Pulp Aging: Fibrosis and Calcospherites

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8.1 Pulp Anatomy During the Aging Process

Dentinogenesis is a constant process as long as a tooth is alive. During the early stages, the daily dentin formation reported is about 10 μm/day, whereas at later stage of crown formation, 4 μm is formed daily. The rate of dentinogenesis decreases slowly, but the phenomenon is still occurring even in the elderly.

Primary dentin formation takes place at early stages, between the beginning of dentinogenesis, when pre-polarized odontoblasts are facing a basement membrane and when the erupted teeth come into contact with their antagonists, when it ends. The initial formation of mantle dentin leads to an atubular dentin, containing high amount of proteoglycans. In the root, two superficial layers have been reported: the granular Tome's layer followed by the hyaline Hopewell-Smith layer. They are characterized by the presence of bent tubules, with minute diameters, large globules, and interglobular spaces.

Secondary dentin formation follows circadian rhythms, characterized by repetitive von Ebner lines separating 4 μm thick dentin layers. At each

4–5 von Ebner lines, at about 20 μm intervals, a more accentuated Owen's line is found, which is less mineralized and displays a higher content of organic matrix. There is no clear-cut explanation for this other periodicity. Because it is centripetal, dentin is formed to the detriment of the close space occupied by the pulp. As long as a tooth is alive, dentin will be produced at a decreasing speed and gradually reduce the space occupied by the dental pulp. Pathologic dentinogenesis, such as *dentinogenesis imperfecta* or *dentin dysplasia*, contributes to reduce dramatically the width of the pulp, especially in the root part, where apparent pulp closure may occur, whereas some remnants are maintained in the coronal pulp.

Tertiary dentin is formed in reaction to a carious lesion or in case of rapid abrasion. The release of cytotoxic molecules of the monomer of restorative resins may also induce the formation of reparative dentin, another name given to tertiary dentin. Facing pulp exposure due to the rapid progress of the carious lesion, reparative dentin formation may protect the pulp from invading bacteria.

Depending on the species, it has been reported that the dentin layer located in the upper part of the pulp chamber increases in thickness more rapidly than the floor of the pulp chamber. This is the case for rat's molar, but the reverse is also seen in the human situation [1]. Dentin deposition along the lateral walls contributes also to reduce the pulp chamber volume, but at a slower rate

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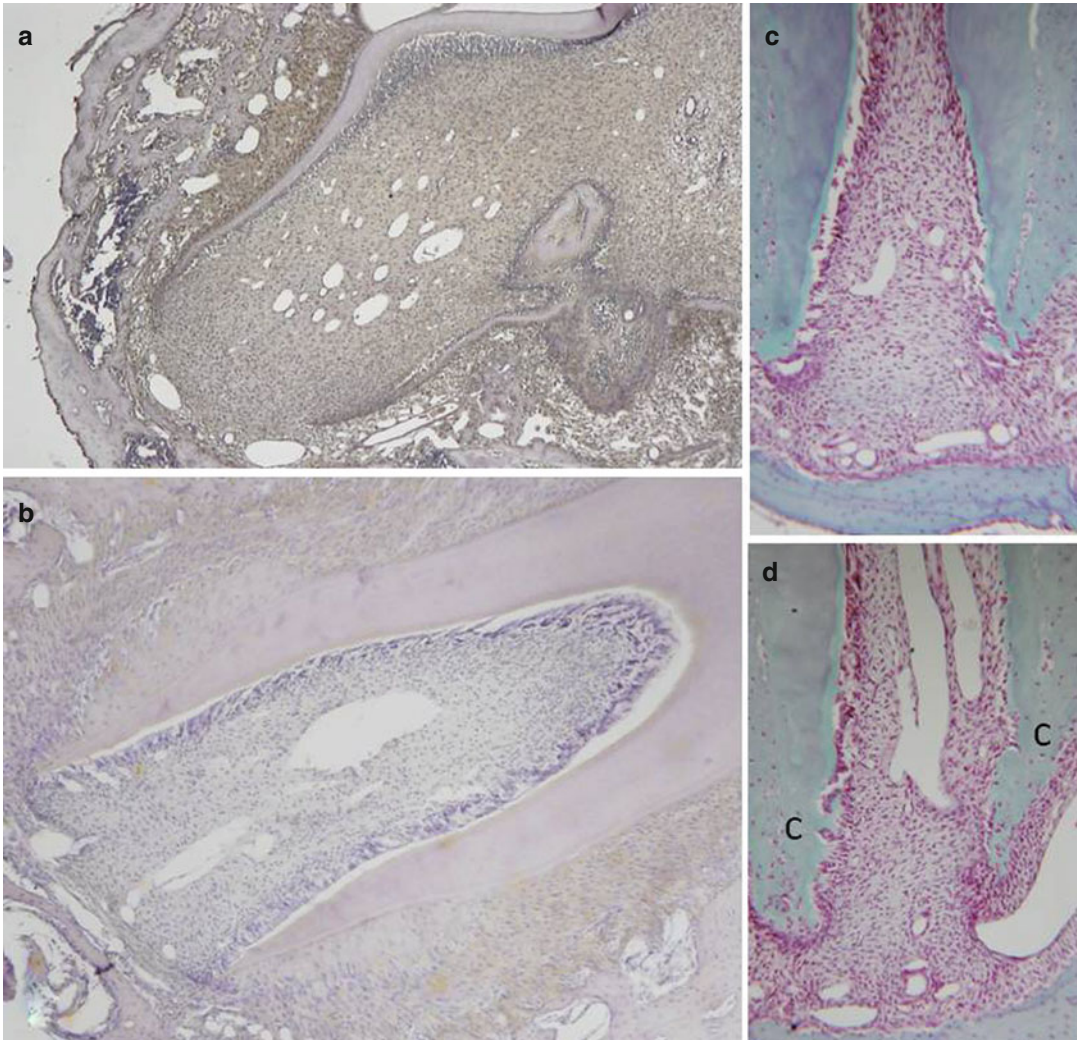
Fig. 8.1 During the formation of the root, the apical part is widely open at the onset of root formation (**a**) and gradually restrained in the three successive stages (**b**–**d**). Cementum (*C*) deposition contributes to apexification

(Figs. 8.1a–d, 8.2, and 8.3). However, it should be noted that pulp horn stays non-mineralized. Other investigations concluded with the lack of difference between the increased formations of dentin in the horn region compared with the floor. The mesiodistal diameter decreased with age, faster between patients age 20 and 40 and slower afterward [2].

In the root, tubular orthodentin formation is seen in the labial and lingual parts, whereas fibrodentin is added in the mesiodistal surfaces of the root canal lumen. This age-related variation gave the root canal lumen an oval profile. The dentin heterogeneity leads to a smaller pulp volume in the elderly (Fig. 8.4).

During aging, the dental pulp is enriched gradually by fibrous bundles of collagen. Noncollagenous proteins located in the so-called ground substance are somehow restricted, whereas the pulp is enriched in lipidic inclusions and pulp stones. The pulp response time is increased in older people, whereas pain intensity decreases.
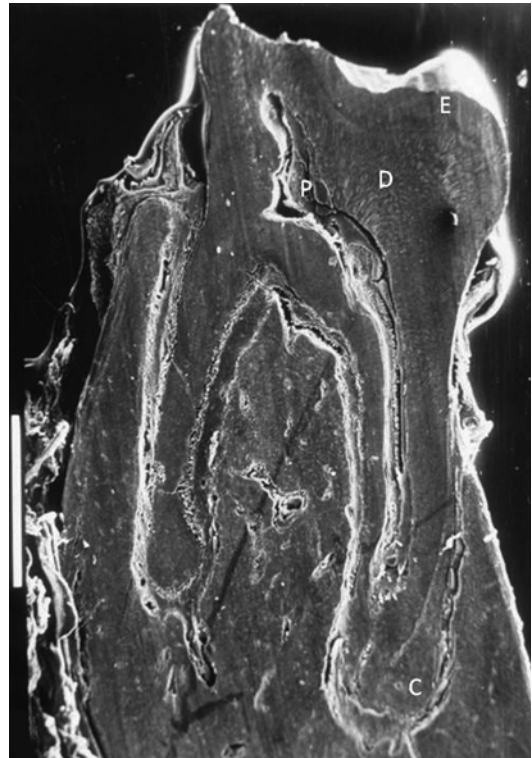
With age *odontometric changes* have been noted with respect to pulp cell density, pulp

Fig. 8.2 At date 21 the full length of the root is achieved, but there is still a gradual reduction in width of the pulp, together with an increased dentin formation in the root
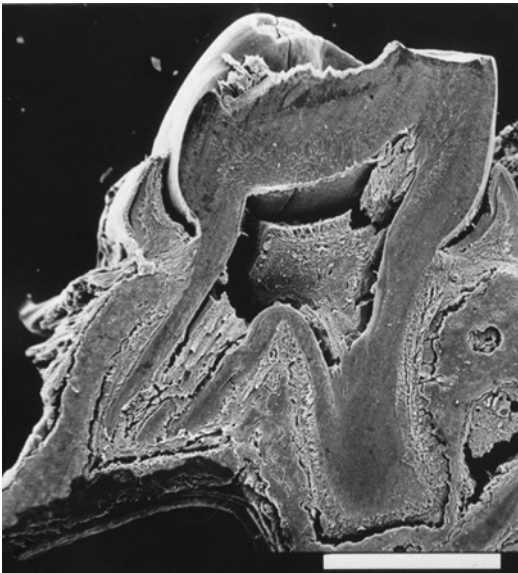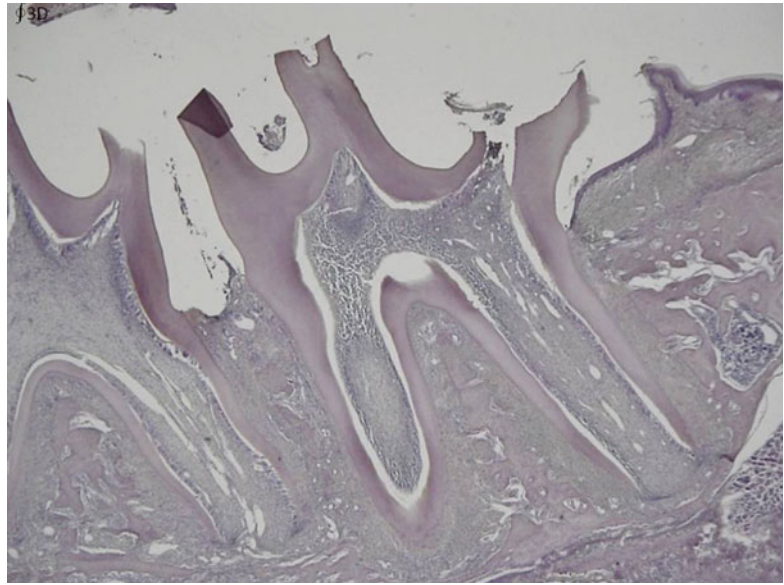

Fig. 8.3 Scanning electron microscopy (SEM). Longitudinal section of a mandibular molar during the formation of roots. Bar, 1,000 μm

Fig. 8.4 SEM. Longitudinal section of a mandibular molar. The roots are totally formed. The dentin (D) thickness is maximal, and the pulp (P) volume is reduced. Cementum (C) extends and is observed in the apical part of the roots

area, and dentinal thickness. Cell density of odontoblasts, sub-odontoblasts, and pulp fibroblasts is decreasing. There is also a decrease of age-related changes in the root, which is more pronounced than in the crown. Cell density in

Fig. 8.5 Three-month-old rat. The apical part of the root is closed
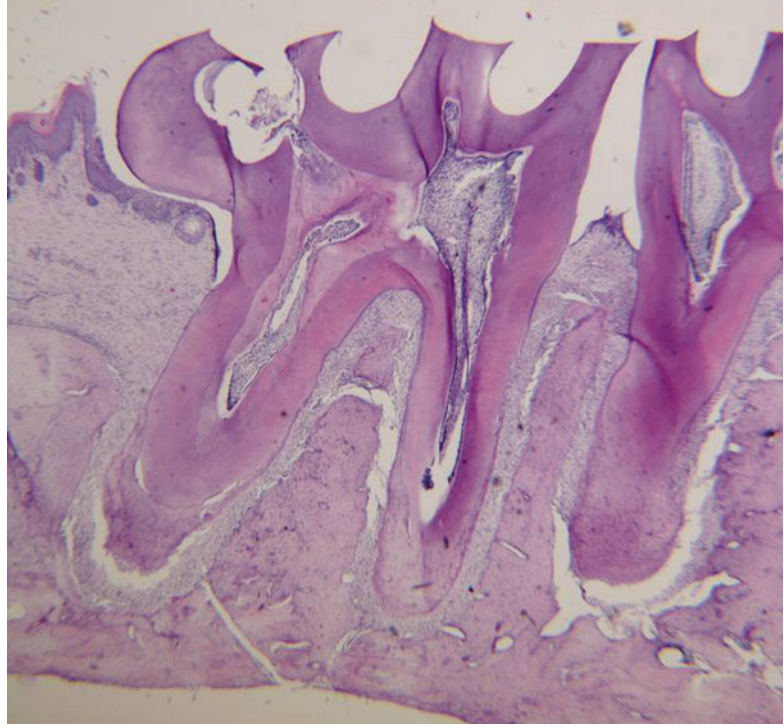
the crown was greater than in the root (Fig. 8.5). Dentinal deposition is greater in the root compared to the crown [3].

8.2 Age Determination

Gustafson's method for age determination from the teeth is based on 6 age-associated parameters evaluated on ground sections [4]. The transparency of radicular dentin and secondary dentin deposition constitute the two major criteria. Cementum apposition, periodontal and root recession, and attrition should also be taken into account.

The age-dependent nonenzymatic changes of D- and L-forms of aspartic acid constitute a reliable and accurate method, tooth dentin being considered as one of the best target tissues. Analysis of osteocalcin and elastin also provides accurate results [5]. A strong correlation was found between the population ages and translucency of dentin. Staining abraded sections with 1 % methylene blue stains the "normal" tubular dentin, whereas sclerotic dentin remains unstained. This is probably due to the precipitation of non-apatitic calcium phosphate within the lumen of the tubules. Intratubular mineralization remains unstained by the dye. The cementum took a dark blue color.

Aging can be distinguished from senescence, defined as an essential irreversible arrest of cell division. Senescent cells are metabolically active but no longer capable of dividing. Replicative senescence is an irreversible loss of division capacity of human cells in vitro after a reproducible number of population doublings. Senescence is the only one possible outcome of a DNA damage response, the other possibilities leading to DNA repair or apoptosis [6]. Senescent cells remain alive and differ from apoptotic cells that are enriched in transglutaminase. They display rigidified thicker cytoskeletal proteins and plasma membrane. After the apoptotic disintegration, apoptotic bodies are engulfed by tubulovesicular endocytic vesicles and degraded inside macrophage lysosomal structures. It differs also from cell cycle arrest.

Aging is characterized by the reduction in length of chromosomes [7]. Telomeres form the end of human chromosomes and senescence-associated distension of satellites (SADS). They shorten with each round of cell division and 50–100 bp are eliminated at each cell division. This mechanism limits the proliferation to a finite number of cell divisions. Telomere extends up to a certain length, and then cells stop dividing. The cells enter in senescence. Telomere shortening limits stem cell function, regeneration, and organ maintenance during aging. It is due to an "end replication problem" of DNA polymerase. In addition, processing of telomeres during the cell cycle and reactive oxygen species may contribute to telomere shortening. The telomerase RNA serves as template for telomere sequence synthesis. The telomerase reverse transcriptase is the catalytic subunit of the enzyme. Active during embryogenesis, telomerase is suppressed postnatally. The gradual loss of telomeres is a regulator for cell life span. Telomerase activity counteracts the gradual loss of telomeres by de novo synthesis of telomere repeats. Telomerase declines by age [8].

Generation of induced pluripotent stem (iPS) cells was obtained by introducing genes encoding pluripotent transcription factors into fibroblasts. Reprogramming of somatic cells of old patients is associated with full re-elongation of telomeres to size comparable with embryonic cells [9].

Beta-galactosidase activity seems to be associated with senescence and with lysosomal dysfunction. As marker, beta-GAL is questionable [10]. However, it is recognized that the senescence β-galactosidase staining was higher in senescent pulp cells than in young cells. They contain greater expression of autophagic proteins (microtubule-associated protein light chain 3 and Beclin 1) than young cells [11].

This is also established for human dental pulp stem cells that STRO-1, nestin, CXCR4, Sox2, nucleostemin, CD90, and CD166, which are considered as well-identified markers, play a role in pulp cell senescence. *Ink4a/Arf* expression is a robust biomarker of aging. The gene locus encodes two tumor suppressor molecules, p16^{INK4a}

and ARF, which are principal mediators of cellular senescence [12]. Generation of *p53$^{+/m}$* mice and other p53 mutants suggests that this gene has a role in regulating organismal aging [13].

Using microarray and RT-PCR, age-related changes in the expression and composition of third molar pulps of young subjects (age group 18–20 years) were compared to older patients (age group 57–60 years).

In young dental pulp, growth factors such as bone morphogenetic protein, TGF, growth factor differentiation family, platelet-derived growth factor α, vascular endothelial growth factor α, and FGF family were highly expressed, suggesting that they regulate the genes controlling cell proliferation and cell differentiation, and were responsible for signaling of many key events in tooth morphogenesis and differentiation.

In the aging pulp, vascular, lymphatic, and nerve supplies decline. Fibroblasts decrease in size and number. A reduction of 15.6 % was scored for crown odontoblasts and 40.6 % in root odontoblasts. The secretory activity was decreased, suggesting that the reparative capacity was compromised. Furthermore, age-related changes include a higher number of collagen cross-linkages, more collagen fibers, lipid infiltration, and calcifications. In the mature tooth, recruitment of dental pulp stem cells allowing their differentiation toward odontoblast cells for dentin bridge formation occurs. The deposition of secondary dentin increases, and the blood, lymphatic, and nerve supply undergo arteriosclerotic changes. This evidences age-related degenerative changes, together with a progressive mineralization. In older dental pulp there was an upregulation of proapoptotic genes like AIFM1, MOAP1, PDCD5, and PDCD7, confirming a possible correlation between apoptosis and the reduction of dental pulp volume. However, the expression in older dental pulp of growth factors like CTGF, FGF1, FGF5, and TGFB1, and genes implicated in the synthesis of collagenous proteins, confirms that, even reduced, the reparative processes were continuous during the entire tooth life [14].

Senescent fibroblasts are flat and display heterogeneous cell shapes. With respect to intracellular proteins, connexin 43 mRNA was

abundantly expressed in young adults (about tenfold higher in young adult) and decreased in aged human dental pulp [15]. Vimentin filaments, parallel with the long axis of the cells, are overproduced by senescent fibroblasts [16]. Expression of Cbfa-1 mRNA, VEGF, and HS27 mRNAs was higher in the adult first molar compared with the young animals [17].

With respect to ECM proteins, osteocalcin expression is reduced in the dental pulp of aged human. Osteocalcin mRNA was decreased in aged human dental pulp [18, 19]. According to some reports, apparently no difference was detectable by immunohistochemistry for type I collagen, osteonectin, and BSP in relationship to the degree of maturation. In contrast, collagen concentration increased as the pulp matured. The ratio of type III to type I similarly increased from 13 % at early stage to 32 % at late stage. The major cross-link dihydroxylsinonorleucine (DHLNL) decreased with age. Hydroxylsinonorleucine (HLNL) and lysinonorleucine (LNL) appeared in insignificant amounts [20]. Collagenase and collagenolytic neutral peptidases showed significantly high activity [21]. Mehrazarin et al. [22] reported a reduced expression of Bmi-1, OC, DSPP, BSP, and DMP-1 compared with replicative senescence, whereas $p16^{INK4A}$ level was increased.

NaF effects are dose dependent. NaF produces large DNA fragments. Higher concentrations reduce the number of viable senescent cells compared with young cells. This suggests that cells become resistant to cytotoxicity of NaF with in vitro aging [23].

8.3 Aging of Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells include both blood and connective tissue cells. Postembryonic, non-hematopoietic bone marrow-derived cells are efficient to undergo multipotent differentiation into osteoblasts, adipocytes, myoblasts, and early progenitors of neural cells as well. They can be isolated from many tissues including the bone marrow, pericytes, and dental tissues. MSCs divide with a donor-dependent average doubling time of 12–24 h dependent. A significant decrease in the growth rate of MSC is observed for aged donors. In some cultures obtained from old mice and measured by 3H-thymidine uptake, the proliferation was more than three times (and even tenfold) what was observed in culture from young animals.

The age-related changes may be due to intrinsic factors or induced by the somatic environment. Dlx3 and Dlx5 are regulators of odontoblastic differentiation. Overexpression of these homeobox domains stimulates osteoblast differentiation while inhibiting adipogenic differentiation of human dental pulp stem cells by suppressing adipogenic marker genes such as C/EBPα, PPARγ, and aP2 levels in preadipocytes [24].

The pulp response to cavity preparation in aged rat molars was evaluated by immunohistochemistry. Heat shock protein (HSP)-25 and nestin were found in odontoblasts, whereas class II MHC-positive cells were densely distributed at the periphery of the pulp, along the pulp-dentin border. They subsequently disappear by 12 h after the preparation of the cavity [25]. Replicative senescence and stress-induced premature senescence (SIPS) intervene in the expression of dentin sialophosphoprotein and dentin matrix-1 and osteogenic markers such as bone morphogenetic protein-2 and protein-7, runt-related transcription factor-2, osteopontin, alkaline phosphatase activity, and mineralized nodule formation [26].

8.4 Apoptosis

A number of gene effects alter apoptotic receptor levels, extrinsic apoptotic pathways through caspases, cytokine effects on apoptotic events, Ca2 +-induced dead signaling, cell cycle checkpoints, and potential effects of surviving factors. Apoptotic potential is decreased in older compared with younger animals.

Fig. 8.6 SEM image of a pulp stone after sawing. The spherically mineralized rings are centered on a vessel lumen
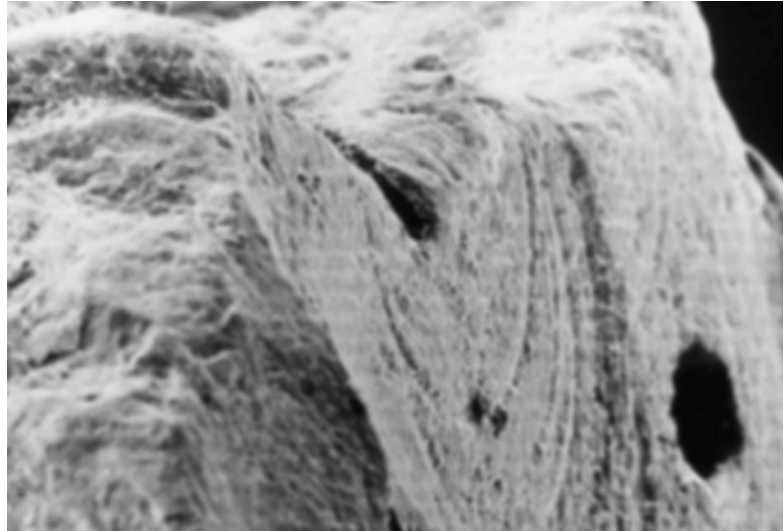
8.5   Pulp Stones or Pulp Calcification

These result from discrete calcifications or they are attached to or embedded in dentin, implicated in more diffuse dystrophic calcifications (Fig. 8.6). They appear either as denticles with a central cavity, probably a vascular vessel, or as compact degenerative calcified tissues occupying the whole dental pulp (Fig. 8.7).

Structurally, they appear as either true or false pulp stone. They may include partially fused calcospherites or almost or completely merged pulp mineralization. They may appear network-like or ridgelike or look like spherically mineralized structures. Amorphous appearances were also detected. True pulp stone appears formed by dentin-like structure, lined by odontoblast-like cells. False pulp stone are formed from degenerating cells, which mineralize, or are surrounded by soft tissue. Stones may be free, or adherent, or eventually embedded within the canal wall (Fig. 8.8a, b). Denticle, formed by epithelial remnants surrounded by odontoblasts, fibrodentin, and dystrophic calcifications, was also found.

In a single pulp, 1–12 stones may occlude the pulp space. They are occurring more often in the
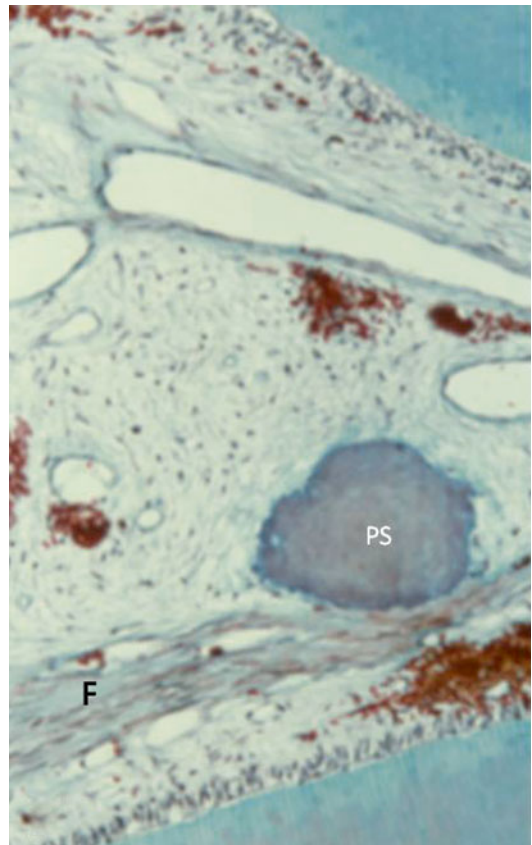
Fig. 8.7 Free mineralized pulp stones (*PS*) form in the central part of the fibrous (*F*) collagen-rich pulp

Fig. 8.8 (**a**) A calciotraumatic line (*CTL*) separates the orthodentin (*upper left*) from the osteodentin structure (*lower right*). (**b**) During aging, attached dystrophic calcifications are seen protruding inside the pulp cavity
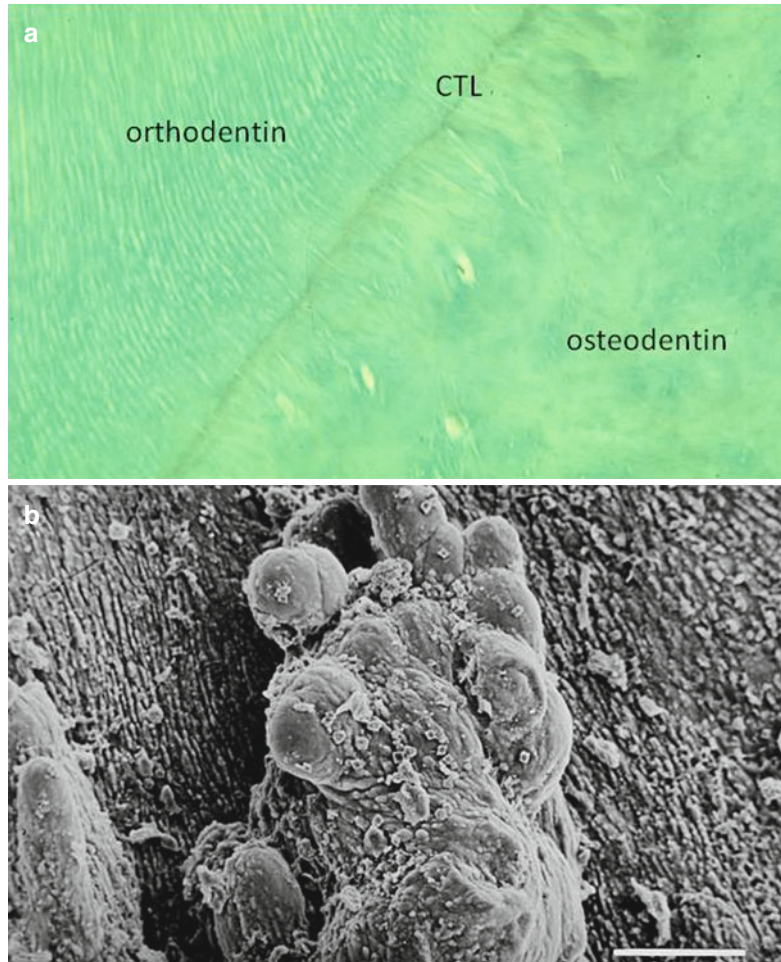
crown, but they may also be present in the root. Total coronal pulp occlusion is found in *dentin dysplasia* and *dentinogenesis imperfecta*. Ninety percent of teeth in those over 40 years of age display pulp calcifications. Many involve apical blood vessels. The gradual calcifying process becomes circumferential in the endoneurium and/or perineurium. Collagenous bundles are associated with the connective tissue surrounding blood vessels and nerves. Cell density is decreasing by half from 20 to 70 years. Fibrous degeneration or pulp atrophy occurs together with fat deposits which are Sudan black positive (lipidic inclusions or lipofuscin-rich structures) [27].

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