Nanobacteria – CNPs the Possibility of Bacterial Association of the Dental Calcifications in Correlation with the Salivary Gland Stones

Aleksova Pavlina

University Dental Clinical Center "St. Panteleimon"- Department of Restorative dentistry and Endodontics, Faculty of Dentistry, University "Ss. Cyril and Methodius", Skopje, R. of Macedonia.

Correspondence: Aleksova Pavlina Department of Restorative dentistry and Endodontics, Faculty of Dentistry, University "St.Cyril and Methodius", Skopje, Republic of Macedonia

Tel. +389 070 260 308 E-mail: pavlinaaleksova@yahoo.com

Abstract: ***Introduction: Nanobacteria is a cytotoxic, sterile-filterable, gramnegative, atypical bacteria that could be detected in bovine and human fluids. It can produce carbonate apatite on its cell wall. Materials and Methods: The present study was analyzed dental pulp stones of the molars, and parallel operative leached salivary gland stones. Qualitative and quantitative chemical analysis was used to determine the ratio between Calcium and Phosphorus given in mg/kg and % (percentages). For the qualitative analysis of the material we used a method of Infrared Spectroscopy, known as FTIR-spectrometry. For the quantitative analysis of the material, we used a method of Atomic-absorption spectroscopy.*** *Control group was samples hydroxyapatite. The analytical methods do not exclude the possible presence of minor quantities of other minerals.* ***Results: This analysis of dental pulp and salivary gland stones showing Ca and P peaks similar to those of hydroxyapatite. Spectrograms in absorbance of the pulp stones and the salivary gland stones were approximately equally. Mainly made up of albumin, which is the most common component of the pulp stones, and as can be seen in the spectra, of the salivary gland stones. With the method of atom-absorbing spectroscopy, we got the following results: pulp stones, the value of Ca and P expressed in mg/kg was 18.501, and 11.001. The value of Ca and P expressed in % was 18.502 and 17.602. Salivary gland stones, the value of Ca and P expressed in mg/kg was 584310 and 232517. The value of Ca and P expressed in % was 58.431 and 23.252. We has presence and*** *minor quantities of other minerals.* ***Discussion: Generally, the apatite formation required elevated Pi and/or Ca2+ levels and AP activity. In the nanobacterial model, apatite was formed at [Ca] 1.8 mM and [Pi] 0.9 mM or less. Values Ca/P ratio in dental pulp stones of this study, is such with values in the nanobacterial model. Two major chemical elements: Ca and P in the sialoliths concentrations 58.43% and 23.25% respectively giving a Ca/P weight ratio of 2.18 which is very close to the weight ratio of pure stoichiometric hydroxyapatite 2.15. Conclusions: With this study we confirm that Nanobacteria or CNPs can be the possibility of bacterial association of dental calcifications and of salivary glands stones.***

Keywords: bacterial association, nanobacteria, calcifying nanoparticles, dental calcifications, salivary glands stones.

1. Introduction

Pathological calcification is a result of different diseases. According to published studies, a common link has been found in a number of diseases, where the presence of a certain form of life is similar to bacteria. They are mineral-producing bacteria which come as a cause of disease in people. The composition of this form is manifested as nanobacteria, now renamed to calcifying nanoparticles – CNPs, i.e. propagating self-replicating nanoparticles. They are an active factor for crystallization of Calcium, Phosphorus, minerals and carbonate apatite.

Nanobacteria or calcifying nanoparticles are 80–500 nm in diameter, typically have coccoid, coccobacillar, or bacillar form. They have hydroxyapatite shell, cellular-membranous structure, central cavity and can form microscopic colonies. Nanobacteria divide by binary fission, fragmentation, gemmation and can form thermoresistant biofilms. They are Gram-negative, can be stained by DNA-specific dyes. Doubling time is 3 days, their metabolism is 10, 000 times slower than in Escherichia coli and calcify under physiological pH [1].

Pulp stones (also called denticles, endoliths) are discrete calcifications and are amongst changes that include more diffuse pulp calcifications such as dystrophic calcification. Stones may exist freely within the pulp tissue or be attached to or embedded in dentine. A single tooth may have from 1 to 12 or even more stones, with sizes varying from minute particles to large masses which occlude the pulp space [2].

2. Materials and Methods

At the clinic for Restorative Dentistry and Endodontics, Faculty of Dentistry, "Ss Cyril and Methodius" University in Skopje, seven dental pulp stones were extirpated from molars of a patient over a period of one month. After their presence was diagnosed with X-rays, the diagnosis pulpits chronic was given. At the Clinic of Maxilofacial surgery, Faculty of Dentistry, University "St. Cyril and Methodius" in Skopje, was analyzed operative leached 10 sialoliths. The analysis was performed in the Institute of Chemistry at the Faculty of Natural Sciences and Mathematics, "Ss Cyril and Methodius" University in Skopje.

Qualitative and quantitative chemical analysis was used to determine the ratio between Calcium and Phosphorus given in mg/kg and % (percentages). For the qualitative analysis of the material we used a method of Infrared Spectroscopy, known as FTIR-spectrometry. For the quantitative analysis of the material, we used a method of Atomic-absorption spectroscopy. Control group was samples hydroxyapatite. The analytical methods do not exclude the possible presence of minor quantities of other minerals; Al, Ba, Cu, Fe, K, Mg (mg/kg and %), Na (mg/kg and %) and Zn.

3. Results and Discussion

This analysis of dental pulp and salivary gland stones showing Ca and P peaks similar to those of hydroxyapatite.

Spectrograms in absorbance of the pulp stones and the salivary gland stones were approximately equally. Mainly made up of albumin, which is the most common component of the pulp stones, and as can be seen in the spectra, of the salivary gland stones.

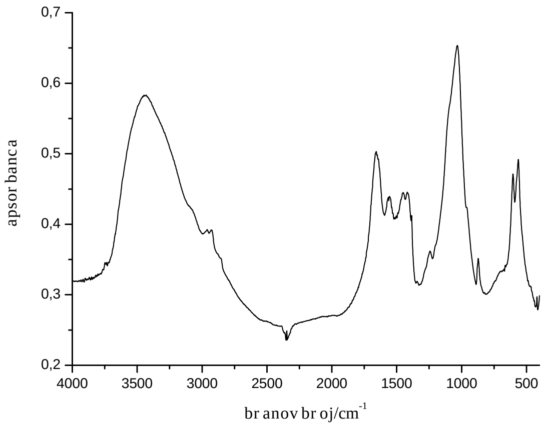

Figure 1: Infrared spectrum in absorbance

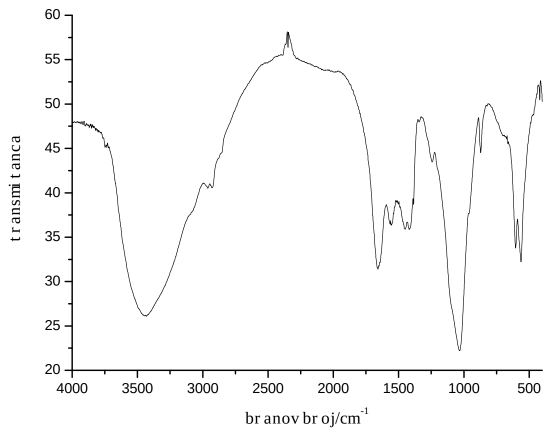

Figure 2: Infrared spectrum in transmittance

Figure 1 and figure 2. Infrared spectrum in absorbance and transmitance **(**Figure 1, Figure 2) of from dental pulp stones, made ap of albumin + phosphate (carbonate apatite).

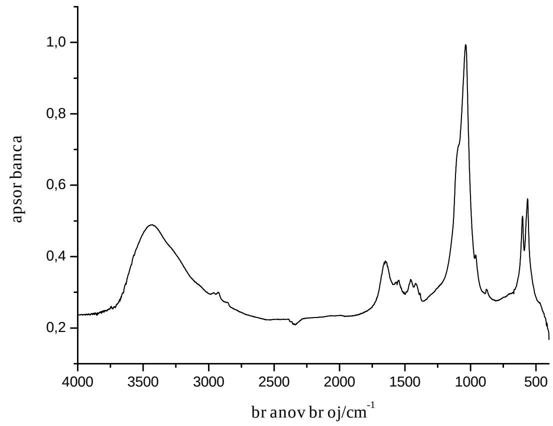

Figure 3: Infrared spectrum in absorbance

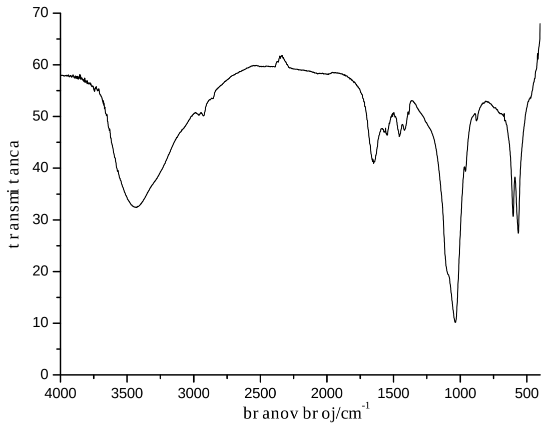

Figure 4: Infrared spectrum in transmittance

Infrared spectrum in absorbance and transmittance (Figure 3, Figure 4) of from salivary gland stone made of albumin + phosphate (carbonate apatite).

Spectrograms in absorbance of the pulp stones and the salivary gland stones were approximately equally. Mainly made up of albumin, which is the most common component of the pulp stones, and as can be seen in the spectra, of the salivary gland stones.

The intensive line in the spectra in transmittance in the high frequency part, as well as the lines in the area around 1600 to 1300 cm-1 show the presence of albumin, and the intensive line in the area around 1000cm-1 and the doublet of lines around 600 cm-1 are the result of the vibration of phosphate ions present in the stones as carbonate apatite (Calcium Phosphate Carbonate). Control hydroxyapatite was correctly identified in the test. This analysis of dental pulp stones and the salivary gland stones, showing Ca and P peaks similar to those of hydroxyapatite.

With the method of atom-absorbing spectroscopy, we got the following results (Table 1 and Table 2):

Table 1: The presence of chemical composition in Dental pulp stones and Salivary gland stones

	Al mg/kg	Ba mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mg %	Na mg/kg	Na %	Zn mg/kg
Dental pulp stones	26.65	9.464	29.92	<1	173.027	1687.643	0.269	1.381	0.138	1404.02
Salivary gland stones	50.20	0.541	27.76	<1	635.244	4785.261	0.479	7727	0.773	86.14

Table 2: Ca and P in mg/kg from Dental Pulp stones and Salivary Gland stones

	Ca mg/kg	P mg/kg	Ca %	P %
Dental pulp stones	185019	110011	18.502	17.602
Salivary gland stones	584310	232517	58.431	23.252

We shouldn't neglect the fact that Calcium, together with Phosphorus, is one of the main components in forming pathological calcifications.

Generally, the apatite formation required elevated P_i and/or Ca^{2+} levels and AP activity. In the nanobacterial model, apatite was formed at [Ca] 1.8 mM and [P_i] 0.9 mM or less [3]. Values Ca/P ratio in dental pulp stones of this study, is such with values in the nanobacterial model. Infrared spectroscopy showing the distinct phosphate absorption signature at 1, 000 to 1, 200 cm21 seen in all four spectra, with inorganic apatite, bone, kidney stones, and nanobacteria [4].

Chemical analysis revealed that the overall composition of biofilm and solid mineral formation was similar to that of bone, except carbonate apatite was formed, as in most extraskeletal tissue calcification and stones, whereas in bone, hydroxyapatite is the prevalent form [1, 5, 6, 7].

The nanocrystalline apatites show a highly organized structure and nanobacteria can act as crystallization centers for the formation of biogenic apatite structures so that they appear as self-propagating calcifying nanobacteria [8, 9, 10, 11].

4. Conclusions

With this study we confirm that Nanobacteria or CNPs can be the possibility of bacterial association of the dental calcifications.

References

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