Comparative Physicochemical Analysis of Pulp Stone and Dentin

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Abstract

Introduction: Odontoblasts are responsible for the synthesis of dentin throughout the life of the tooth. Tooth pulp tissue may undergo a pathologic process of mineralization, resulting in formation of pulp stones. Although the prevalence of pulp stones in dental caries is significant, their development and histopathology are poorly understood, and their precise composition has never been established. The aim of the present study was to investigate the physicochemical properties of the mineralized tissues of teeth to elucidate the pathologic origin of pulp stones. **Methods:** Areas of carious and healthy dentin of 8 decayed teeth intended for extraction were analyzed and compared. In addition, 6 pulp stones were recovered from 5 teeth requiring root canal treatment. The samples were embedded in resin, sectioned, and observed by scanning electron microscopy and energy-dispersive spectroscopy. X-ray diffraction was performed to identify phases and crystallinity. X-ray fluorescence provided information on the elemental composition of the samples. **Results:** Pulp stones showed heterogeneous structure and chemical composition. X-ray diffraction revealed partially carbonated apatite. X-ray fluorescence identified P, Ca, Cu, Zn, and Sr within dentin and pulp stones. Zn and Cu concentrations were higher in pulp stones and carious dentin compared with healthy dentin. **Conclusions:** Pulpal cells produce unstructured apatitic mineralizations containing abnormally high Zn and Cu levels. *(J Endod 2016;42:432–438)*

Key Words

Carious dentin, copper, inflammation, pulpal calcification, zinc

Dental pulp and dentin act as a single physiological unit usually named the dentin-pulp complex. At the periphery of the dental pulp, dentinogenesis is the main biological activity of the odontoblasts, which are also mechanosensitive and immunocompetent cells (1). Odontoblasts are postmitotic cells but remain active throughout their lifetime. Odontoblasts secrete primary dentin during early tooth development, and this creates the first pattern of the tooth. When the tooth becomes functional, secondary dentinogenesis replaces primary dentinogenesis as the same odontoblasts secrete dentin at a lower rate (2).

Tertiary dentinogenesis is a third process mediated by odontoblasts and may be categorized into 2 types, reactionary and reparative. Reactionary dentinogenesis is the process whereby dentin is secreted in response to a local stimulus that reactivates the resting odontoblasts (3). Reparative dentinogenesis occurs when odontoblast cells die, thus initiating a complex regenerative process that allows the formation of reparative dentin after recruitment of progenitor cells, their differentiation into odontoblast-like cells, and activation of mineralized tissue secretion. These 2 types of pulp responses are quite well-described (4).

In addition to dentinogenesis in its varied forms, the occurrence of central pulpal calcifications has been reported in several clinical observational studies (5). Pulp stones are usually clinically identified on routine radiographic examination and in close association with deep restorations or repeated mild tooth injury over time. The frequency of occurrence of such calcifications is unclear. Reported rates vary from 4% (6) to 78% (7). It is commonly accepted that at least 50% of all teeth present 1 or more mineralized stones (8). Pulp stones have been observed in the dental pulp in all age groups, albeit with increased frequency in older age groups and insulted pulps (9).

The etiologic factors in pulp calcification formation are not well-understood. Several mechanisms have been proposed (8). Calcifications may develop around an area of damaged pulp tissue (eg, degenerating cells, blood thrombi, or collagen fibrils). Calcium phosphate crystals may also be deposited within the mineralizing cells. Calcifications replace the normal components of the pulp and might generate an inflammatory environment (8).

Different forms of pulp stones can be found: entrapped, adherent, or free within the pulp tissue (10). Their diameters vary from 50 μm to several millimeters (11). These calcifications may fully seal the pulp chamber volume and thereby complicate endodontic treatment. The calcifications are described as true (resembling dentin), false (composed of localized masses of calcified material), or diffuse (often found

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near blood vessels) (12). Their mineral phase consists of typical carbonated hydroxyapatites (13); however, their precise composition has never been established (14).

More knowledge on the crystallographic structure and physicochemical composition of pulp stones would contribute to a better understanding of their formation and the pathogenesis of pulp calcifications. In normal and ectopic mineralizations, the accuracy of physicochemical analysis has been demonstrated (15–18). The present study aimed to provide a detailed comparative description of the mineral phase that is in the pulpal calcification. To this end we used x-ray diffraction (XRD) and x-ray fluorescence (XRF) spectrometry and compared the mineral composition of pulp stones with their ultrastructure by using healthy (19, 20) and carious dentin (21) of the same teeth as references.

Materials and Methods

Collection of Samples

Healthy and Carious Dentin. Human freshly extracted permanent molars were obtained from patients requiring extractions after consulting in the emergency department (Hopital de la Pitié Salpêtrière). Selected teeth were unrestored but presented carious diseases with either occlusal or proximal active lesions. A carious zone and a healthy zone (free from caries) were isolated from each specimen. Caries involving more than half of the crown dentin (on a periapical radiograph) were excluded from the study. Eight teeth were selected from 8 different patients (3 women and 5 men, aged 20–73 years).

Pulpal Calcifications. Pulp stones were retrieved from teeth scheduled for endodontic treatment. In total, 6 pulpal calcifications had sizes ranging from 1.2 mm to 2.5 mm (mean size, 1.7 mm ± 0.4 mm). They were obtained from 5 patients (3 women and 2 men, aged 37–68 years).

Human sample collection complied with the Helsinki Declaration. According to French law on human research (Law 2007–1110, article 1211–2), tooth and pulpal sample collection (surgical waste) is allowed unless a patient objects; all patients were informed of the use of the samples and gave their consent.

Preparation of Samples. After collection, specimens were gently cleaned, and exogenous material was removed. Samples were rinsed with a phosphate-buffered saline solution at pH 7.4 (Invitrogen, Carlsbad, CA) and fixed with 4% formaldehyde in the same phosphate-buffered saline buffer (Electron Microscopy Sciences, Hatfield, PA). Samples were then dehydrated in a graded series of ethanol solutions (75% for 2 days, 90% for 2 days, and 100% for 2 more days). Samples were embedded into light-cured acrylate-resin (Technovit 7200 VLC; Heraeus Kulzer, Hanau, Germany) or not (for surface characterization).

Blocks were cut into 150-μm-thick slices by using a low-speed diamond saw (Isomet Low Speed Cutter; Buehler, Düsseldorf, Germany) under constant water irrigation. Surfaces of samples were polished by using increasing grit polisher disks.

Scanning Electron Microscopy

Each sample was sputter-coated with a 5-nm layer of platinum (SC7640 sputter coater; Quorum Technologies, Guelph, ON, Canada). A SUPRA 40 scanning electron microscope (Carl Zeiss, Oberkochen, Germany) was used to observe the microstructure. This field-effect gun microscope operates at 0.5–30 kV. Observations were made by using an Everhart-Thornley secondary electron (SE) detector at 5 keV. Polished section samples were observed with a backscattering electron (BSE) detector at 15 keV.

Energy-dispersive Spectroscopy

Sputter-coated samples were prepared. Imaging and microanalysis were performed on an SU-70 Hitachi SEM-FEG (Tokyo, Japan) and an X-Max 50 mm^2 Oxford EDX (Oxford Instruments, Concord, MA) detector.

X-ray Diffraction

Phase identification and crystallinity of the dental mineral part were evaluated by XRD. Experiments were carried out with a molybdenum rotating anode x-ray generator (Rigaku RU-H2R; Rigaku, Tokyo, Japan) coupled with multilayer W/Si optics delivering a focalized and monochromated (λ = 0.711 Å) x-ray beam of 800 μm × 1 mm size onto the sample. X-ray images were recorded with a MAR345 (@MAR Research, Hamburg, Germany) detector placed at a distance of 200 mm from the sample. Acquisition time for each measurement was 30 minutes. Diffraction diagrams were obtained by processing radial intensity integration of each image, and then the positions of the diffraction peaks were compared with reference files from the Joint Committee on Powder Diffraction Standards database.

X-ray Fluorescence

XRF allows precise determination of the elemental composition of the sample. Experiments were carried out with a molybdenum rotating anode x-ray generator (Rigaku RU200) coupled with multilayer W/Si optics delivering a focalized and monochromated (λ = 0.711 Å) x-ray beam of 150 μm × 150 μm size. Fluorescence spectra were measured with an energy-dispersive detector (SDD detector @Ketek), with a time acquisition of 1500 seconds each. XRF analysis was performed with PyMca software (22).

Statistical Analysis

Statistical comparisons were made by using the Wilcoxon-Mann-Whitney test (GraphPad Prism, LaJolla, CA). Data are expressed as the mean ± standard deviation.

Results

Representative scanning electron microscopy (SEM) images of the samples are shown in Figure 1, which were obtained by using the SE (Fig. 1*A* and *B*) and BSE modes (Fig. 1*C–F*). Pulp stones displayed a heterogeneous structure with smooth and regular surfaces (Fig. 1*A*). At high magnification, spherical calcifications of various sizes were identified on the surface, which could be elementary structures capable of aggregating and forming a stone core (Fig. 1*B*). In sections, the chemical structure was highly heterogeneous, showing alternating dark and light areas (Fig. 1*C*). The higher electron backscattering coefficient of the light areas indicates they are either composed of heavier chemical elements or more mineralized than the darker areas. Tubular areas were evident in regions sparse in pulp stones, suggesting the existence of tubular dentin-like matter (high magnification of blue box) (Fig. 1*D*). In other areas, concentric structures were formed by successive layers of material of different electron densities (Fig. 1*E*), showing a disorganized matrix pattern (high magnification of red box) (Fig. 1*F*). Localization of heavy elements was also performed by using energy-dispersive spectroscopy (EDS) (Fig. 1*G* and *H*). Zn (at energy of 8.63 keV) was detected in the core of pulp stones. The light areas previously mentioned did not show any significant signal of trace elements (eg, points 2, 3, and 6). The Pt peaks were induced by sputter-coating.

Two-dimensional XRD patterns for healthy dentin (Fig. 2*A*), carious dentin (Fig. 2*B*), and pulp stone (Fig. 2*C*) are illustrated in