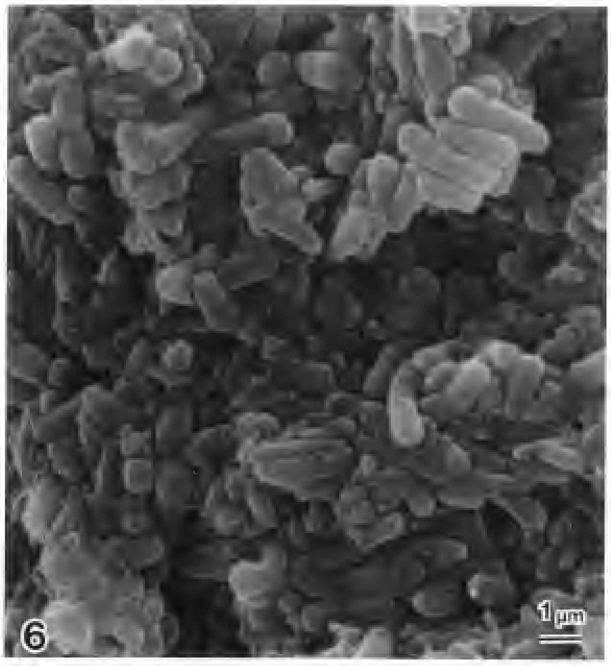


Fig. 6. Aggregation of rod-shaped microorganisms maintaining a solid structure in a fractured surface of supragingival calculus. Untreated with NaOCl and analysed by the SEM-EDX.

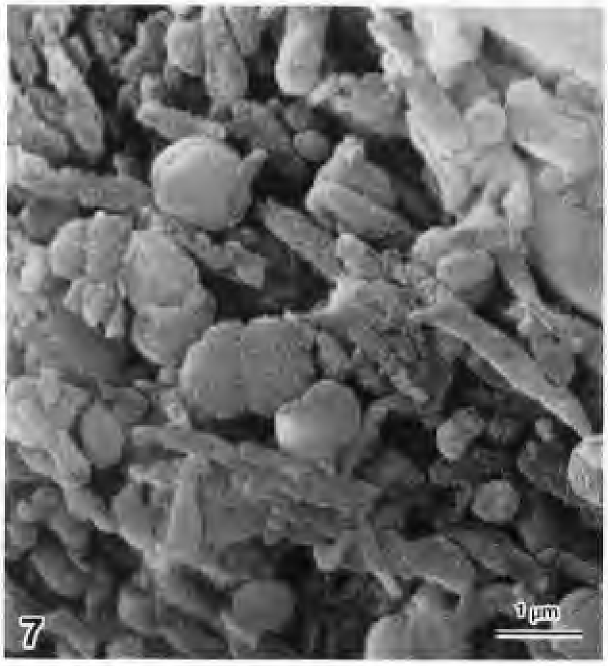


Fig. 7. NaOCl-undissolved microorganisms in a fractured surface of subgingival (marginal) calculus. Treated with NaOCl.

Fig. 8. FE-SEM image of a part of a NaOCl-undissolved microorganism. Arrowheads show minute hexahedrally based or pseudocuboidal crystals in a fractured surface of supragingival calculus. Treated with NaOCl.

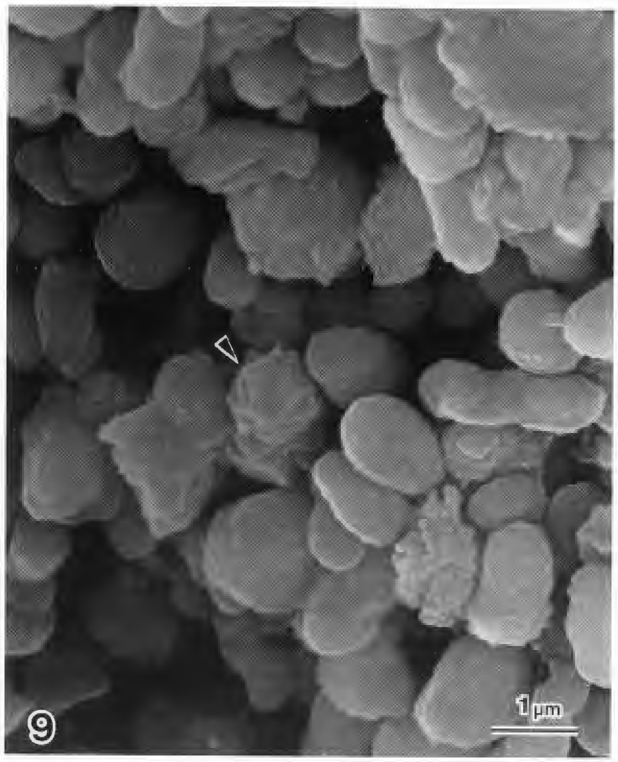


Fig. 9. Aggregation of NaOCl-undissolved microorganisms contaminated by a rocky-pile shaped microorganism (arrowhead) in a fractured surface of supragingival calculus. Treated with NaOCl.

However, in the regions of marginal ledge-type calculus exposed to the gingival pockets and in the outer layers of deep subgingival calculus of spiny deposits (Figs. 14 - 16), the bacillus-shaped deposits were frequently observed.

These deposits were morphologically distinguished from various microorganisms in supragingival (Figs. 5 and 6) and marginal ledge-type calculus (Fig. 7). The specimens of Figures 5 and 6 were not followed by the procedure of NaOCl-treatment and critical point drying with CO_2. When these microorganisms were analysed by the SEM-EDX, calcium (Ca) and phosphorous (P) were always detected. In Figure 6; a fractured mass including solid rod-shaped microorganisms, the Ca and P concentrations were 31.46±1.21 and 14.95±0.62 weight %, respectively. The Ca/P molar ratio was 1.63±0.02. In Figure 5; a natural surface layer containing stunted and teared filamentous microorganisms, sulphur (S) was also detected. The concentrations of Ca, P, and S were 3.16±3.54, 1.92±2.15, 0.55±0.19 weight %, respectively. The Ca/P molar ratio was 0.90±0.32.

Microorganisms maintaining a solid structure shown in Figure 6 possessed smooth surfaces. The surfaces of microorganisms undissolved by NaOCl showed numerous granular (Fig. 7) or fine needle-like structure under a high resolution FE-SEM (Fig. 8). In some cases, they coexisted with minute hexahedrally based or pseudocuboidal crystals attached to them (Fig. 8, arrowheads). These crystals measured approximately 0.1 - 0.3 μm in length.

A careful investigation of a number of calculus pieces treated with NaOCl under the SEM revealed a rocky-pile shaped microorganism in aggregations of NaOCl-undissolved microorganisms possessing smooth or granular surfaces (Fig. 9, arrowhead). Their diameters were approximately similar to each other. The hexahedrally based crystals of rocky-pile shaped microorganisms measured about 0.3 - 0.5 μm in length, whereas some of the bacillus-shaped deposits coexisted with NaOCl-undissolved microorganisms of a filamentous or long rod-like form with microvillus surfaces of almost the same

Fig. 10. Co-existing of a NaOCl-undissolved microorganism and bacillus-shaped deposits in a fractured surface of supragingival calculus. Treated with NaOCl.

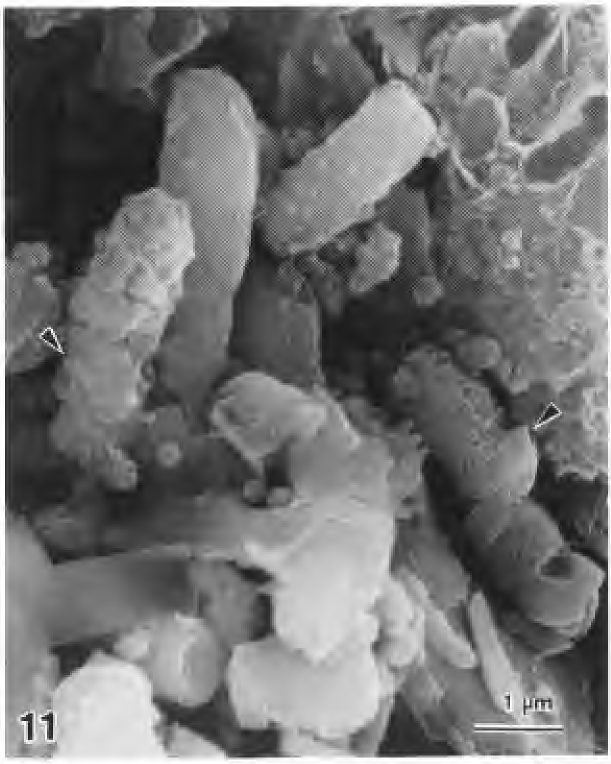
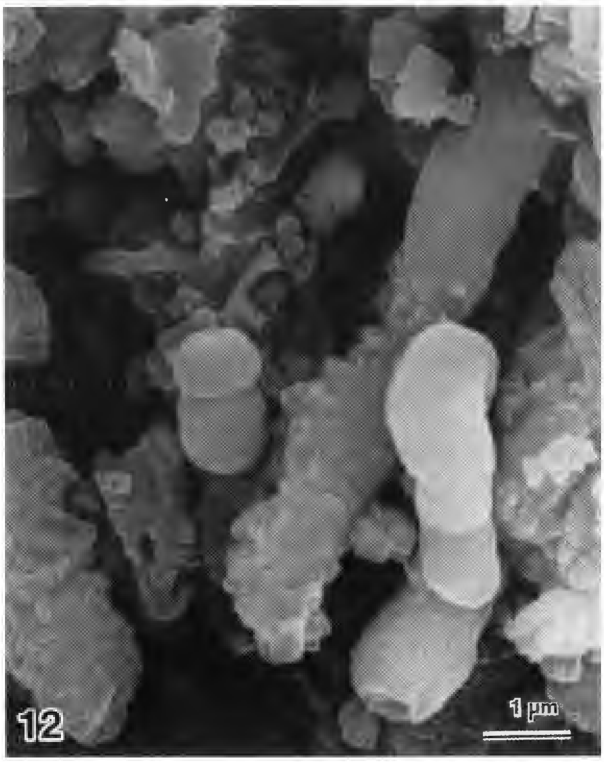


Fig. 11. Hexahedrally based crystals in NaOCl-undissolved microorganisms in a fractured surface of subgingival (marginal) calculus. Arrowheads show the crystals. Treated with NaOCl.



size (Fig. 10). The hexahedrally based crystals measured approximately 0.3 - 1.0 µm in length. Moreover, hexahedrally based crystals fused with the granular surfaces of NaOCl-undissolved microorganisms of a rod-like form that surrounded them were observed (Fig. 11, arrowheads). These crystals measured approximately 0.2 - 0.5 µm in length.

In some specimens, filamentous microorganisms, probably in a semi-removal state with NaOCl, were partially replaced by hexahedrally based crystals (Fig. 12). The crystal size was approximately 0.1 - 0.4 µm in length. Figure 13 is the higher magnified FE-SEM image of a rocky-pile shaped microorganism similar to that of Figure 12. The minute pseudocuboidal crystals measuring approximately 0.1 - 0.3 µm in length were clearly visible.

Fig. 12. Filamentous microorganism partially replaced by minute hexahedrally based crystals in a fractured surface of subgingival (marginal) calculus. Treated with NaOCl.

Fig. 13. FE-SEM image of a part of a rocky-pile shaped microorganism similar to that of Figure 12. Treated with NaOCl.

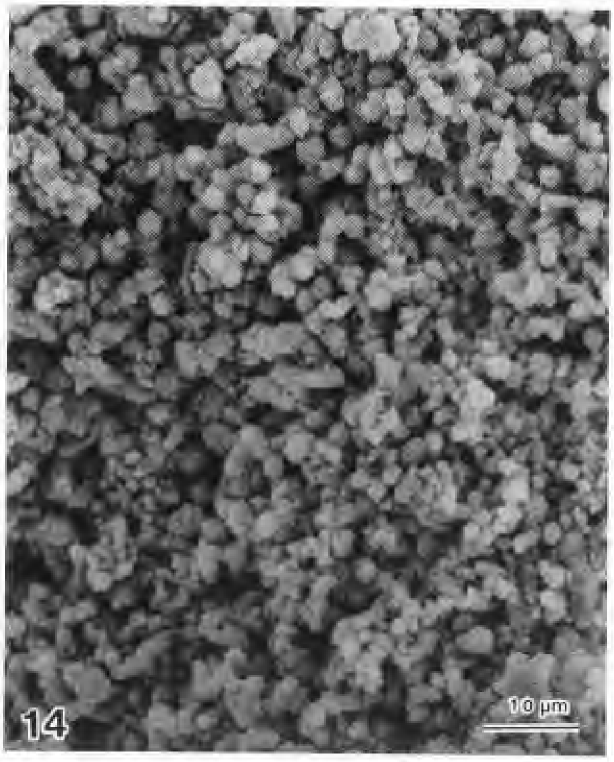


Fig. 14. Aggregation of bacillus-shaped deposits in an outer surface layer of deep subgingival calculus. Treated with NaOCl.

For the purpose of the following TEM studies, aggregations of the bacillus-shaped deposits were confirmed by the SEM in the pieces of deep subgingival calculus of spiny deposits untreated with NaOCl and coated with carbon. Some of the pieces were treated with NaOCl and then observed by the SEM (Figs. 14 and 15). The surface calculus layers were more or less occupied by the bacillus-shaped deposits (Fig. 14), although the crystal shape showed a somewhat rhombic modification measuring about 0.5 - 1.0 μm (Fig. 15, arrowheads). The undecalcified ultrathin sections were observed as a link of several rhombohedrally based crystals measuring approximately 0.05 - 0.2 μm in length (Fig. 16, arrowheads). The

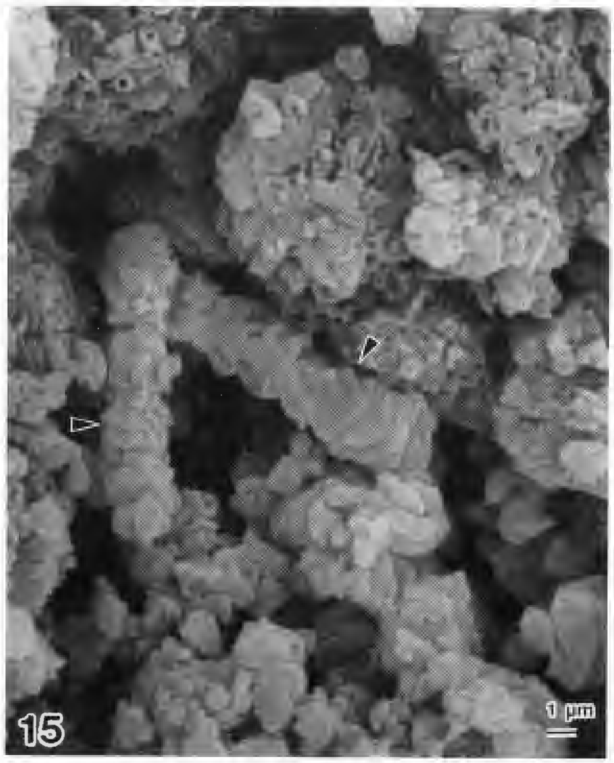


Fig. 15. Bacillus-shaped deposits (arrowheads) composed of rhombic crystals in an outer surface layer of deep subgingival calculus. Treated with NaOCl.

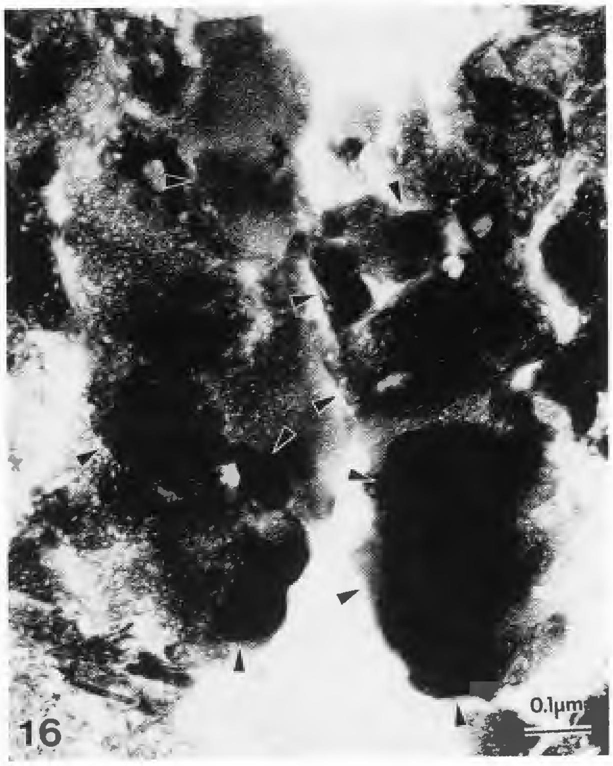


Fig. 16. TEM image of bacillus-shaped deposits showing several rhombohedrally based crystals (arrowheads) in deep subgingival calculus. One crystal similar to them was analysed by the SEM-EDX (Table 1).

Table 1. Comparison between Mg-containing whitlockite and bacillus-shaped deposits

	Mg-whitlockite	Bacillus-like deposits SEM-EDX analysis (***)	Bacillus-like deposits TEM-EDX analysis
Mg weight%	1.9*	3.05 (2.75±0.44)	2.59
Molar Ca/P	1.29-1.43**	1.29 (1.28±0.03)	1.38
Molar Ca+Mg/P	1.43-1.50**	1.50 (1.48±0.02)	1.54
Molar Mg/Ca	0-0.11**	0.14 (0.18±0.02)	0.12

*: Jensen and Rowles (1957)
**: Keppler (1965);
Calvo and Gopal (1975)
(***): Kodaka et al. (1988)

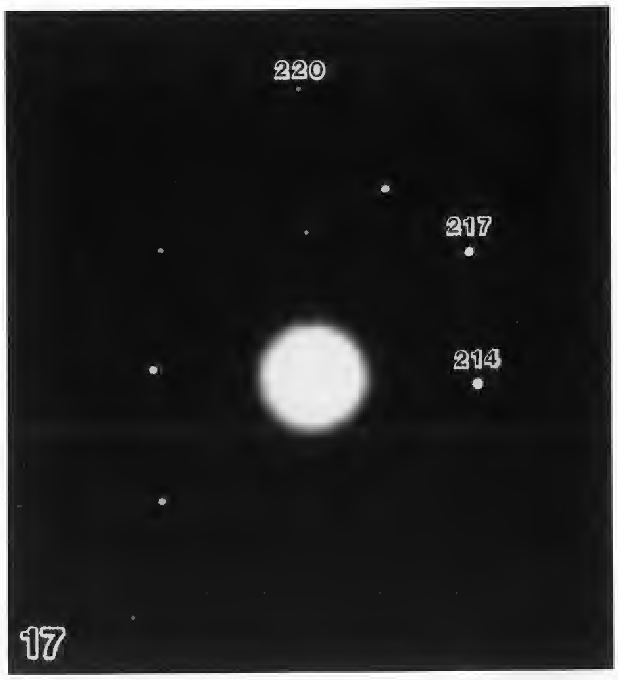


Fig. 17. Electron diffraction pattern of whitlockite in a rhombohedrally based crystal similar to those of Figure 14. The [214], [217], and [220] planes are clearly seen.

electron diffraction pattern of a crystal similar to those of Figure 16 showed the [214], [217], and [220] planes (Fig. 17). Thus, the rhombohedrally based crystals were identified as whitlockite.

When hexahedrally based crystals of the bacillus-shaped deposits (Figs. 1 - 4, 9 - 15) were qualitatively analysed by the SEM-EDX, magnesium (Mg) as well as calcium (Ca) and phosphorus (P) was always detected. The SEM images of Figures 1 and 2 were from the sample which had been quantitatively analysed in our previous SEM-EDX study [Kodaka et al., 1988]. The data are shown in Table 1. In the SEM-EDX analysis of the bacillus-shaped deposits shown in Figure 3 (arrowhead), the Ca, P, and Mg concentrations showed 30.68, 18.53, and 3.05 weight %, respectively. The Ca/P molar ratio was 1.29, the Ca+Mg/P was 1.50, and the Mg/Ca was 0.14 (Table 1).

Under quantitative analysis of the TEM-EDX, the Ca, P, and Mg concentrations of a rhombohedrally based crystal similar to those of Figure 15 showed 35.65, 19.98, and 2.59 weight%, respectively. The Ca/P molar ratio was 1.38, the Ca+Mg/P was 1.54, and the Mg/Ca was 0.12 (Table 1).

Discussion

It is known that sodium hypochlorite (NaOCl) removes organic substances such as pellicle and plaque attached to dental enamel, but calcified materials such as cabonate-hydroxyapatite and enamel crystals are morphologically unaltered [Holma et al., 1970; Fejerskov et al., 1984]. In human dental calculus, Lustmann et al., [1976], Nakagawa [1981], and Kodaka et al. [1988] observed the inorganic structures using SEM with NaOCl-treatment. In the present study the bacillus-shaped deposits composed of hexahedrally based crystals found in human dental calculus were unaltered in shape and size following NaOCl-treatment, although traces of amorphous substance was removed from the crystal surfaces (Figs. 2 and 4). Hence, calculus deposits undissolved by NaOCl should be recognized as fully calcified materials in dental calculus.

It has been reported that the intracellular calcifying microorganisms have main constituents of fine needle-shaped hydroxyapatite [Rizzo et al., 1962; Takazoe et al., 1963; Ennever and Creamer, 1967; Sidaway, 1980; Ruzicka, 1984]. In the SEM images calcifying microorganisms undissolved by NaOCl were composed of fine sandy-grain crystals [Nakagawa, 1981] and their surfaces showed a smooth, fine granular [Lustmann et al., 1976], microvillus, or needle-like structure (Figs. 6-10). Hence, these microorganisms undissolved by NaOCl will be the intracellular calcifying microorganisms.

In specimens untreated with NaOCl and dried in the air, the aggregation of microorganisms maintaining a solid structure (Fig. 6) showed a large amount of Ca and P; the Ca/P molar ratio of 1.63±0.02 was similar to that of 1.67 in hydroxyapatite [Rowles, 1964; Schroeder and Bambauer, 1966]. On the other hand, the natural surface layer of supragingival calculus containing stunted and teared microorganisms (Fig. 5) showed a small amount of Ca and P; moreover, sulphur (S) was also detected. It means that the lower concentrations of Ca and P in the samples depicted in Figure 5 are detected either from the underlying calcified layer or the aggregation of microorganisms itself; however, the detection of sulphur suggests that the microorganisms are a main constituent of dental plaque which is scarcely calcified. Judging from the above-mentioned discussion, microorganisms maintaining a solid structure as shown in Figure 6 are probably the intracellular calcifying microorganism.

In our previous study [Kodaka et al., 1988], hexahedral calcium phosphate crystals containing magnesium (Mg) in human dental calculus were identified as calculus whitlockite in shape with Mg content and molar ratios of Ca, P, and Mg [Frondel, 1941, 1943; Jensen and Rowles, 1957; Keppler, 1965; Newesely, 1965; LeGeros et al., 1973; Calvo and Gopal, 1975; Santos and González-Díaz, 1980]. The crystal size measured approximately 0.1 - 3 µm in length. In the present study hexahedrally based crystals forming bacillus-shaped deposits measured approximately 0.1 - 1.5 µm in length.

As can be seen in Table 1, Jensen and Rowles [1957] reported that calculus whitlockite contained about 1.9 weight % magnesium, whereas in rock whitlockite Keppler [1965] and Calvo and Gopal [1975] revealed that the Ca/P molar ratio was 1.29 - 1.43, the Ca+Mg/P was 1.43 - 1.50, and the Mg/Ca was 0 - 0.11. Recently, LeGeros et al. [1988] reported that synthetic Mg-substituted whitlockite showed a cuboidal form with the Ca/P molar ratio of 1.26 - 1.47, and that in human dental calculus cuboidal crystals showing the Ca/P molar ratio of 1.41 - 1.57 were identified as Mg-substituted whitlockite by electron probe microanalysis (EDX).

The bacillus-shaped deposits shown in Figures 1 and 2 had already been analysed in the previous SEM-EDX study [Kodaka et al., 1988]. According to the SEM-EDX data (Table 1), the mean of the Mg concentrations was 2.75 weight %, and the means of the Ca/P, Ca+Mg/P, and Mg/Ca molar ratios were 1.28, 1.48, and 0.16, respectively. In this study the SEM-EDX data of the bacillus-shaped deposits shown in Figure 3 (Table 1) were analogous to the previous data.

The Ca/P molar ratios analysed by the SEM-EDX in the present and previous studies (1.29; 1.28±0.03) and by the TEM-EDX in the present study (1.38), the Ca+Mg/P molar ratio in the SEM-EDX (1.50; 1.48±0.02), and the Mg/Ca molar ratio in the TEM-EDX (0.12) were similar to those of Mg-containing whitlockite, although the other results were somewhat different from those of Mg-containing whitlockite (Table 1). Certainly, the rhombohedrally based crystals of the bacillus-shaped deposits in the TEM image (Fig. 16) gave the electron diffraction pattern of whitlockite (Fig. 17).

The SEM and FE-SEM observations (Figs. 1 - 4 and 9 - 15) strongly suggest that various rocky-pile forms of the bacillus-shaped deposits composed of hexahedrally based crystals are based on the filamentous and rod-like forms (bacillus) of oral microorganisms.

Takazoe [1961] reported that oral filamentous microorganisms (Leptotricia), identified as Bacterionema matruchotii by Takazoe et al. [1963], calcified themselves under suitable medium conditions and their main constituents were tricalcium phosphate (maybe whitlockite) and hydroxyapatite. Killian and Ennever [1975] and Boyan-Salyers et al. (1978) described that B. matruchotii, which is known as one of the intracellular calcifying microorganisms [Ennever, 1960], crystallized not only hydroxyapatite but also whitlockite itself under some calcifying medium containing Ca and Mg. Takazoe and Itoyama [1980] noted that in a sample of calcifying B. matruchotii the Ca/P and Mg/Ca molar ratios were 1.35 and 0.16 by analytical transmission electron microscopy. The ratios resembled those of Mg-containing whitlockite [Keppler, 1965; Calvo and Gopal, 1975] and the bacillus-shaped deposits (Table 1).

Rizzo et al. [1963] and Sidaway [1978, 1979, 1980] revealed that various genera of microorganisms derived from human dental calculus calcified themselves, and showed that in filamentous and rod-like forms of these calcifying microorganisms there were Actinomyces, Bacterionema, Bacteroides, Eikenella, Fusobacterium, Haemophilus, and Propionibacterium.

It is, therefore, considered that the intracellular matrices of some filamentous and rod-shaped microorganisms in human dental calculus will be replaced by Mg-containing whitlockite showing hexahedrally based shapes in suitable calculus environments where there is a small increase of pH and a supply of Mg [Newesely, 1965; Knuuttila et al., 1980; Driessens, 1982]. The formation patterns are probably divided into two cases; one is derived from calcified microorganisms on which the hexahedrally based crystals of whitlockite will precipitate (Fig. 11), and the other is based on viable and/or non-viable microorganisms which will calcify by the deposition of whitlockite (Figs. 9, 10, and 12). The latter case may be similar to the intracellular calcification of microorganisms deposited by hydroxyapatite.

Mg-containing whitlockite was a predominant component in subgingival calculus [Jensen and Danø, 1954; Grøn et al., 1967; Sundberg and Friskopp, 1985] and whitlockite was a main component in the homogeneous portions of supra- and subgingival calculus showing high calcification [Kani et al., 1983]. Judging from the above-mentioned previous studies and the present results, the bacillus-shaped deposits composed of Mg-containing whitlockite will never form the surface layers exposed to the oral cavity, but at the interface between calculus and teeth, in the intra-spaces of supragingival and marginal ledge-type calculus, and in the deep gingival pockets.

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Discussion with Reviewers

J. Theilade: Are you sure the crystals are not artifacts as a result of the preparatory procedures as for example the NaOCl traeatment?
Authors: Yes. The NaOCl treatment has been used for SEM studies of calcified tissues by many workers. Holma et al. [1970] and Fejerskov et al. [1984] reported fine calcified structures in human tooth enamel following NaOCl treatment, and moreover, Holma et al. [1970] demonstrated that cabonate-hydroxyapatite treated with NaOCl was morphologically unaltered compared with the untreated mineral. In SEM studies of dental calculus, Lustmann et al. [1976], Jones [1987], Kodaka et al. [1988], and other workers made use of NaOCl. In this study, the bacillus-shaped deposits observed in untreated specimens (Fig. 2) were fundamentally composed of hexahedrally based crystals, although they were more or less covered with traces of amorphous or organic substance compared with NaOCl treated specimens (Fig. 4).

R. Legeros: What is meant by the conclusion of the authors, "the intracellular matrices of some filamentous and rod-shaped microoganisms in human dental calculus will be replaced by Mg-containing whitlockite in suitable calculus environment"?
Authors: We observed the bacillus-shaped deposits at the interface between calculus and teeth, in the intra-spaces of supragingival and marginal ledge-type calculus, and in the deep subgingival calculus. These sites, which are

unexposed to the oral cavity, may experience such changes in conditions as a small increase in pH and in a supply of Mg, so that Mg-containing whitlockite will be deposited in oral microorganisms [Newesely, 1965; Knuuttila et al., 1980; Driessens, 1982].

J. Theilade: Is it possible that recrystallization could have occurred in the calculus samples during the one month storage in 10 % formaldehyde at pH 6.5?

Authors: No, we do not think so, because we have also found the bacillus-shaped deposits in unfixed calculus samples treated with NaOCl. This method was suggested by Dr. A. Carrassi. If recrystallization could occur, the samples had to be decalcified at a time by formaldhyde. The whitlockite phase at physiological pH, however, is more stable than OCP and as stable as apatites [Driessens, 1982]. In addition, OCP crystals showing various plate-like forms in the SEM were commonly detected in marginal ledge-type calculus fixed in formaldehyde at pH 6.5 by the SEM-EDX [Kodaka and Ishida, 1984].

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