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SCANNING ELECTRON MICROSCOPIC STUDY OF PULP STONES IN HUMAN PERMANENT TEETH

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Abstract

Scanning electron microscopy was used to examine pulp stones which are small calcified formations found in the coronal and/or radicular part of the dental pulp. Pulp stones range considerably in size and shape. Most are round or oval but others can be irregular and may correspond to a reduced duplicate of the pulp chamber anatomy. Both free and attached pulp stones were observed. The surface aspect was variable and frequently exhibited large resorption zones. Three characteristic features were observed on fractures : a) no characteristic organization where the mineralized mass is compact and homogeneous, b) a concentric architecture around an initiating central core, and c) a linear orientation along the pulp axis showing mineralized fibres and vessels. The findings suggest that the presence or absence of tubules should not be the sole factor for denticle classification since tubules can be also observed in "false" pulp stones. Cellular oval lacunae connected by long extensions were also found. Various stages of mineralization were seen, in particular, a deposition of fine needle shaped crystals on a collagenous matrix, and the fusion of numerous small calcospherules to the mineralized mass.

KEY WORDS : Pulp stone, denticle, dental pulp calcification, human permanent teeth, scanning electron microscopy.

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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 (HILL, 1934 ; HALL, 1968 ; SAYEGH and REED, 1968 ; BERNICK and NEDELMAN, 1975). They may also be present in permanent teeth that are erupted or impacted and free of pathology (THOMAS, 1940 ; LANGELAND and LANGELAND, 1965 ; NITZAN et al., 1986). Calcified masses have been also observed in deciduous teeth (KURODA and TAKEDA, 1986 ; YAACOB and HAMID, 1986 ; DARD et al., 1988 ; ARYS et al., 1989a et b).

KRONFELD (1933) classified pulp calcifications histologically into "true" and "false" pulp stones according to their resemblance to tubular dentine. "True" pulp stones contain dentinal tubules. They are rare and are mainly found in the apical portion of the radicular pulp. "False" pulp stones are more common and have an atubular structure.

Denticles can be also classified according to their relationship to dentine. Pulp stones located within the pulpal tissue are termed "free" denticles, whereas those bound to the dentinal wall are termed "attached". Others can also be entirely surrounded by dentine and are said to be "embedded".

Most histological studies have been performed in light microscopy (WILLMAN , 1934 ; HILL, 1934 ; THOMAS, 1940 ; KEREBEL, 1950 ; BURSTONE, 1953 ; JOHNSON and BEVELANDER, 1956 ; JAMES, 1958 ; SAYEGH and REED, 1968 ; SUNDELL et al. 1968 ; STENVIK and MJOR, 1970 ; MATENA, 1972 ; SELTZER et al., 1977 ; EAKLE and BELFIELD, 1983 ; MOSS-SALENTIJN and HENDRICKS-KLYVERT, 1983, 1988 ; TAGO, 1985 ; BEER et al., 1986 ; KURODA and TAKEDA, 1986 ; NITZAN et al., 1986 ; YAACOB and HAMID, 1986). Pulpal calcifications have been also studied in polarized light (CAVANHA, 1967 ; FOREMAN, 1984) and in transmission electron microscopy (APPLETON and WILLIAMS, 1973 ; PLACKOVA and VAHL, 1974 ; TAGO, 1985 ; DARD et al., 1988). Only a few studies on intrapulpal mineralizations have been conducted under a scanning electron microscope (SELTZER et

al., 1977 ; AOBA et al., 1980 ; MERIDA FUENTES, 1980 ; DE RYSKY et al., 1981 ; BINUS and STIEFEL, 1983 ; FOREMAN, 1984 ; SCOTTI and MORABITO, 1986 ; ARYS et al., 1989a).

The present study was designed to investigate macroscopic and microscopic aspects of human intrapulpal mineralized bodies under a scanning electron microscope to obtain new and detailed morphological information.

Materials and Methods

One hundred and fifty eight erupted and unerupted permanent teeth were extracted in patients with no evidence of general pathology. The teeth were stored in 10 per cent neutral-buffered formalin and were radiographed on highly sensitive X-ray films (KODAK, Industrex M) using a dental X-ray generator (MATIC TROPHY, France) in order to detect pulp stones. The films were exposed at 60 kVp, 10 mA, 50 cm focal distance with a 12 sec exposure time.

Teeth evidencing pulpal calcifications were then longitudinally fractured and the exposed mineralized bodies were removed from the connective tissue. Among the more voluminous free pulp stones, some specimens were mechanically fractured under a binocular microscope. Attached pulp stones were cleared of the surrounding connective tissue in order to observe their surface and their relationship with the dentinal wall.

In addition, 17 denticles were extracted during clinical endodontic procedures on various human teeth and were immediately placed in a cacodylate buffered glutaraldehyde solution.

Some pulp stones were submitted to 10% sodium hypochlorite in order to eliminate residual soft tissue.

All specimens were rinsed in running tap water, dehydrated in gradient alcohols, critical-point dried and sputter-coated with gold prior to examination in a Cambridge Stereoscan 600 or a Jeol 25 S III scanning electron microscope.

Results

External macroscopic and microscopic features

The data show that intrapulpal mineralized bodies vary considerably in shape, size and localization. Most are round or oval (Fig. 1 and 5) but can also exhibit a more complex morphology, at times reflecting a reduced duplication of the pulp chamber anatomy. Pulp stones found in the coronal pulp of upper molars can thus have a tripod morphology, each extension corresponding to the beginning of the three radicular canals (Fig. 2). Others, coming from the large palatal root of the upper molars or a distal root of the lower molars, present a tapered rod-like shape with a wide coronal base and become thinner toward the apical region (Fig. 3). A thin non-mineralized radicular pulpal filament can be seen emerging at the apical end of the elongated denticles (Fig. 4).
All the previously described pulp stones were isolated from the pulpal tissue and therefore classified as free pulp stones. Some, although non-adhering to the dentinal wall, were difficult to collect because they were fully trapped in the radicular pulp chamber. They were clearly visible after longitudinal fracture of the tooth. Figure 5 presents an example of an oval free denticle caught in the radicular canal of a canine.

Observation of the longitudinally fractured teeth with attached pulp stones serves to study their relationship with their dentinal base (Fig. 6). The external features of the calcified body differ from the dentine where the openings of the dentinal tubules are visible. However the boundary between the two mineralized structures could not always be determined accurately.

All the pulp stones exhibit a wide range of surface features. Generally, the surface is fairly smooth and compact, especially in round or oval pulp stones. In contrast, irregularly shaped pulp stones generally present a rough surface with domes of various sizes and lacunae of different depths, and the pulp stone as a whole exhibits a thin leaf or scale-fish microrelief (Fig. 7).

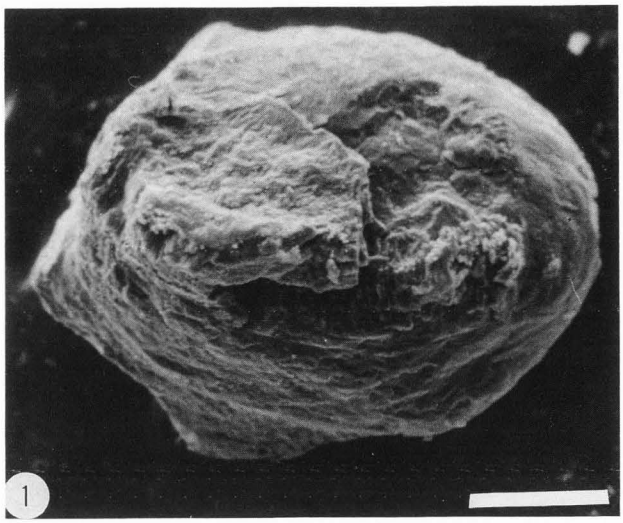
In addition to these features, resorption areas varying in size are very often found at the surface. These regions are characterized by the presence of lacunae of different sizes and depths, separated by a network of small irregular ridges (Fig. 8). At higher magnification, the collagen fibres exposed by the resorption process are clearly visible (Fig. 9). These resorption areas are particularly frequent on voluminous pulp stones, and especially on the surface opposite the dentinal wall. Figure 10 presents an example of a denticle resorption area facing the dentine and the narrow space between the two mineralized structures.

Figure 1 : A free, round mineralized nodule showing a fairly smooth surface. Bar = 200 µm.

Figure 2 : A very irregular free pulp stone found in the pulp chamber of an upper molar. Its general configuration is a reduced homothetic duplication of the anatomy of this region of the pulp chamber. The entry of two out of three radicular canals are visible (→). As a whole, its mass appears budded. Bar = 1 mm.

Figure 3 : Mounting of four photographs of a free, long needle-like denticle found in the palatal canal of an upper first molar in which the remaining pulp was particularly fibrotic. Four holes are roughly equidistant and a longitudinal tubular structure runs along the axis of this denticle (→). Bar = 1 mm.

Figure 4 : Extremity of an elongated pulp-stone showing a thin residual pulpal filament. Bar = 1 mm.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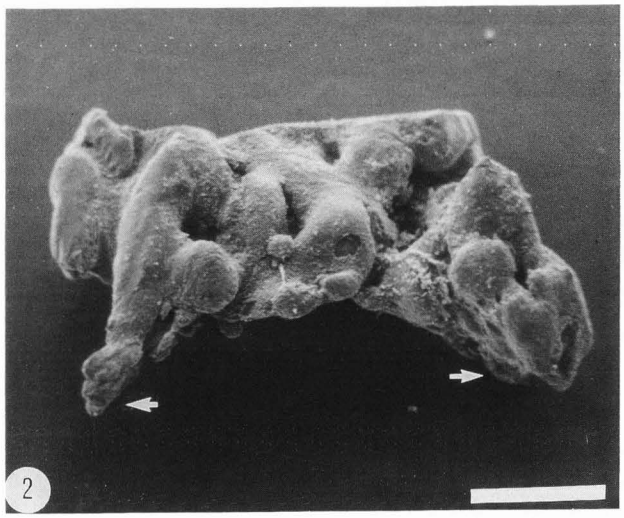
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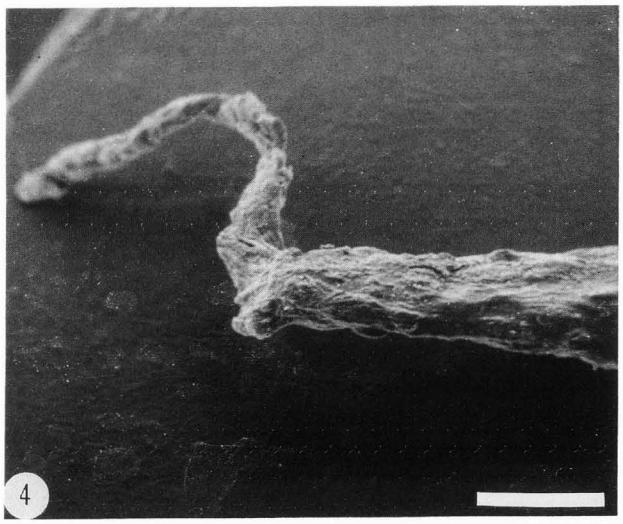
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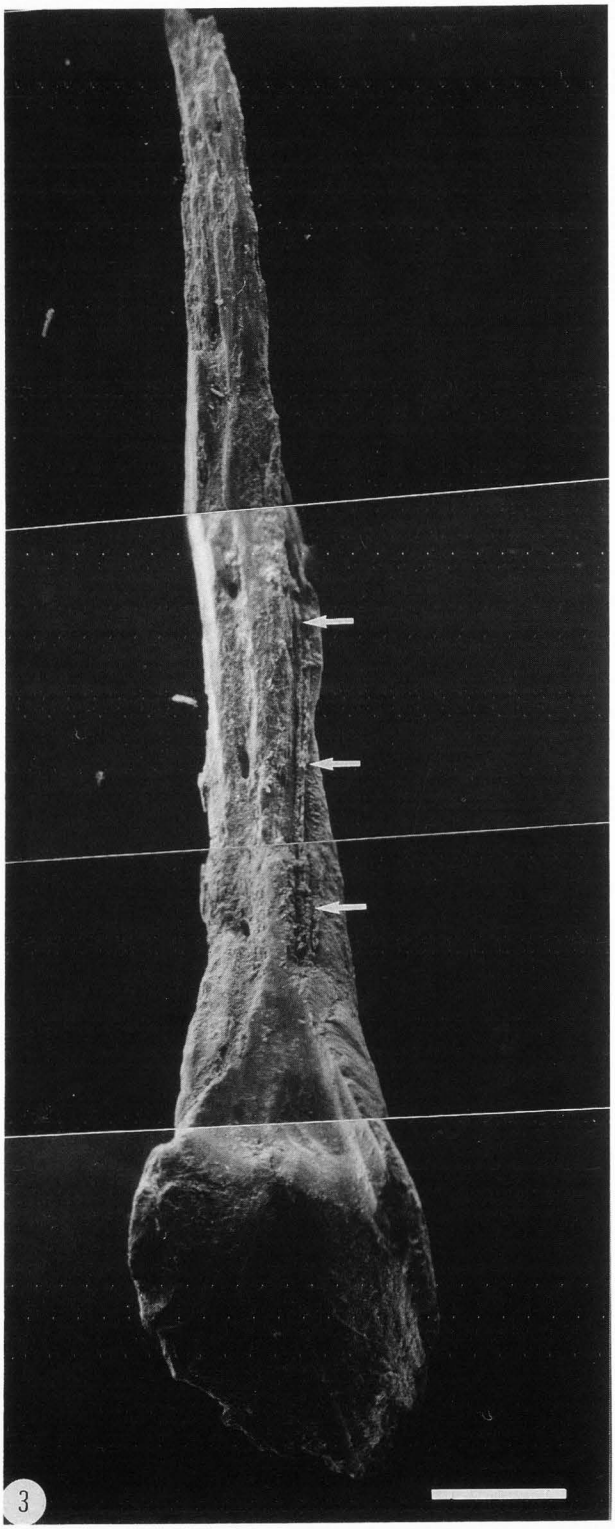
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Internal macroscopic and microscopic features on fractures

Fractured pulp stones can exhibit three types of internal architectural features.

The first type is represented by pulp stones without an organized architecture, the fractured surface is homogeneous and compact. Only one case of attached denticle entirely traversed by numerous parallel tubules similar to those of dentine was observed here.

The second type displays concentric layers organized around a central nidus in the "onion skin" fashion often reported in light microscopic studies (Fig. 11). These pulp stones were classified as "false" and are considered to be atubular. However, the external layers of some denticles present irregular and scattered tubules showing a well-defined peritubular ring, bifurcations and accessory canalicules similar to those of dentine (Fig. 12). Occasionally, the fracture plane passes through the denticle center completely, thus revealing a smooth and compact nodular mass constituting the initiating core of the pulp stone (Fig. 13). Its homogeneous structure contrasts with the surrounding layers but its true nature and composition cannot be identified by the scanning electron microscope.

The third type consists of elongated radicular pulp stones which after fracture display a linear organization along the longitudinal axis of pulpal nerves and vessels (Fig. 14). At higher magnification, mineralized fibres and vessels are clearly visible and numerous small globular bodies near them can be observed (Fig. 15). They occasionally exhibit tubules in their widest coronal portion, similar to those of dentine although less numerous (Fig. 16). In contrast to the irregular and more or less anarchically disposed tubules found in the concentric pulp stones described above, these tubules are more highly ordered since they are parallel and arranged along the longitudinal axis of the pulp stone.

In some cases, fractured denticles show cellular lacunae. These are oval in shape and have extended canalicules (Fig. 17) which may connect them to one another (Fig. 18).

In some areas, a loose organic network is visible, and its fibrils show no particular orientation. At higher magnification, the characteristic collagenous striation is apparent (Fig. 19).

At proximity, a downy aspect predominates resulting from the deposition of fine needle shaped crystals on the collagen fibrils (Fig. 20).

In pulp stones with tubules, the mineralization process takes place around the tubule openings, the crystals are located around the tubules with no particular orientation. Some tubules are already surrounded by a smooth and compact ring similar to peritubular dentine (Fig. 21).

Another aspect of the mineralization of denticles is illustrated in figure 22 where many small globular bodies are seen lying on the surface. Most of these calcospherules are well individualized but others coalesce with the mineralized mass.

These different structural features correspond to various stages of mineralization of the pulp stones.

Discussion

Pulp stones may exhibit a wide variety of sizes and shapes. In most cases they are round or ovoid, but may also present a very irregular morphology. The largest may even reproduce an internal cast of the pulp chamber portion where they were found. When they are located in the radicular pulp canal and blocked between the dentinal walls, they constitute an obstacle which is more or less negotiable by endodontic instruments, thus rendering a correct root canal filling difficult or even impossible.

After fracture, the pulp stones present three different internal structures.

The laminated disposition is the result of progressive concentric growth of the mineralized mass by apposition of successive lamellar layers giving the whole structure an onion skin aspect frequently described in the literature. JOHNSON and BEVELANDER (1956), FOREMAN (1984), TAGO (1985), NITZAN et al. (1986) only observed this type of false concentric pulp stone in their studies.

Figure 5 : This large oval denticle is situated in the middle root of a canine almost entirely obliterating the radicular pulp chamber (PC). The fracture plan has preserved the integrity of the pulp stone (PS) which appears to protrude between the dentinal walls. (D = dentine). Bar = 1 mm.

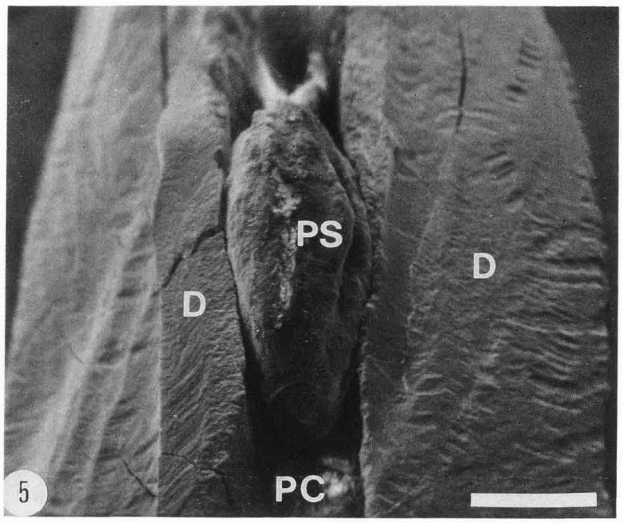
Figure 6 : Example of an attached pulp stone (PS) located in the root canal (RC). Its peduncle is large and its junction with the dentinal wall is hard to distinguish (→). Bar = 400 µm.

Figure 7 : Very irregular pulp stone surface consisting of protuberances and depressions surrounded by scale-fish formations. Bar = 200 µm.

Figure 8 : A large resorption area at the surface of a hypochlorite-treated voluminous denticle. It is characterized by the presence of numerous lacunae, each corresponding to a large resorbing cell. Bar = 100 µm.

Figure 9 : Fibrillous pins (*) raised up from the surface in a resorption area where the fibrous matrix has been revealed. Bar = 20 µm.

Figure 10 : Area of pulp stone resorption facing the dentinal wall where longitudinal tubules are clearly visible. A gap remains between the pulp stone (PS) and the dentine (D) where no resorption process is visible. Bar = 100 µm.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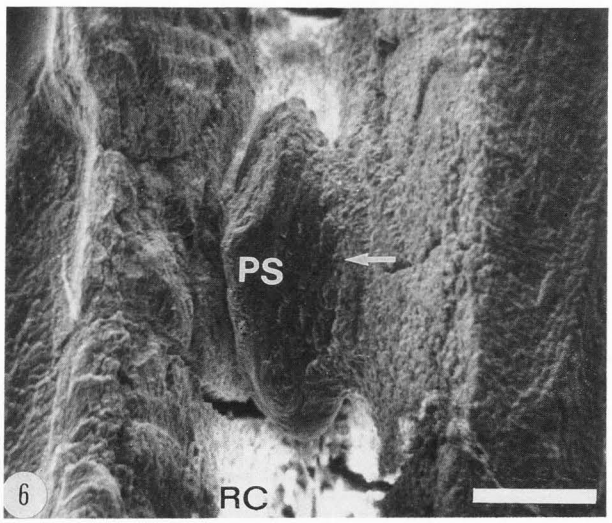
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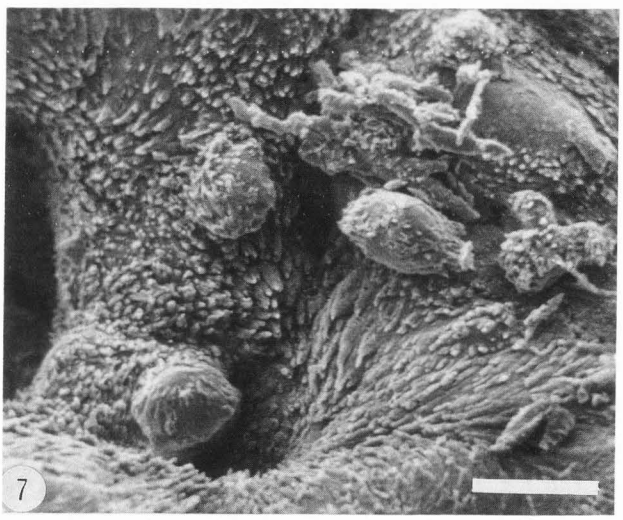
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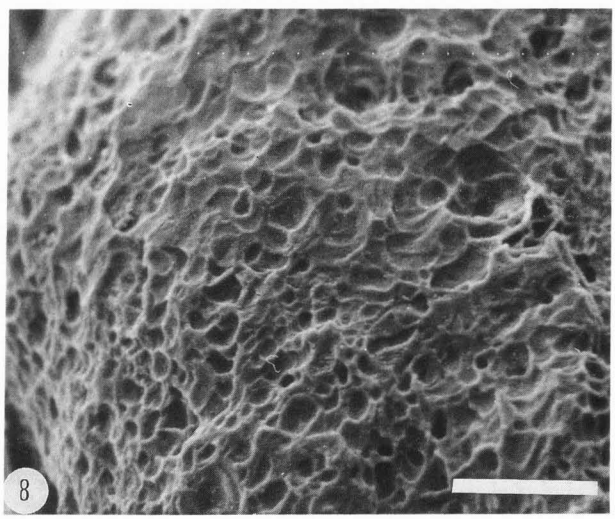
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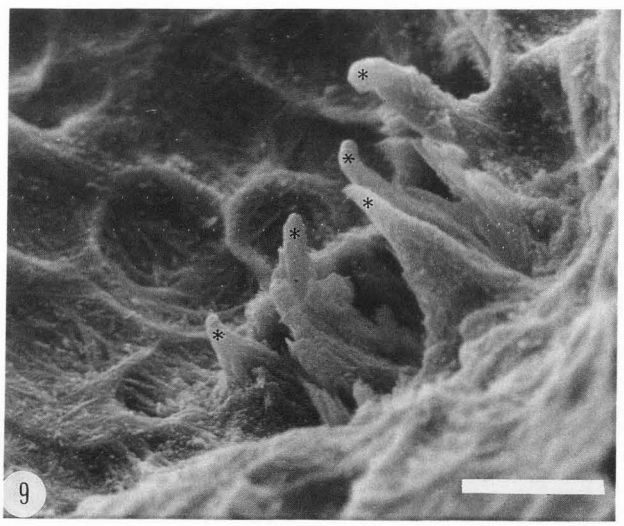
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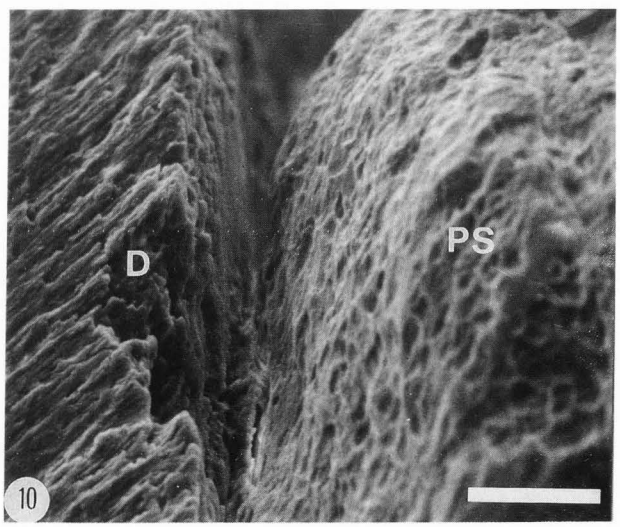
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Nevertheless, all denticles do not exhibit a concentric organization. In many cases, the fractured surface is compact and homogeneous in the scanning electron microscope. However, some authors in light or transmission electron microscope studies occasionally describe a radial organization of the peripheral collagenous fibrils perpendicular to the surface and undergoing mineralization (BAUME, 1980 ; TROWBRIDGE and KIM, 1987).

The third category, seldom reported in the literature, is the linear mode. The pulp stone is elaborated along the axis of the pulpal collagenous fibres and blood vessels. This arrangement has been particularly well described in the case of diffuse calcifications which are small islands of mineralization appearing in light or transmission electron microscope that are closely associated with blood vessels and nerves, or collagen bundles. EAKLE and BELFIELD (1983), FOREMAN (1984) and ARYS et al. (1989a) underlined that these small calcified spicules can coalesce in large pulp stones similar to those observed in this study. In most recent histological textbooks, pulpal calcified bodies are divided into two separate groups : pulp stones and diffuse calcifications (PROVENZA, 1972 ; CAPPUCCINO and SHEEHAN, 1978 ; TORNECK and TEN CATE, 1981 ; OSBORN and TEN CATE, 1983 ; MJOR, 1983, 1986 ; SELTZER and BENDER, 1984 ; MOSS-SALENTIJN and HENDRICKS-KLYVERT, 1985 ; WALTON et al., 1985 ; AVERY, 1986). However, the present data suggest that diffuse calcifications do not constitute a specific category but may simply represent the first observable stage of a mineralization process which could lead to the formation of large longitudinal denticles.

The exact nature of the central core observed in some pulp stones which probably generates the mineralization process cannot be identified accurately by the scanning electron microscope. The core could initiate from a blood clot formed after a vascular thrombus, associated with disturbances in vascular circulation observable even in young pulps (SAUNDERS and ROCKERT, 1967 ; SUNDELL et al., 1968 ; RUSHTON et al., 1970). Alternatively, it could be made up of collagen fibres or degenerated or necrotized cells (SELTZER and BENDER, 1984 ; AVERY, 1986 ; TROWBRIDGE and KIM, 1987 ; GROSSMAN et al., 1988 ; TORNECK, 1989). These elements could induce local alkalinity attracting calcium salts (SUNDELL et al., 1968 ; SMULSON and SIERASKI, 1989).

Resorption areas varying in size were frequently observed at the surface of the pulp stones, suggesting that they do not solely undergo a growth process. Note that in the case of voluminous denticles, only the surface facing the dentinal wall is resorbed and a narrow gap persists between dentine and pulp stone. It is difficult to determine which factor induces this local selective resorption. Inflammation which is often the case in dental tissue resorption, does not appear to be involved. Rather, inflammatory processes associated with the denticle are rarely described in light microscopy (BEER et al., 1986) and in addition there is no way of assessing whether the pulp stone itself is the etiological factor of the inflammation or whether it is purely a simple pulpal inflammation independent of denticle presence. Further, resorption only affects the pulp stone, since no comparable processes were observed here on the dentinal wall facing the denticle. Thus, the tooth apparently preserves its integrity by forming an odontoclastic cell barrier, as reflected by the presence of numerous resorption lacunae, each corresponding to a resorbing cell. When the resorbing capacity is bypassed, the pulp stone may fuse to the dentine by progressive growth. As long as the secondary and/or tertiary dentine apposition progresses, the pulp stone is gradually embedded into the dentinal tissue.

Direct observation in this study of cellular lacunae inside the denticle mass is not sufficient to assess whether they are the location of pulp cells trapped during the denticle growth or, on the other hand, whether they correspond to cells involved in the elaboration of pulp stone mass. However, the long extensions frequently connecting the lacunae are similar to the osteocyte lacunae network and may be one indication that they play a role in denticle formation. These internal denticle cells could play a major role in the synthesis of a collagenous matrix which is frequently seen in the non-mineralized zones of pulp stone.

Figure 11 : Fracture of a spherical free pulp stone showing the concentric layers yielding the classic "onion skin" aspect. This kind of formation is classified as a false pulp stone. Bar = 100 µm.

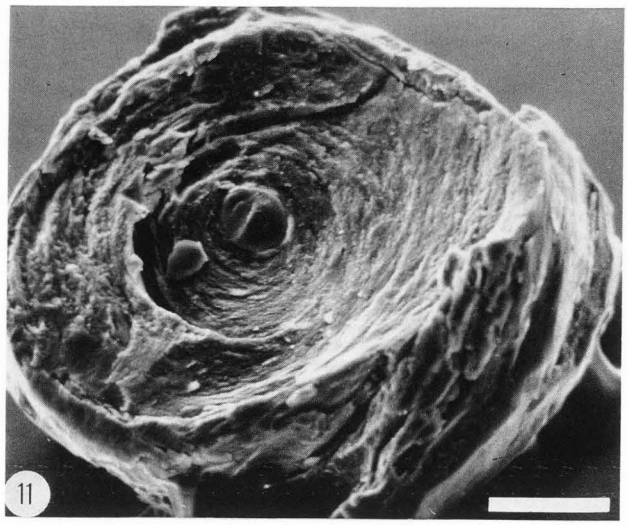
Figure 12 : High magnification of the periphery of the previous denticle showing a longitudinaly fractured tubule with a branching canalicule (*) and openings of lateral ramifications (→) very much like what is found in dentinal tubules. Bar = 4 µm.

Figure 13 : The central nucleus of this concentrically laminated denticle appears extremely dense and homogeneous compared to the surrounding granulous layers. Bar = 20 µm.

Figure 14 : Elongated pulp stone longitudinally fractured and submitted to hypochlorite exhibiting a linear and fibrillar organization along the radicular axis. Bar = 200 µm.

Figure 15 : Higher magnification showing a group of calcified vascular vessels (V) with spherical nodules at their surface. Bar = 20 µm.

Figure 16 : Numerous longitudinal and parallel tubules (→) lying in the coronal part of a fractured elongated pulp stone. This wide base corresponds to the most calcified and compact portion of the elongated pulp stone. Bar = 20 µm.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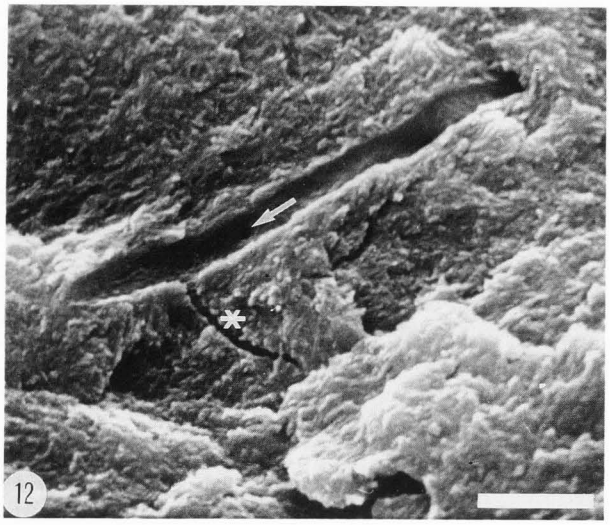
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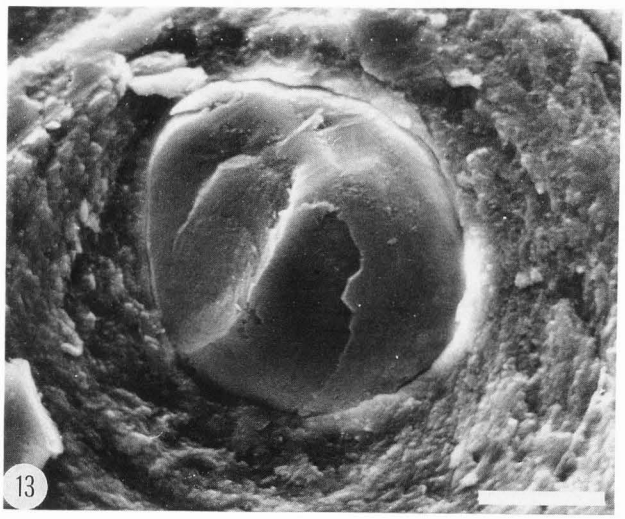
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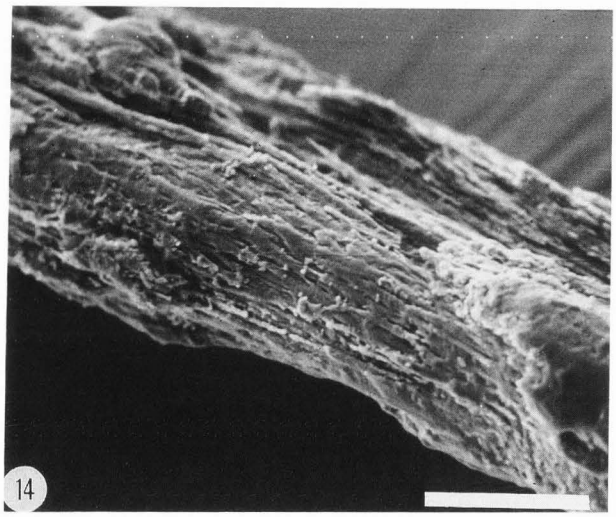
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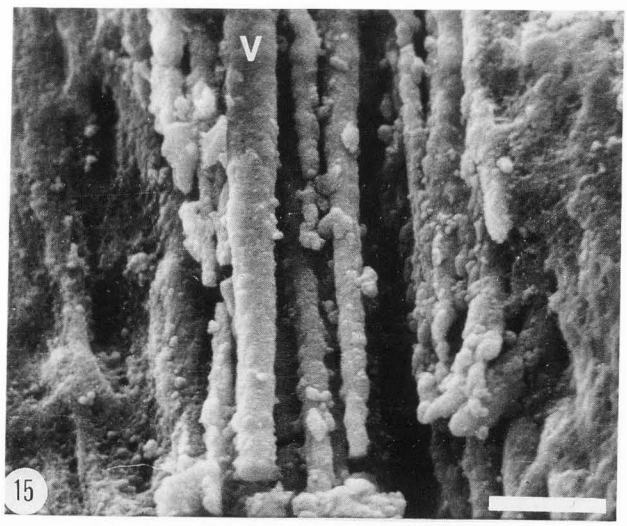
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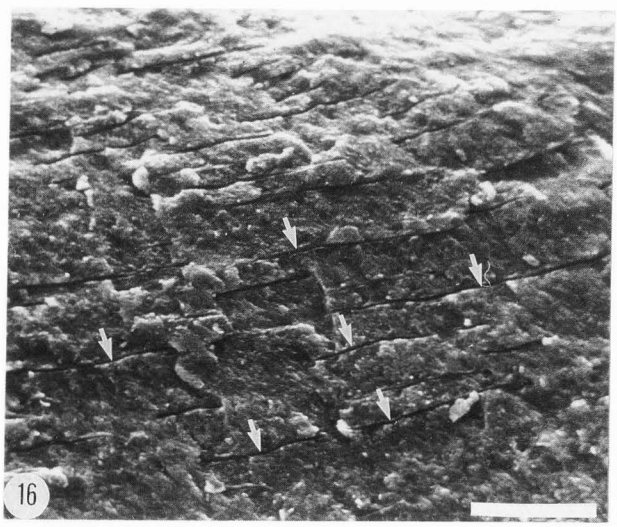
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The calcospherules observed in pulp stone areas in the active mineralization stage have already been reported in scanning electron microscopy by AOBA et al. (1980) and in transmission electron microscopy by DARD et al. (1988). They are smaller than the dentinal calcospherites observable at the mineralization front after elimination of the predentine.

The needle-shape crystals observed in this scanning electron microscopy study have also been described in transmission electron microscopy at the denticle periphery by APPLETON and WILLIAMS (1973), PLACKOVA and VAHL (1974), TAGO (1985), DARD et al. (1988). These authors consider they correspond to hydroxyapatite.

MOSS-SALENTIJN and HENDRICKS-KLYVERT (1983, 1988) have argued that the customary classification into true and false denticles is improper and should no longer be used since most of the calcified bodies observed in their light microscope study are composed of a combination of tubular dentine and atubular calcified material. They suggest another classification of calcified bodies based upon their mode of genesis. They distinguish "denticles", formed after an inductive interaction between pulp tissue and intra-pulpal cell remnants of the Hertwig epithelial root sheath, from "pulp stones" formed around foci of calcifying pulp tissue components. They suggest that the term "denticle" which is often used indiscriminately for "pulp stone" in the literature, should be reserved for calcified bodies with a central cavity filled with epithelial cell remnants. In this study, no mineralized mass of this type was observed but when pulp stones were mechanically fractured, the fracture plane rarely passed through the center of the structure. Moreover, the denticles described by these authors were small bodies varying from 180 to 260 µm in size whereas the smaller pulp stones observed in this scanning electron microscopy study measured about 400 µm.

Our study nevertheless confirms that the presence or absence of tubules, which is the basis of historical classification into true and false pulp stones cannot serve as the sole criterion for classification. Tubules similar on all points to those found in the dentine were frequently observed here in concentric pulp stones which are classified in the false type and considered as being atubular. However, these tubules are not very numerous. In most cases, they are scattered and do not exhibit any particular orientation except in the case of longitudinally oriented denticles where they are parallel to the general axis of the structure.

In this study, only one case of pulp stone entirely crossed by numerous tubules and thus exhibiting an architecture similar to dentine was observed. Importantly, this denticle was attached to the dentinal wall. Some authors contend that true pulp stones do not exist and argue that these structures simply correspond to dentinal excrescences connected to the dentinal wall by a peduncle varying in thinness which thus appear to be free in the pulpal tissue on serial histological sections. RUBACH and MITCHELL (1965), SAYEGH and REED (1968) and FOREMAN (1984) posit that true pulp stones are artifacts. However, the numerous observations of fractured human teeth made in this laboratory with scanning electron microscope have never pointed to the existence of such "dentinal pearls" on the dentinal wall of the pulp chamber (KAQUELER et al., 1989).

To sum up, pulp stones are polymorphous and atypical calcifications located within the pulpal tissue which does not mineralize in normal conditions. There are other examples in the human body of abnormal individualized calcified masses : salivary calculi, kidney stones, gallstones... But in these cases, the formation process differs since it bears more resemblance to a precipitation of mineral salts within an organ, a gland or its excretory canal. In the case of pulp stones, the elaboration mechanism is more complex since it does not consist of a simple precipitation of inorganic elements but involves a mineralization process similar to those observed in bone or dentine. As a general rule, there is a first stage consisting of the formation of a collagenous matrix which mineralizes in the second stage. Thus pulp stones are not only a mere histological curiosity but rather an useful model of heterotopic mineralization. In particular, it would be of interest to study their mineral micro-composition in order to specify their constitution, and to determine whether there are significant variations in the composition of the different pulp stone categories for purposes of comparison with other mineralized dental tissues.

Figure 17 : Empty cellular lacuna (CL) with a long canalicule within the denticle mineralized tissue. Bar = 10 µm.

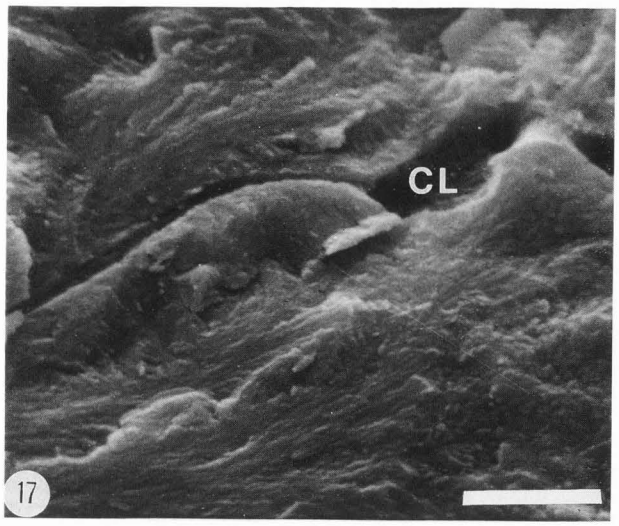
Figure 18 : Two adjacent lacunae connected by a lateral ramification (→) reminiscent of osteocyte or cementocyte lacunae. Bar = 10 µm.

Figure 19 : Detail of a fibrous matrix at high magnification allowing for observation of the collagenous striation. Bar = 4 µm.

Figure 20 : Irregular deposition of fine crystals masking the collagen matrix. Bar = 10 µm.

Figure 21 : Tubular denticle area undergoing mineralization showing crystal deposition around the tubule openings. One tubule (→) exhibits a regular and more mineralized collar. Bar = 20 µm.

Figure 22 : Numerous calcospherules lying on the surface of a pulp stone. These small concretions are part of the mineral apposition process. Bar = 10 µm.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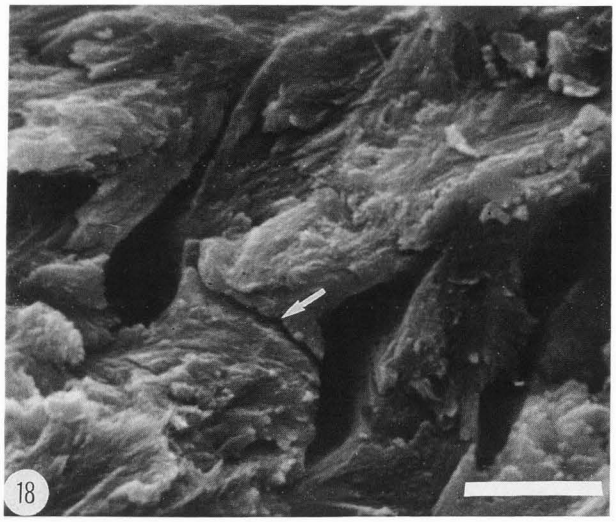
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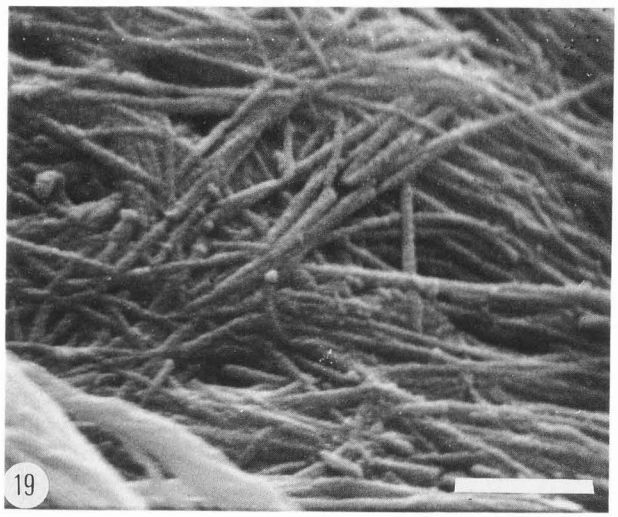
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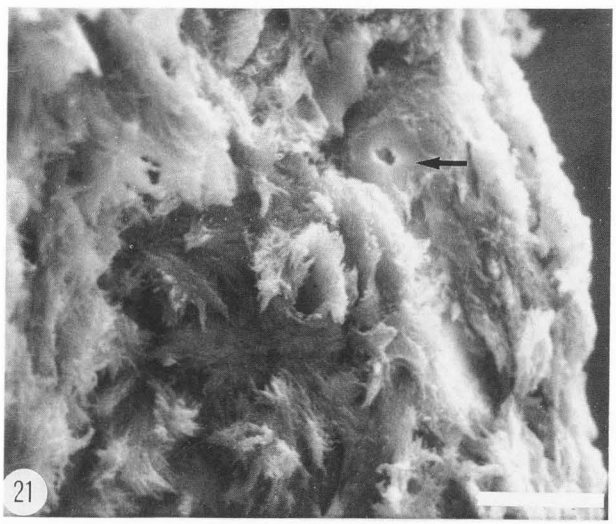
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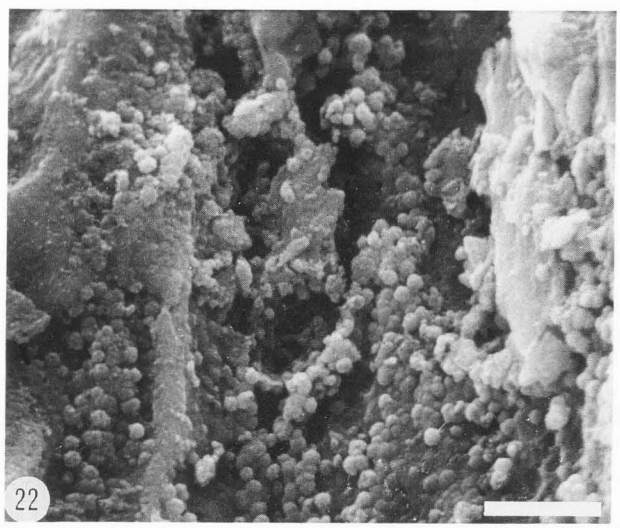
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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.