Observations of the Surface of Dental Calculus
Using Scanning Electron Microscopy

by

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Introduction

Dental plaque on man's teeth, when left to take its own course, calcifies and becomes dental calculus in several days. The calculus formed near the cervical portion of a tooth has been studied for many years as a cause of inflammation of periodontal tissue. There have been many morphological studies done on the components of bacteria in dental calculus. Many of these studies were made by using a histochemical method [1–3], microradiography [4–6], transmission electron microscopy [7–13] or scanning electron microscopy [14–19].

The authors report here on the interesting results about the morphology of calculus obtained by observing the surface structures of calculus by scanning electron microscopy.

Generally speaking, calculus is classified into supragingival and subgingival calculus. The calculus which the authors observed was all supragingival.

Materials and methods

For material, 30 extracted bimaxillary anterior teeth, diagnosed as untreatable because of chronic marginal periodontitis were used.

The materials were fixed with a 1% glutaraldehyde solution buffered with 0.15M sodium cacodylate (pH 7.3) for 24 hours. The materials were then soaked in 10% NaOCl (10% sodium hypochlorite) or 1N NaOH (1N sodium hydroxide) for one hour at 60°C in an incubator for the purpose of removing the organic substances contained in the plaque and calculus on the surface of the teeth. After being washed with distilled water for ten minutes and dehydrated with ethyl alcohol, the materials were immersed in amylacetate and dried by the critical point drying method. Ion sputter coating with gold paradium was then given to the surface of the materials. Observations were made with an HFS-2S scanning electron microscope.

Results

Plaque adhered to the calculus. No differences were observed in the results of treatment with NaOCl or NaOH, the treatment given in order to remove the organic substances contained in the plaque and calculus of the materials.
Fig. 1  Spongy-like dental calculus. The bacteria itself did not calcify but the surrounding matrix did.

Fig. 2  Higher magnification of Fig. 1. Some of the portions formed by contact with the tubular holes were left uncalcified and formed a foramen.  F: foramen
The surface of the calculus showed a variety of structures, many of which were found to be formed in groups having a certain range and each group consisted of those with the same structures. Most of the structures were spongy-like, observed as a large number of tubular holes 0.7–0.8 μ in size, and the tubular holes were at right angles with the surface of the tooth (Fig. 1). The tubular holes were found to be almost the same in size as the shorter diameter of the bacteria. It was observed that the bacteria itself did not calcify but that the surrounding matrix did. Some of the portions which were formed by contact with the tubular holes were left uncalcified and formed a foramen (Fig. 2 F).

The calcified pattern inside the tubular holes was observed as not flat but having an irregular granular structure. The calcification of the bacteria itself was also observed, sections of which seemed to be filled irregularly with granular material in various sizes, surrounded by a narrow space (Fig. 3 S).

Bacteria in the process of calcification were also observed, though not in many cases. They were composed of small rod-shaped crystals of various sizes arranged radially from the center toward the direction of the cytoplasmic membrane. The intervals of these crystals were irregular (Fig. 4). Plate-like, leaflet-like and cube-like crystals were also observed. The plate-like crystals were about 0.2–0.3 μ thick and showed a regular plate-like structure having the same direction (Fig. 5).

The leaflet-like crystals were about 0.5 μ thick, and their structure and arrangement were very irregular (Fig. 6). Some of the cube-like crystals showed a wide
range of the same structure and some were among leaflet-like crystals (Fig. 7). The cuboidal crystals were irregular in size and were about 0.2–1.3 μ in thickness.

The spongy-like spherical mass was observed on the surface of the spongy-like calculus mentioned before and its sizes were very irregular. The spongy-like spherical mass resulted from calcification which took place around the bacteria (Fig. 8) and leaflet-like crystals adhered to them, increasing their diameter (Figs. 9 and 10).

Discussion

Dental plaque, more or less, adheres to the surface of calculus. It is considered that the bacteria and the matrix, of which plaque consists, calcify with time and that the amount of calculus increases accordingly. The authors observed that the surface structure from which the organic substances were removed had various structures in addition to the calcification of the bacteria and matrix. Sponge-like calculus (Figs. 1 and 2) was observed most frequently. The size of the tubular holes almost corresponded with that of the bacteria. This means that only the matrix had calcified while the bacteria had not. Many reports [16,18] on similar structures, observed with the use of a scanning electron microscope, have been made, but there are no detailed reports on the surface structures with calcification of the surroundings of the bacteria. The surface structure observed by the authors was not flat but irregularly granulated. It was considered to have calcified not by being in touch

Fig. 4 Bacteria in the process of calcification. They were composed of small rod-shaped crystals of various sizes arranged radially from the center toward the direction of the cytoplasmic membrane
Fig. 5  Plate-like calculus shows regular arrangement

Fig. 6  The leaflet-like calculus shows irregular arrangement
Fig. 7 Some of the cube-like crystals exist in leaflet-like calculus.

Fig. 8 Dental calculus of sponge-like spherical mass
Fig. 9  Leaflet-like crystals adhered to the spongy-like calculus

Fig. 10  Higher magnification of Fig. 9
with the cytoplasmic membrane but that the cell wall above the surface calcified. There was a space that remained uncalcified between the calcified bacteria and the calcified matter around them as shown in Fig. 3, and it is considered to be a part of the cell wall in filamentous bacteria as observed with a transmission electron microscope [20]. The structure of calcified bacteria in carious dentin observed by KOMURO [21] is the same as this. Thus, the surface structure of the calculus observed by the authors is considered to be a calcified structure of the part of the glycocaryx which exists in the cell wall of the bacteria. The crystal patterns of the bacteria themselves were also observed. They seemed to be buried in the sponge-like calculus and cross sections showed irregular small granules with spaces around them. The authors observed some bacteria in the process of calcification. They were different from the calcified bacteria shown in Fig. 3. The crystals, in small rod shapes, radiated from the center of the bacteria toward the cytoplasmic membrane and the whole area was considered to be calcified by filling the spaces between the crystals.

As to whether the bacteria or the matrix calcifies first, when bacteria calcify to form calculus, is a controversial question. RIZZO et al. [22] and TAKAZOE et al. [23] reported that formalin-killed bacteria calcified faster than living bacteria. LIE et al. [20] observed the calcification of Bacterionema matruchotii in vitro with a transmission electron microscope and found intracellular and extracellular calcifications. It is considered that calcification varies depending on the condition and the species of bacteria. The authors’ observations could not clarify which calcified first, the bacteria or the matrix, when dental calculus was formed.

It is known that there are two kinds of the dental calculus, A-center formed by the bacteria themselves and B-center formed without direct adherence of the bacteria [18]. Plate-like, leaflet-like and cube-like crystals are considered to belong to those of B-center. Their crystals were observed by LUSTMANN et al. [18] with a scanning electron microscope but the authors could not see the needle-like crystals they had observed. They also observed cuboidal crystals the same size as the ones observed by the authors. In addition, the authors observed leaflet-like crystals which were formed on the calculus that had the structure of sponge-like spherical masses with rather large diameters as a whole. This means that the B-center calculus was formed on the A-center calculus.

**Conclusions**

The authors have come to the following conclusions from observations, made with a scanning electron microscope, of the surface of calculus from which organic substances had been removed.

1) Various forms were observed on the surface of the calculus and many of them, which were of the same structure, were seen as a group in a certain area. The sponge-like calculus was observed most often. Plate-like, leaflet-like and cube-like crystals were also observed.

2) The surface structure, considered to be formed by the calcification of the surroundings of the bacteria, was not flat but irregularly granular.

3) Cross sections of calcified bacteria appeared to be filled with irregularly granulated substances with narrow spaces around them. The bacteria which were in the
The process of calcification consisted of large and small crystals in small rod shapes radiating out from the center of the bacteria toward the cytoplasmic bacteria membrane.

References