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ELECTRON PROBE MICRO-ANALYSIS OF HUMAN DENTAL PULP STONES

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Abstract

The mineral composition of ten human dental pulp stones presenting various morphological aspects has been studied by electron probe micro-analysis. The denticles were composed of two major chemical elements : Ca and P with mean concentrations 32.12% and 14.69% respectively giving a Ca/P weight ratio of 2.19 which is very close to the weight ratio of pure stoichiometric hydroxyapatite (2.15). The concentration of some other elements was much lower (0.88% for F ; 0.75% for Na ; 0.51% for Mg). The other analysed constituents (K, Cl, Mn, Zn, Fe) were present at trace concentrations. The mineral composition of sound human dentine from one tooth containing a pulpal calcification was also analysed for comparative purpose.

Key Words : Wavelength dispersive spectrometry (WDS), elemental microanalysis, dental pulp stone.

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Introduction

Dental pulp stones, or denticles, are irregular calcified bodies present in the coronal and/or radicular part of the dental pulp. They can be classified according to their relation with the surrounding dentinal wall. The free pulp stones are entirely surrounded by pulp tissue, attached pulp stones are partly fused with the dentine, and embedded pulp stones are entirely surrounded by dentine (Avery, 1986). According to their histological structure, they are also classically divided in "true" and "false" pulp stones. "True" denticles contain tubules similar to those of irregular dentine and "false" denticles do not exhibit any tubular structure but often present concentric layers of calcified tissue (Kronfeld, 1933). However, tubules can be also observed in so-called "false" pulp stones (Le May and Kaqueler, 1991) and this usual classification is challenged by some authors proposing a classification based upon the mode of genesis of the calcifications (Moss-Salentijn and Hendricks-Klyvert, 1983, 1988).

Although pulp stones have been extensively studied morphologically, their origin is still obscure and little is known about their chemical composition. Johnson and Bevelander (1956), in an histochemical study of pulpal calcifications, showed that the organic matrix consists of reticular connective tissue fibers and of a ground substance containing glycoproteins and acid polysaccharides. The inorganic phase, according to the electron diffraction analyses of Appleton and Williams (1973) and Plackova and Vahl (1974), would consist of hydroxyapatite. In a diffractometric investigation, De Rysky et al. (1981) found that pulp stones contain hydroxyapatite with a low degree of crystallinity. The mineral phase of a pulp calcification has been also studied by Aoba et al. (1980) who found by X-ray microbeam diffraction, electron spin resonance spectroscopy, X-ray energy dispersive spectrometry (EDS) and chemical analysis that calcium salts are deposited in the form of apatite, possibly carbonate containing apatite. They detected also a very high concentration of iron (Fe) at the periphery of the calcified mass. For these authors, this iron may be a degradation product from erythrocytes after pulpal hemorrhages and can play a role in the formation of pulp calcification since iron may induce

mineralization in connective tissues. However, it must be pointed out that their study, although dealing with numerous analytical methods, has been carried out on a single case of pulp stone.

The aim of this work was to study the inorganic composition of two denticle types by wavelength dispersive spectrometry (WDS) in order to provide a more detailed mineral characterization. The WDS micro-analyser allows qualitative and quantitative elemental analysis of small volumes in situ by comparing the emission of characteristic X-ray radiations of each element selected within the sample and a standard under the same experimental conditions. This non-destructive technique enables the simultaneous observation of the specimen surface in a light optical microscope or a scanning electron microscope (SEM) and the precise choice of the point to be analysed.

Materials and Methods

Nine free pulp stones entrapped during endodontic treatments on various human permanent teeth were used in this study in addition to a voluminous free denticle included in the coronal pulp chamber of an unerupted but extracted right mandibular third molar (Fig. 1). This denticle was selected since a previous morphological study of a pulp stone present in the impacted left third molar from the same patient had revealed a mass entirely traversed by numerous tubules similar to those of dentine and therefore corresponding to the "true" type according to Kronfeld's classification. In order to compare the composition of this expected dentine-like pulp stone and its surrounding mineralized tissues, the tooth was longitudinally fractured and the pulp tissue extirped under a binocular dissecting microscope in order to remove the denticle. The crown and the denticle were both prepared for analytical study.
The characteristics of the samples are summarized in Table 1.

Table 1. Characteristics of the analysed pulp stones.

Sample n°	01	02	03	04	05	06	07	08	09	10
Size in mm	8.7	1.9	1.5	2.0	1.4	2.2	2.5	1.2	1.8	1.5
Number (n) of analysed points	44	18	30	40	20	24	20	20	14	30
Sex (M = male, F = female) and years of the patients	M 41	M 38	M 47	M 29	M 33	M 26	F 58	M 36	M 22	F 16
Tooth	6	7	$\overline{7}$	3	$\overline{4}$	6	$\overline{6}$	5	6	$\overline{8}$

The samples were embedded in araldite resin and ground sagitally, after polymerisation, toward a plane cross-secting the pulp stone so that the analysis could be made either at a central or peripheral point. They were then progressively polished with diamond pastes down to a 1 μm grain in order to obtain a smooth, flat surface. They were then coated with carbon in a vacuum evaporator and analysed in an electron microprobe (SX 50 or Camebax Microbeam, Cameca, France) operating at 15 kV accelerating voltage with a 10 nA specimen current. Characteristic X-rays were measured for ten elements : calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg), potassium (K), chlorine (Cl), fluorine (F), manganese (Mn), zinc (Zn), iron (Fe) with four wavelength dispersive spectrometers each one containing a diffracting crystal: pentaerythritol (PET), lithium fluoride (Li F) and thallium acid phthalates (TAP 1 and TAP 2) . The comparison standards used for quantitative analysis were Durango apatite for Ca, P and F, albite for Na, diopside for Mg, orthoclase for K, scapolite for Cl, zinc sulfite for Zn, synthetic crystals of Mn Ti O_3 for Mn and Fe_2 O_3 for Fe. Raw X-ray data were acquired at each analysis point and a PAP correction applied to give percentage analysis. The data correction procedure PAP (Pouchou and Pichoir, 1991) uses a X-ray emission model, based on an improved description of Φ (ρ z) ionization functions. The electron beam, with a spot diameter of 10 μm, was focused at different points on the polished surface of the sample visualized either with a co-axial high resolution light optical microscope or with a Cameca SEM.

The statistical analyses of the data were carried out using the Student's t-test at the 95 and 99% confidence interval.

Results

Morphological study

The observation with the SEM or with the light microscope showed that the calcification (n° 10) obtained from the impacted third molar was traversed by numerous tubules and looks like a "true" pulp stone (Fig. 2). The other denticles did not exhibit so many tubules, most of them did not present a peculiar architecture, the polished surface being compact and homogeneous ; two samples (n° 3 and 4) showed a concentrical organization around an initiating central core (Fig. 3) and one elongated pulp stone (n° 1) exhibited a linear organization along the longitudinal axis of radicular pulpal nerves and vessels.

Analytical study

The wavelength dispersive spectrum (Fig. 4) is very sensitive and allows the visualization of peaks of some minor elements (Mg and Na). However, the values of the other trace elements are too low to be represented on the spectrograph or will require too long counting times incompatible with the integrity of the biological samples.

The mean concentrations of the ten selected elements obtained by WDS for the ten pulp stones and for dentine of the third molar are summarized in Table 2.

The data showed that the pulp stones were composed of two major elements : calcium and phosphorus. The calcium concentration varied from 30.31% to 33.21% by weight with a mean concentration of 32.12%. For the phosphorus, the values ranged from 14.09 to 15.29% with a mean concentration of 14.69%. The Ca/P weight ratio has

Figure 1 : Radiograph of an extracted lower third molar showing a voluminous free calcification (➧) in the coronal pulp chamber. Bar = 2 mm.

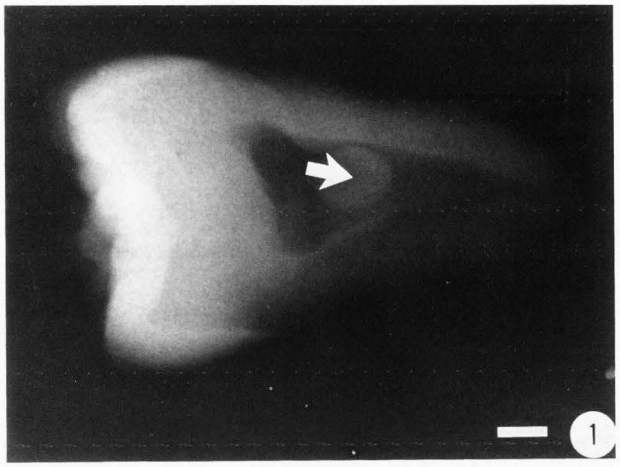
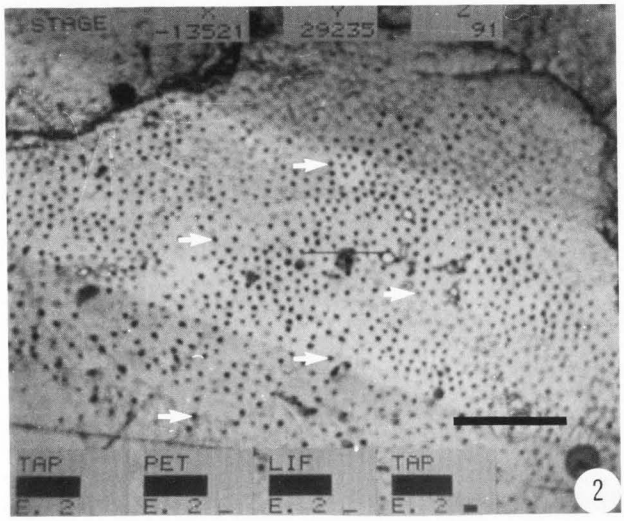
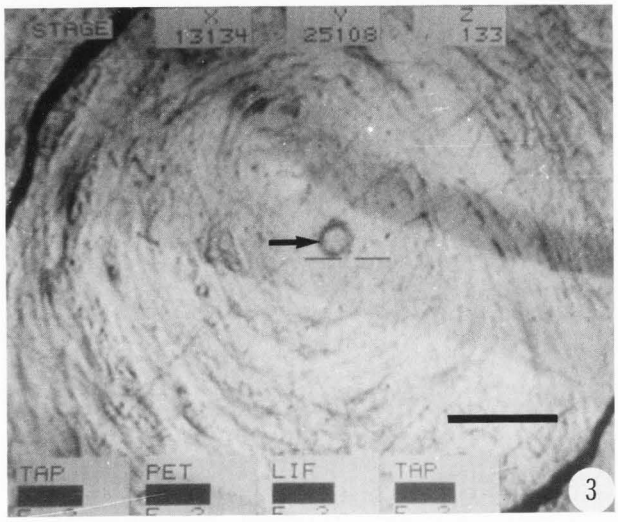


Figure 2 : Light micrograph of the inner aspect after polishing of the non-demineralized intra pulpal calcification presented in figure 1 showing numerous dentine-like tubules (→). Bar = 50 μm.

Figure 3 : Light micrograph of a non-demineralized polished free pulp stone showing concentric layers organized around a central nidus (→). Bar = 50 μm.

Figure 4 : WDS spectrum of a pulp stone showing peaks for minor elements Mg (Kα) and Na (Kα) beside the third and fourth order lines of Ca (Kα), the second and third order lines of P (Kα) and the second order line of P (Kβ).





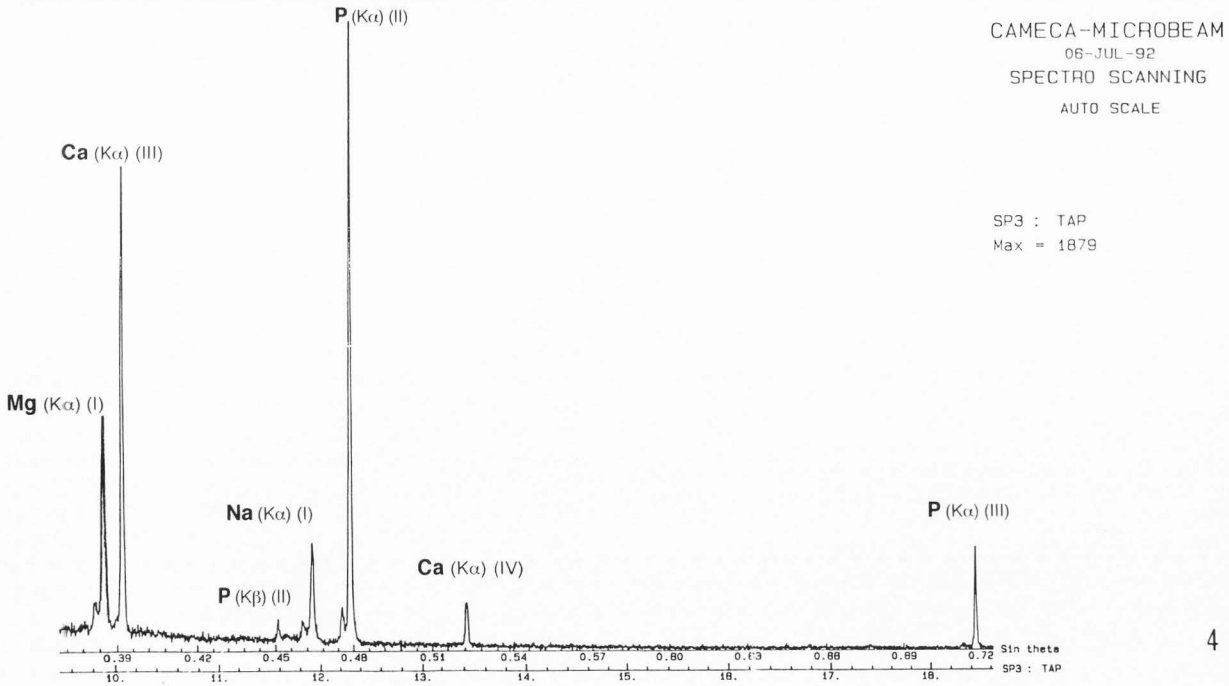


Table 2. WDS microanalysis of pulp stones and dentine of the impacted third molar. Mean concentrations (Weight Per Cent ± Standard Error of the Mean*) of the selected elements.

	Pulp stones (10 samples)	"False" pulp stones (9 samples)	"True" pulp stone (1 sample)	Dentine (1 sample) n = 20
Ca/P	2.19 ± 0.03	2.20 ± 0.02	2.07	2.07 ± 0.01
Ca	32.12 ± 0.31	32.30 ± 0.29	30.55	30.53 ± 0.40
P	14.69 ± 0.12	14.68 ± 0.13	14.79	14.77 ± 0.21
Na	0.75 ± 0.10	0.76 ± 0.11	0.63	0.61 ± 0.03
Mg	0.51 ± 0.04	0.48 ± 0.03	0.79	0.60 ± 0.01
K	0.05 ± 0.02	0.05 ± 0.02	0.02	0.01 ± 0.00
Cl	0.04 ± 0.01	0.03 ± 0.00	0.06	0.08 ± 0.01
F	0.88 ± 0.05	0.89 ± 0.05	0.79	0.83 ± 0.04
Mn	0.03 ± 0.01	0.03 ± 0.01	0.02	0.03 ± 0.01
Zn	0.10 ± 0.02	0.10 ± 0.03	0.09	0.05 ± 0.02
Fe	0.03 ± 0.00	0.03 ± 0.01	0.03	0.01 ± 0.00

* The S.E.M. is based on the number of pulp stones in columns 1, 2 and 3, and on the number (n) of analysed points in column 4.

been calculated for each pulp stone and varied from 2.07 to 2.27 with a mean value of 2.19.

The average WDS concentrations of the Ca and P in dentine of the impacted third molar were 30.53% and 14.77% respectively, giving a Ca/P weight ratio of 2.07. The pulp stone which presented a morphological structure similar to dentine i.e. "true" denticle (sample n° 10) and another pulp stone (sample n° 9) which, on the opposite, did not exhibit tubules had a Ca/P weight ratio identical to that of dentine. The mean Ca/P weight ratio of the other eight pulp stones was 2.22.

The microanalysis of all the studied pulp stones revealed a concentration of 0.88% for F, 0.75% for Na and 0.51% for Mg. The other elements were only detectable under trace concentrations. The concentration of Fe was very low in all the microanalysed sample sites and in many analysed points, the quantity present was probably below the detection threshold.

In the two samples (n° 3 and 4) which presented a concentric organization, the spot was scanned from the center to the periphery on several radial lines for each structure in order to detect any progressive variation in the concentration of the analysed elements. However, the data showed that there was no statistically significant difference in the values.

One of the pulp stones (sample n° 1) which had an elongated shape with a longitudinal morphology was linearly scanned from its widest coronal base to its thinnest apical extremity. The Ca/P weight ratio was 2.22 in the coronal third, 2.14 in the middle third and 2.17 in the apical third.

When comparing the elemental composition between "true" (n° 10) and "false" pulp stones, there is a statistically significant difference at the 1% level ($p<0.01$) for three elements : Ca, Mg and Cl.

Discussion

The observation of the inner aspect of polished pulp stones confirms our previous work in scanning electron microscopy on fractured pulp stones which showed three modes of organization : a linear morphology, a concentric architecture or no peculiar organization (Le May and Kaqueler, 1991). However, this morphological classification does not correspond to differences in the inorganic composition of pulp stones since no major variation in the concentration of the studied elements was found between the three categories.

Since a spot by spot analysis does not give the accurate composition of the whole sample, the average values given here may not reflect the right concentrations. They can however be considered as being very close to them since no considerable variation has been observed within a single pulp stone.

Pulp stones contain two major chemical elements : calcium and phosphorus with a mean weight ratio of 2.19 which is slightly higher than the weight ratio of pure stoichiometric hydroxyapatite (2.15). Our value is lower than the Ca/P molar ratio of 1.86 (weight ratio of 2.40) found by Aoba et al. (1980) by chemical analysis of a pulp stone after dry-ashing but is almost identical to the value given by Vahl et al. (1973) who found by microprobe analysis of pulpal calcifications a Ca/P molar ratio of 1.70 (2.20). For Posner and Tannenbaum (1984), a Ca/P in excess of the pure hydroxyapatite ratio is due to some combination of missing phosphate ions, supplanted by carbonate and/or adsorbed calcium ions. The present electron probe micro-analysis confirms the previous studies which all indicated that pulp stones contain hydroxyapatite (Appleton and Williams, 1973 ; Vahl et al., 1973 ; Plackova and Vahl, 1974 ; Aoba et al., 1980 ; De Rysky et al., 1981). Recently, Arys et al. (1989) reported after microprobe analysis that brushite with a Ca/P weight ratio of 1.4 was present in peculiar kinds of spherulitic calcifications found in the pulp of primary molars.

Since the aim of this work was not to determine the accurate composition of dentine, the values given here for this mineralized tissue must only be considered as indicative since based upon an unique sample. They have been calculated in order to compare with the composition of the denticle localized in this tooth. Thus this calcification which presents numerous tubules, has a composition similar to that of dentine. Its Ca concentration is significatively lower than the Ca concentration in "false" pulp stones. It contains higher concentrations of Mg and Cl. The differences between the other elements (P, Na, K, F, Mn, Zn, Fe) are not significant. Its Ca/P weight ratio (2.07) is lower than for the other "false" pulp stones except one (sample n°9) which does not present tubules and has the same Ca/P weight ratio value. Although, this calcification found in an impacted third molar was oval and had a regular external aspect, it can not however totally be excluded that it could not represent a "real true pulp stone" but a rudimentary portion of interradicular dentine resulting of a developmental anomaly in the

radicular furcation area as frequently observed in mandibular third molar.

The pulp stones analysed contain other minor elements (Na, Mg, K, Cl, F, Mn, Zn, Fe) which could be acquired through pulpal vascularisation and might reflect the particular individual diet throughout life as in dentine (Posner and Tannenbaum, 1984). It is not possible to say if they are substituted for calcium or phosphorus in the lattice of hydroxyapatite or are part of other constituants. Like for other mineralized tissues, sodium and magnesium are the main minor ions. Concerning the fluoride content, it is reasonable to think that its concentration in pulp stones is largely dependent of the drinking water and external supply.

Since there is no other published study about the elemental composition of pulp stones, comparison can only be made with the dentine mineral phase. However, this comparison is difficult since the values reported in the literature for the dentine composition vary considerably. These variations are due to the differences in sampling preparations, the analytical techniques used and the biological variations from one tooth to another (Driessens, 1982). Verbeeck (1986), after a review of different studies on the dentine mineral content, provided average concentrations of 26.9% for Ca, 13.2% for P, 0.6% for Na, 0.8% for Mg, 0.06% for Cl, 0.02% for K. These values are close to those found in our study for dentine.

Our study does not confirm the results of Aoba et al. (1980) by EDS analysis who showed in the vicinity of one pulp stone surface, a peak of iron almost twice higher than the Ca peak. This peak could be an artifact. Thus Roomans (1988) had underlined that spurious peaks in the spectrum during EDS microanalysis may be considerable. More specifically, when these peaks are dealing with metallic elements and arising at the periphery of the sample, they may be due to the specimen holder or parts of the microscope.

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Discussion with Reviewers

T. Kodaka: Authors said that element values in Table 2 were close to those of dentine (Verbeeck, 1986). However, Ca and P in Table 2 were clearly higher than Verbeeck's and our EDS data in human dentine, although the other element values were similar to Verbeeck's data. Have you any comments about it?

Authors: It is well known that there is a great variability in the mineral content of human dentine. Thus, if the concentrations of Ca and P found in our study of an impacted third molar (30.53% and 14.77%

respectively) are higher than Verbeeck's data (26.9% and 13.2%), on the other hand, they are lower than the data of Driessens (1982) who reported mean concentrations of 37% for Ca and 18% for P in human dentine.

T. Kodaka: In Table 2, element values other than Ca and P were similar to those of a "true" pulp stone showing dentinal tubules. Have you any comments about it?
Authors: "True" pulp stones have been said to be relatively similar to reparative tertiary dentine since they are probably synthesized by identical pulpal cells. This fact could explain the similarities in the mineral compositions.

T. Kodaka: Mg-containing whitlockites often distribute in human several calculi and dentinal tubules of attrited dentine. Authors showed that Mg value in "false" pulp stones was higher than that in a "true" pulp stone. Have you any comments about Mg-whitlockite in "true" pulp stones?
Authors: Mg-containing whitlockite has effectively been identified in human calculi and attrited dentine. It is quite possible that pulp stones also contain this element. However, a higher concentration of Mg in "true" pulp stone compared to "false" pulp stones (and not the contrary) does not allow us to assert to the presence of Mg-containing whitlockite and further investigations using other analytical methods would be necessary to specify this point.