Sedimentary rocks in our mouth: Dental pulp stones made by Nanobacteria

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ABSTRACT

The mechanisms of dental pulp stone formation are still largely unknown. Pulp stones are mainly composed of carbonate apatite. Only few experimental reports have elucidated the potential of some selected bacteria to produce apatite under *in vitro* conditions using special calcification media. The tested stone forming bacteria were, in fact, often better known for their cariogenic potential. Our preliminary work with 18 dental pulp stones from Turkey, selected only by severity (size) of the stone formation, indicated the presence of nanobacterial antigens in the demineralized stones. Furthermore, high incidence of kidney stones and gall stones in the patient group and in their parents was found. This raises the implication that nanobacteria may enter the body also via oral route, in addition to the parenteral and transplacental routes.

The role of nanobacteria in dental pulp stone formation was further studied by following nanobacterial colonization and mineral formation on human tooth in vitro. Two molar teeth, one having pulp stone and one without, were vertically cut into two pieces, sterilized by autoclaving and incubated with or without nanobacteria in DMEM. Electron microscopic observations indicate that nanobacteria can cause apatite stone formation on tooth surface. The severe form of dental pulp stone formation might be associated with nanobacteria. This form of dental disease results in loss of teeth due to osteolytic processes. This addresses the necessity for a study on unconventional mineral-forming bacteria as a cause for human diseases.
Keywords: Nanobacteria, mineralization, dental pulp stones, denticles, apatite

1. INTRODUCTION

Pulp stones or denticles are polymorphous mineralized bodies of various sizes occasionally found in the pulpal connective tissue of human teeth.' Their etiology remains unclear although they have been frequently associated with aging or pathology of the pulp.2"4 They may also be present in permanent teeth that are impacted free of pathology for a long time.5 Although pulp stones have been extensively studied morphologically, their origin is still obscure and little is known about their chemical composition. In an histochemical study of pulpal calcifications, it has been shown that the organic matrix consists of reticular connective tissue fibers and of a ground substance containing glycoproteins and acid polysaccharides.6 The mineral phase of pulp calcification has been studied with X-ray energy dispersive spectrometry (EDX) and chemical analysis, and proven that calcium salts are deposited in the form of apatite, possibly carbonate containing apatite.7 Actually, there is not big difference between the chemical structure of a tooth and denticles.8 Bone and tooth formation in the body have similar mechanisms9 which have many unanswered questions. Apatite formation in the body except in tooth and bone is called pathologic biomineralization, e.g., dental pulp stones, kidney stones, joint calcifications.
Interestingly, also environmental apatite stones have almost the same chemical composition as in bone and dentine. Recently, bacteria have been implicated as factors in biogeochemical cycles for mineral formation in aqueous

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sediments. 10T Nanobacteria is the smallest cell walled, apatite forming bacteria isolated from mammalian blood and blood products. 12"14 Their small size (0.05-0.3 *fim),* and unique properties make their detection difficult with conventional microbiological methods, m nanobacteria-infected mammalian cells, electron microscopy revealed intra- and extracellular acicular crystal deposits, stainable with von Kossa staining and resembling calcospherules found in pathological calcification. 15 Recently, we detected them with immuno logical methods and culture from human kidney stones (Ciftcioglu et al, in this issue).

Only a few studies (published in English) on dental pulp stone formations have been conducted under a scanning electron microscope (SEM). The purpose of the present study was to investigate ifnanobacteria participates in the dental pulp stone formation. The design of the study was to culture nanobacteria on a healthy tooth, without dental pulp stone, and compare the results with those obtained from a tooth having dental pulp stone. Mineral formations were observed under SEM. Additionally, an epidemiological screening was carried out on the possible correlation between dental pulp stone and kidney stone disease, and other bodily calcifications in 18 patients using a questionnaire.

2. CORRELATION BETWEEN DENTAL PULP STONES AND OTHER STONE FORMATION IN THE BODY

18 patients were randomly selected from a private dental practice in Turkey based upon their periodontal problems caused by severe pulp stone formation. Collected pulp stones were stored in PBS containing 0.05% NaNa at +4°C. The samples were demineralized in IN HCl for 10 min at room temperature, neutralized with NaOH and potassium phosphate buffer, and immunostained by using and-nanobacteria monoclonal antibodies. 1 N HCl treatment of the samples did not effect the epitopes recognized by our monoclonal antibodies. The immunostaining revealed positive, small cocci at various concentrations in all samples. Specificity of the staining was further proven with negative staining results with three different monoclonal antibodies detecting nonrelevant antigens.

The results, we obtained from the patient questionnaire, were showing high incidence of kidney stones and gall stones in both patients and their parents (Table 1.).

	Patients (9M+9F)	Mothers	Fathers
Kidney stones	5/18 (28%)	3/18 (17%)	6/18 (33%)
Urinary sand	6/18 (33%)	1/18 (6%)	0/18 (0%)
Gall stones	2/18(11%)	7/18 (39%)	3/18 (17%)
Tissue calcifications	1/18(6%)	5/18(28%)	1/18(6%)

Table 1. The presence of calcification and stone formation in the patients with dental pulp stones, and their parents.

It has been shown that there is an increase in calculus formation on teeth among the laboratory animals whenever common drinking water was given, which suggests that transferring the flora from one animal to an other.22 In addition, it had been reported that erythromycin strongly inhibited calculus formation, whereas chloramphenicol, and penicillin did not." This suggests that the organisms involved in calculus accumulation may be very specific. These findings may bring one possible explanation to the results shown in Table 1, indicating high incidence for stone formation and calcification in the family members.

3. NANOBACTERIA CAUSE DENTAL CALCULI FORMATION IN VITRO

In our SEM observations of a tooth with dental pulp stones (Fig. 1A), at high magnification, mineralized fibers, and numerous small globular bodies near them were observed (Fig. 1B). SEM observations made by the other scientist gave similar results."2 There was no calcospherules observed in the control tooth (Fig. 1 C and D).

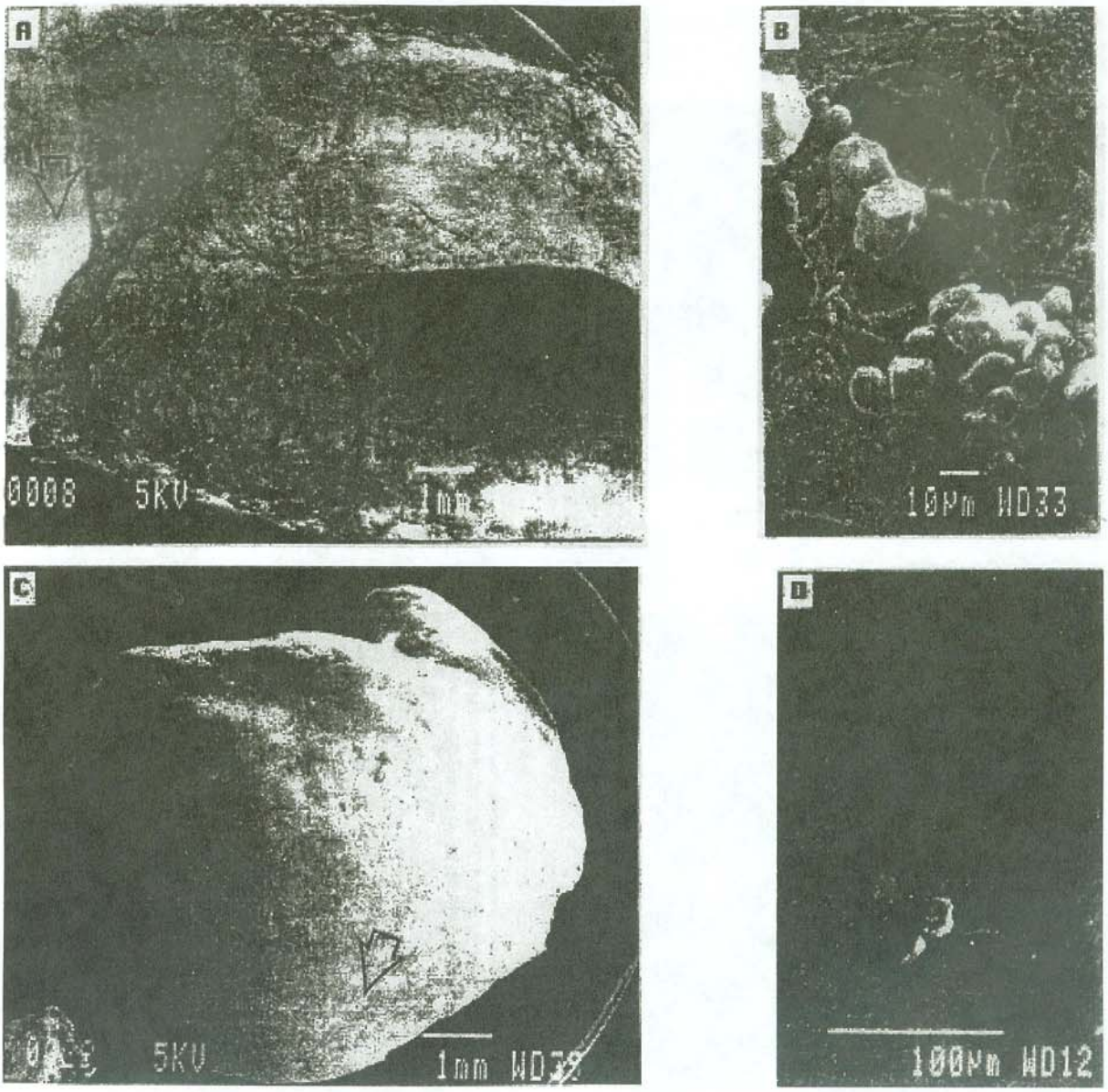

Figure 1. SEM images of teeth with (A and B), and without (C and D) dental calculi. The tooth shown in (A) was extracted because of periodontal problems, and bone desorption caused by severe dental pulp stone formation. Higher magnification from the area shown by arrow depicts round, and fibrous calcification (B). The tooth shown in © was extracted because of an orthodontic problem. This tooth was autoclaved and exposed to DMEM culture medium for one month, in cell culture condition. No crystallization on the surface was observed (D) Shows the higher magnification of the area marked with an arrow in ©. The vertically cut other half of the same tooth was used for the experiment described in Fig. 2.

When we exposed a healthy tooth to SF-nanobacteria culture for one month, SEM revealed voluminous mineral formation, resembling dental pulp stones, on the surface of the tooth (Fig. 2 A, B, D, and E).

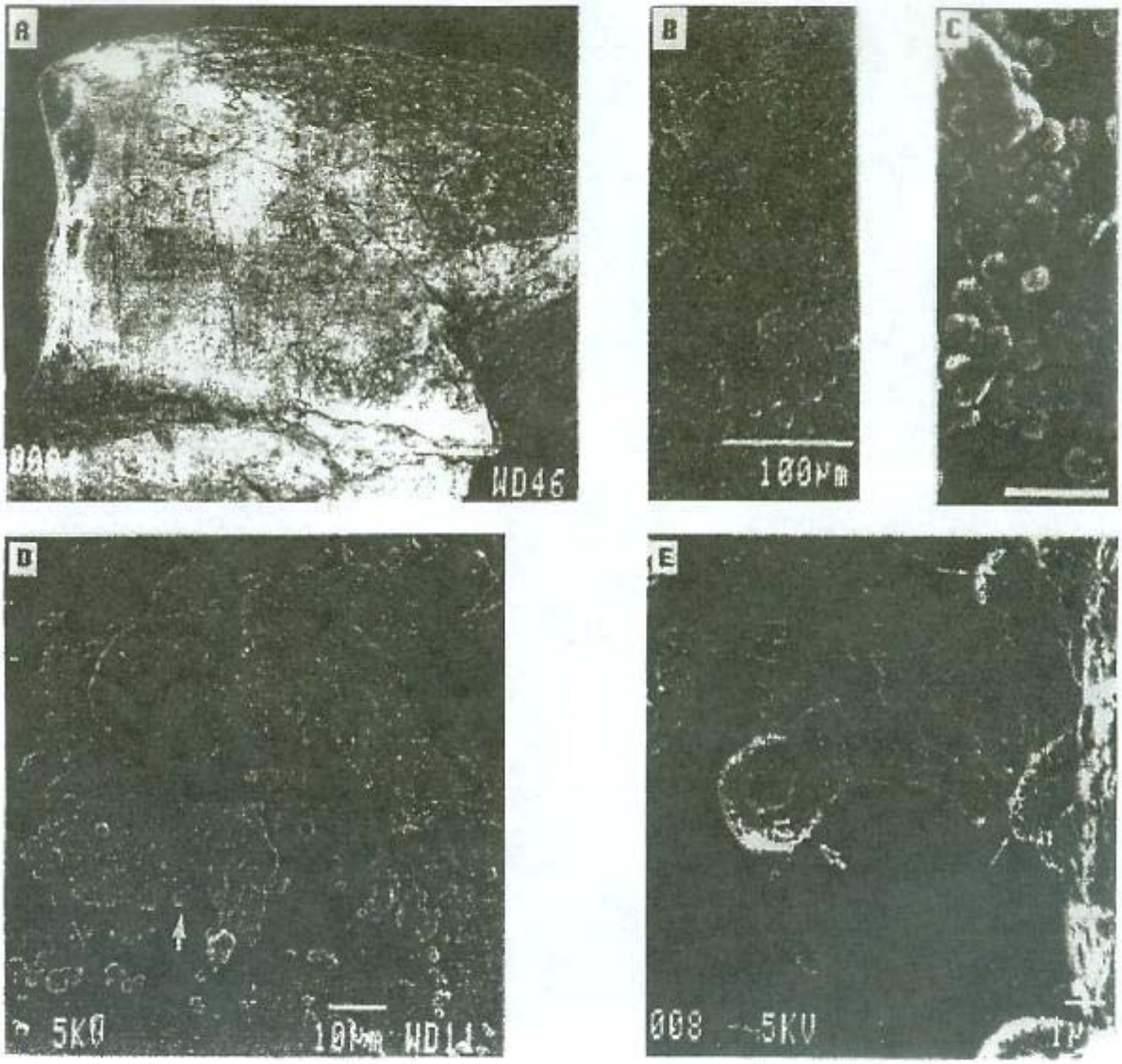

Figure 2. SEM micrographs of the healthy tooth shown in Fig 1C and D after autoclaving, and incubating with SF-nanobacteria for one month in cell culture conditions. (A) General image showing th surface of the tooth, higher magnification to an area shown by the arrow is seen in (B). (C)Nanobacteria cultured for 3 months, and adhered to cell culture vessel; bar is 1µm. (D) An area in the same tooth having voluminous pulp stone that appeared after SF-nanobacteria exposure for one month. (E) Higher magnification of the same area shown with big arrow in (D). Small arrows show the growth of SF-nanobacteria on the surface of calculi.

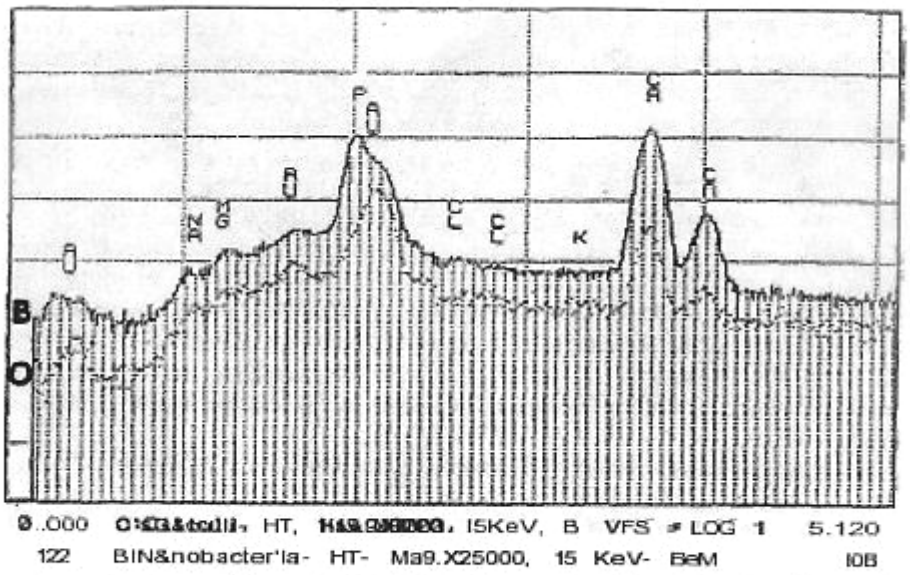

Figure 4. Energy dispersive X-ray microanalysis (EDX) of human dental calculi (0) and SF-nanobacteria (B).

5. CONCLUSION

Our data indicates that dental pulp stones are associated with apatite forming nanobacteria. Further studies are needed to prove pathogenic role of nanobacteria in dental diseases, and to find the correlation between dental pulp stones and the other stone formation in the mammalian body.

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