The Later Calcification of the Bacterial Molds in the Human Dental Calculus Formed by the Extracellular Calcification

TETSUO KODAKA* and MASAYUKI YAMADA

Second Department of Oral Anatomy, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan

(Chief: Prof. Shohei Higashi)

Abstract: In the bacterial molds of human dental calculus formed by the extracellular calcification, whitlockite and octacalcium phosphate crystals as well as apatite crystals were occasionally precipitated in the later stages of calculus formation. Thus, the deposits would be similar to the calculus formed by both the intra- and extracellular calcifications.

Keywords: human dental calculus, extracellular calcification, bacterial molds, later calcification, scanning electron microscopy

As is well known, there exist the intra- and extracellular calcifications of oral microorganisms in human dental calculus1-5). The crystals are biological apatites including hydroxyapatite, Ca-deficient and carbonate apatites1-8), although oral microorganisms were occasionally replaced by Mg-containing whitlockite crystals9-11). The formation processes will be roughly divided into 3 cases: I to III1-5,11,13); case I: the intra- and the extracellular calcifications occur at almost the same time, case II: the intracellular calcification first occurs followed by the extracellular calcification, and case III: the extracellular calcification first occurs.

In case III, the bacterial molds will be retained during the time of the calculus formation, although the deposits of case III would become like a calculus of case I in the later formation stage. In this study, crystals deposited within the bacterial molds were investigated with scanning electron microscopy (SEM).

Materials and Methods

Supra- and subgingival dental calculi attached to human teeth, which had been used in our previous studies,9-11,10,14) were also used in these observations. The fractured calculus were treated with 10% sodium hypochlorite (NaOCl) for 1 hour in order to remove organic debris9-12, 14,15). This was followed by rinsing in running water for 1 hour and drying in the air. The samples were observed with a Hitachi S-430 SEM at 20 kV accelerating voltage after coating with a 10 to 15-nm platinum-palladium layer.

Results

Figure 1a is an example of the dental calculus formed mainly by the extracellular calcification. Many microorganisms were dissolved with NaOCl so that the bacterial molds clearly appeared, while some microorganisms were retained because they were formed by the intracellular calcification. In dental calculus which might be formed by both the intra- and extracellular calcifications, the calculus was densely composed of fine needle or sandygrain-shaped crystallites in the intracellular as well as in the extracellular region (Fig. 1b).

Figures 2 to 4 are the regions formed by the extracellular calcification. Long needle-shaped (Figs. 2, 4), small plate-shaped (Figs. 3, 4), and hexahedrally based crystals (Figs. 3, 4) were loosely existent in the bacterial molds, al-
Figs. 1–4. Scanning electron micrographs of dental calculus fractured and treated with NaOCl. Arrow: bacterial mold, Double arrows: calcified microorganism, AP: long needle-shaped crystals, OCP: small plate-shaped crystals, WH: hexahedrally based crystal. Bars=5 µm (1a) and 1 µm (1b, 2–4).
though some of the molds were retained as an empty space.

Discussion

These SEM observations indicate that the crystals within the bacterial molds (Figs. 2-4) have been deposited in the empty spaces of the molds, after dental calculus was roughly formed by the extracellular calcification.

We have investigated calcium phosphate crystals in human dental calculus by using SEM and energy-dispersive X-ray microanalysis9-14). From previous studies1-8) and our data9-10), long or thin needle-shaped1-8,11,14), plate-shaped4,6-8,10,11,14), and hexahedrally based crystals6-11,13) are, respectively, identified as biological apatites (AP), octacalcium phosphates (OCP), and Mg-containing whitlockites (WH). That is, we suggest that their crystals found within the bacterial molds should be AP, OCP, and WH.

According to Newesely6), Schroeder4), Driessens7) and LeGeros8), AP, OCP, and WH crystals are formed in different conditions; that is pH ranges, ratios of Ca to P, sources of a small amount of Mg or fluoride, and others. Therefore, their crystals are probably precipitated in the bacterial molds during the different stages of calculus formation, even when the crystals are existent in the same bacterial molds. Such a crystallization will cause the calculus deposits formed by the extracellular calcification to be of a higher density. In conclusion, the deposits could be similar to the calculus formed by both the intra- and extracellular calcifications.

References


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