The Pulp Reaction Beneath the Carious Lesion

11

Michel Goldberg

Abstract

The carious lesion may develop at a slow rate. In such case, they are involved in the formation of reactionary (or tertiary) dentin. Rapidly progressing carious lesions lead to the formation of an atubular dentin of the osteodentin type or to the complete absence of tertiary dentin. This rapidly progressing lesion management often leads to pulp necrosis, followed by the formation of a periapical lesion. Type I, III, V, and VI collagens are associated with phosphorylated and non-phosphorylated proteins form a loosely network. Combined with proteins, CS-4 and CS-6 and KS appear as proteoglycans. MMPs, TIMPs, and other proteases are involved in matrix components degradation. Reactionary dentin results from the synthetic and secretory activities of altered odontoblasts. The dentinal layer is mostly tubular and deposited beneath a calciotraumatic line. Reparative dentin is under the control of pulp cells. It appears as an osteodentin, with osteoblast-like cells being residing in osteocyte lacunae, with tiny interconnections between cells. The dentin matrix proteins contains collagen type I, phosphophoryn (PP), and dentin sialoprotein (DSP), all of which play crucial roles in the dentin mineralization process. Apical cells formed a niche of stem/progenitor cells sliding from the apex toward the coronal pulp where they differentiate. Arrested caries contribute to the formation of reactionary dentin. Rapidly progressing carious lesions lead to the formation of an atubular dentin. Pulp capping induces the formation of reparative dentin. After a mild caustic effect, the pulp surface undergoes necrosis due to the alkaline of calcium hydroxide (pH 12), followed by new matrix formation and the mineralization of a dentinal bridge. The cells proliferate and form a dense extracellular matrix. The cells display

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odontoblast-like morphology by day 14. The mineralization of the reparative bridge is uncompleted. The bridge showing many interruptions is due to the presence of tunnels and pulp remnants. However, the reparative dentin contributes to occlude the pulp exposure. Pulp stones are either adherent to the pulp walls, or isolated within the pulp, forming calcospherites around blood vessels.

11.1 Extracellular Matrix of Sound Pulp: Composition

Primary dentin characterizes the dentin formed during odontogenesis. Secondary dentin starts to be formed while the tooth is still embedded in the jaws and is continuous after the tooth is erupted. The tubules in primary and secondary dentins form a continuum. Secretory odontoblasts and the so-called Hoehl's cell layer form primary and secondary dentins. Tertiary dentin generated in response to nonphysiological stimuli, such as caries or cavity preparation, is formed only by mesenchymal tissue and is formed by "secondary" odontoblasts, which are actually pulp cells. They are activated after primary odontoblasts have been destroyed (Mjör 2009). Dentin matrix results from the secretion of specific cells (odontoblasts and Hoehl's cells exclusively) (Figs. 11.1, 11.2, 11.3, and 11.4). In addition, dentin is composed of a mineral phase (70 %), an extracellular matrix (20 %), and water (10–12 %), formed by partially free water and bound water.

Type I collagen is the major fibrous component of the dentin matrix, but pulp matrix also contains significant amounts of type III collagen (Figs. 11.4, 11.5, and 11.6). Fibronectin and proteoglycans are also present in the dental pulp (osteoadherin/osteomodulin) (Linde 1985). *Type III collagen* constitutes 28 % of the pulp (Shuttleworth et al. 1978). It may form up to 42.6 % type III of the total collagen (see table 11.1, 41 %). *Type V collagen* comprised two different molecular species of $[a1(V)]_2$ a2(V)a2 (V)a3(V), the ratio of which represented, respectively, 56,41 and 2 % of the total collagen (Tsuzaki et al. 1990). Type VI (0.5 %) is associated with microfibrillin.

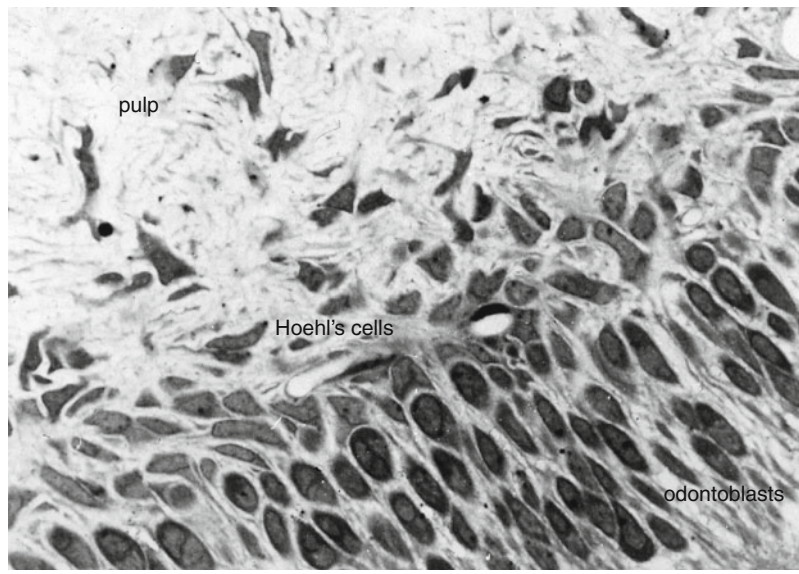


Fig. 11.1 Odontoblasts and Hoehl's cells are located at the periphery of the pulp

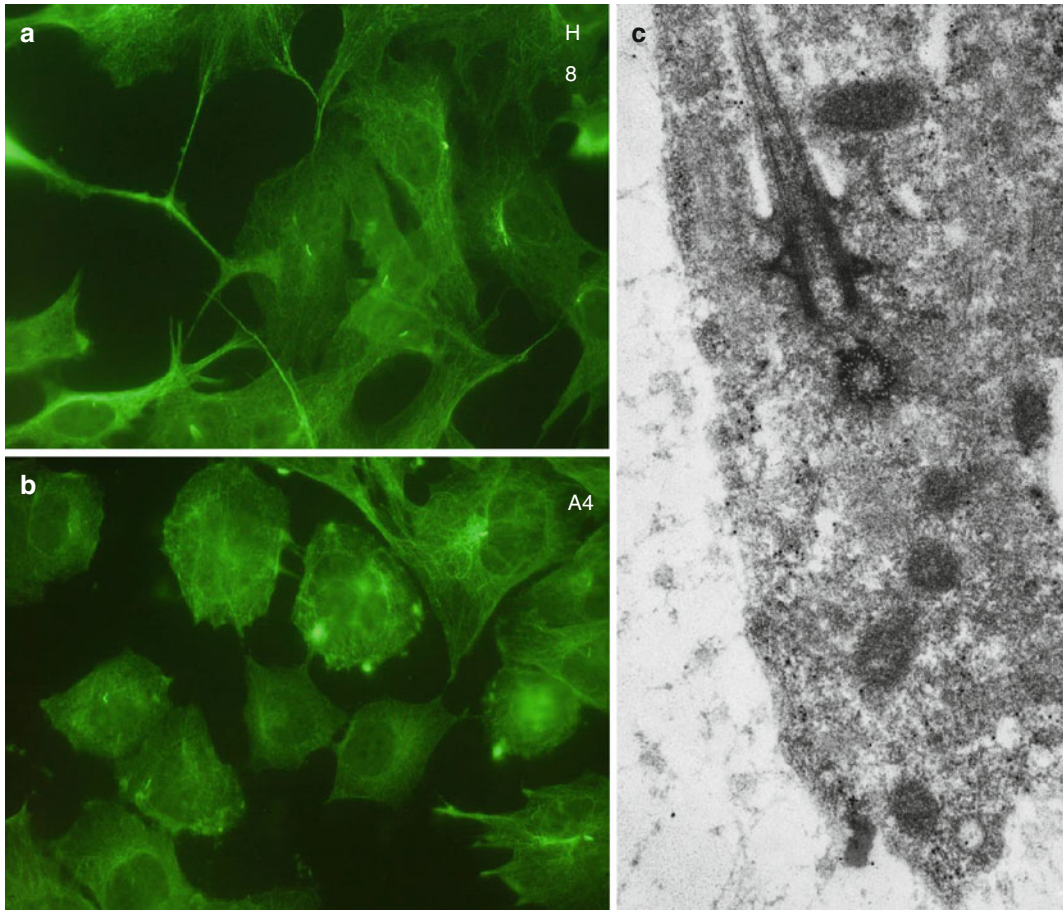


Fig. 11.2 Immunohistochemical visualization of alpha acetyl tubulin, a component of microtubules, in H8 (**a**) and A4 (**b**) cell lines. In c = a pulpoblast displays a cilium and basal body

Collagen degradation is regulated by matrix metalloproteinases (MMPs). TNF-α, ΙΛ – 1β, and ΙΛ – 6 ανδ ΤΓΦ – β1 πλαψ ρολε ιν χολλαγεν δεγραδατιον are mediated by pulp fibroblasts (Wisithphrom and Windsor 2006) (Fig. 11.6).

Phosphorylated and non-phosphorylated proteins: Osteocalcin, dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE) have been detected in pulp cell cultures. DSPP and MEPE expressions are regulatory pattern of DPCC with stem cell characteristics. DSPP is processed by protease (BMP-1) into three major domains: dentin sialoprotein (DSP), dentin glycoprotein (DGP), and dentin phosphoprotein (DPP). Expression of full-length *Dspp mRNA* by quantitative real-time polymerase chain reaction analysis was significantly higher in odontoblasts than in pulp (Yamamoto et al 2015). DSPP-derived proteins in porcine pulp are expressed at both the protein and mRNA levels.

Tenascin and fibronectin were found by immunohistochemistry at the periphery of the pulp, next to the odontoblasts of normal human dental pulp.

Glycosaminoglycans (GAGs) and proteoglycans (PGs) are present in the dental pulp. GAGs are formed by chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, and hyaluronic acid. CS-4 and CS-6 are the major glycosaminoglycans, hyaluronic acid and keratan sulfate being presented in minor amount (Rahamtulla 1992).

Decorin, biglycan, and fibromodulin are CS PGs (Goldberg et al. 2005, 2006) (Fig. 11.4).

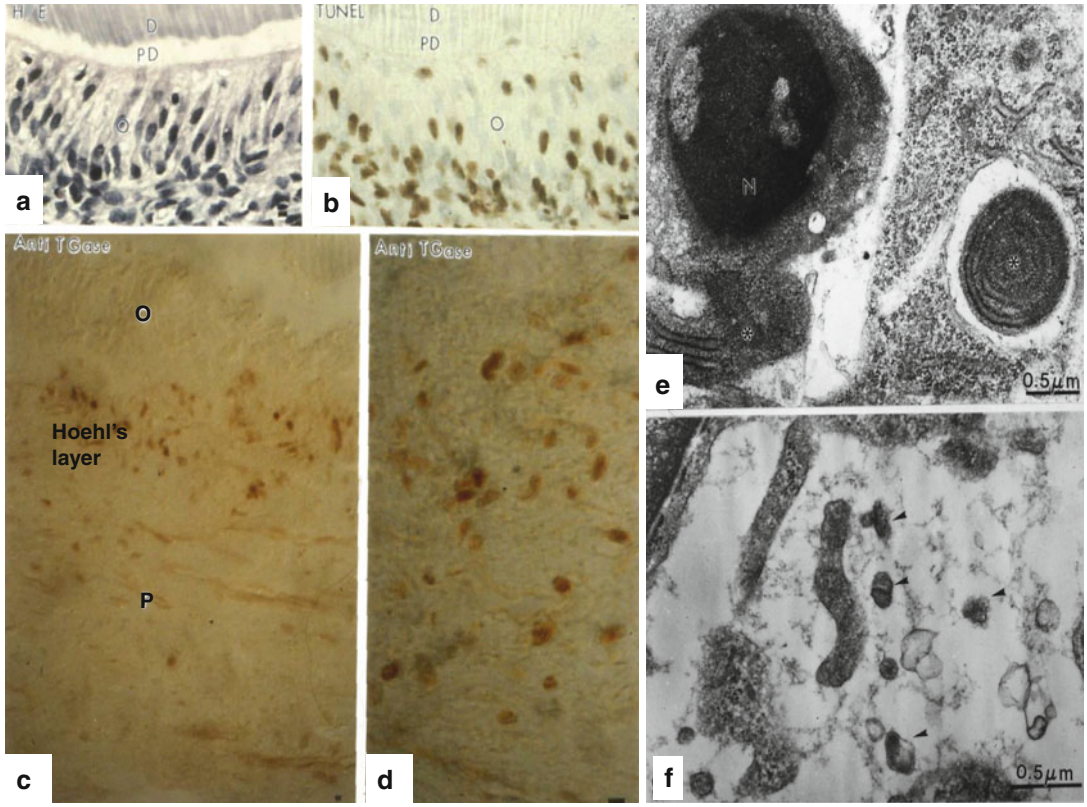


Fig. 11.3 (**a**, **b**) (**a**) Hematoxylin-eosin staining of a control semi-thin epon section. b: after TUNEL staining apoptotic cells (colored in *dark brown*) are located in the odontoblast (*o*) layer. *PD* predentin, *D* dentin. (**c**, **d**) An antibody raised against the apoptotic marker the anti-transglutaminase (anti-TGases) stains cells located in the Hoehl's layer, but not odontoblasts (*O*) or pulp (*P*) cells. (**e**) Chromatin condensation in the nucleus (*left* part of the figure) and isolated rough endoplasmic reticulum (*asterisk*) inside another pulp cell (*right* part of the figure). (**f**) Apoptotic bodies (*arrowheads*) after the apoptotic destruction of the cell

Versican, a proteoglycan aggregate, has also been extracted from the dental pulp. Versican, hyaluronan, and link protein form ternary aggregate structures in the rat dental pulp (Shibata et al. 2000).

Metalloproteinases (MMPs) and tissue-specific inhibitors (TIMPs) are implicated in the extracellular matrix degradation (Sulkala et al. 2004). cDNA microarray demonstrated the high level for MMP-13 (collagenase-3) and a lesser expression of MMP-16 (MT3-MMP) and TIMP-1, especially during caries progression. During rat tooth eruption, a disintegrin and metalloprotease with thrombospondin type 1 motifs (ADAMTS) is implicated in cleaving proteoglycans such as aggrecan, versican, and brevican. ADAMTS1, ADAMTS4, ADAMTS5, and versican were expressed in dental pulp cells (Fig. 11.5). Dental pulp cells are involved in both production and degradation of versican with secreting ADAMTS1, ADAMTS4, and ADAMTS5 (Sone et al. 2005).

11.2 Cells

The pulp periphery: At the outer border of the pulp, odontoblasts and the so-called Hoehl's cells form continuous layers. These postmitotic cells have the capacity to undergo terminal differentiation.

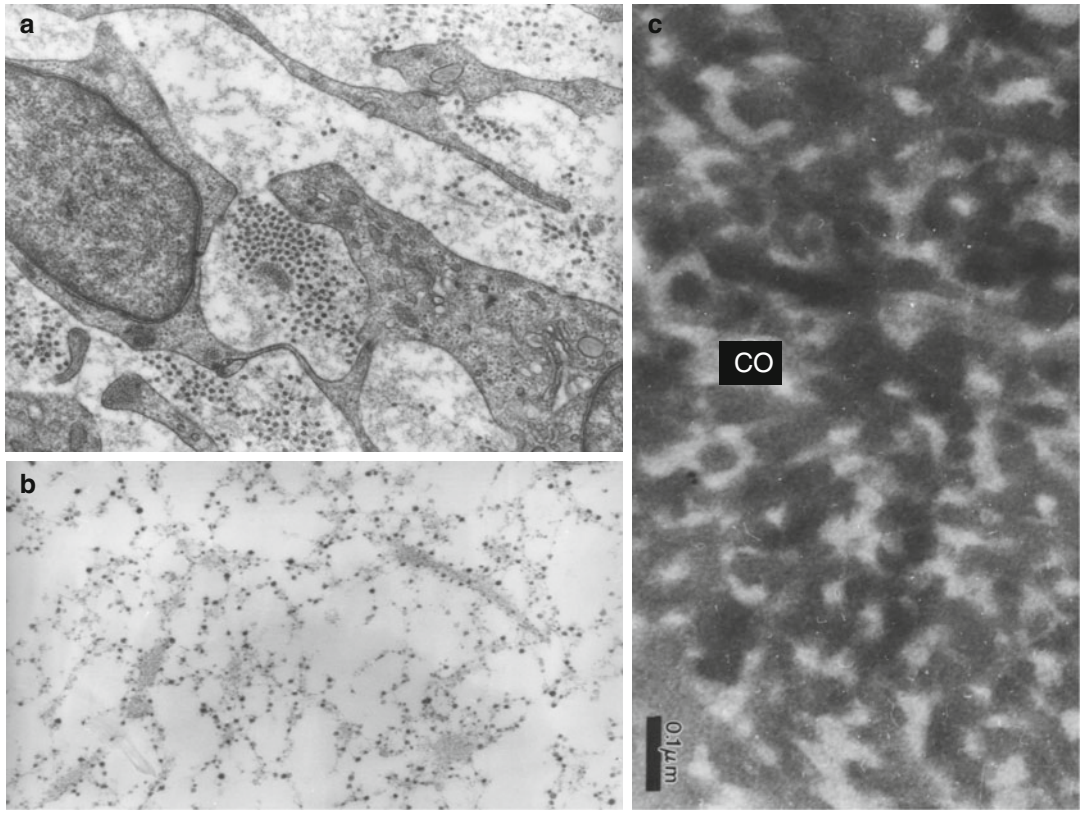


Fig. 11.4 (**a**) Pulpoblasts and collagen fibrils displaying different diameters. (**b**) Histochemical staining of glycosaminoglycans appearing as electron-dense granules aligned along collagen fibrils and pulpoblast processes. (**c**) Alcian blue stained predentin after rapid freezing freeze substitution. The collagen fibrils appear electron lucent; intercollagenic spaces are electron dense and stained by the cationic dye

Odontoblasts are implicated in the synthesis of collagen and noncollagenous extracellular matrix components. Some ECM proteins are phosphorylated (SIBLINGs), whereas others are non-phosphorylated. ECM components are implicated in predentin and dentin formation, followed by dentin mineralization. Due to a fixation artifact, the formation of a cell-free layer results in from fixation and dehydration. A cell-free area underlines odontoblasts and Hoehl's cells, which do not appear on sections after adequate fixative perfusion. Fenestrated capillary loops infiltrate the layer formed by odontoblasts and Hoehl's cells but do not cross the terminal junctions located between the distal odontoblast cell bodies nor penetrate within the predentin. In contrast, axons infiltrate the odontoblastic layer and penetrate into the predentin. A few axons penetrate into dentin tubules and occupy the inner dentin but are found only in the inner 150 micrometers (Fig. 11.1).

Pulp cells are present in the dental pulp. Fibroblasts (pulpoblasts) and fibrocytes are the prominent cells, with variable cell density. They are elongated, with thin spinous processes. A few macrophages, plasmocytes, mast cells, and leukocytes have been also identified. Pulpoblasts contain dense cytoskeletal proteins, including microfilaments, intermediary filaments, and microtubules. Immunostaining of alpha acetyl tubulin revealed the presence of microtubules (Fig. 11.2a, b) associated in cilium and basal corpuscle (Fig. 11.2c).

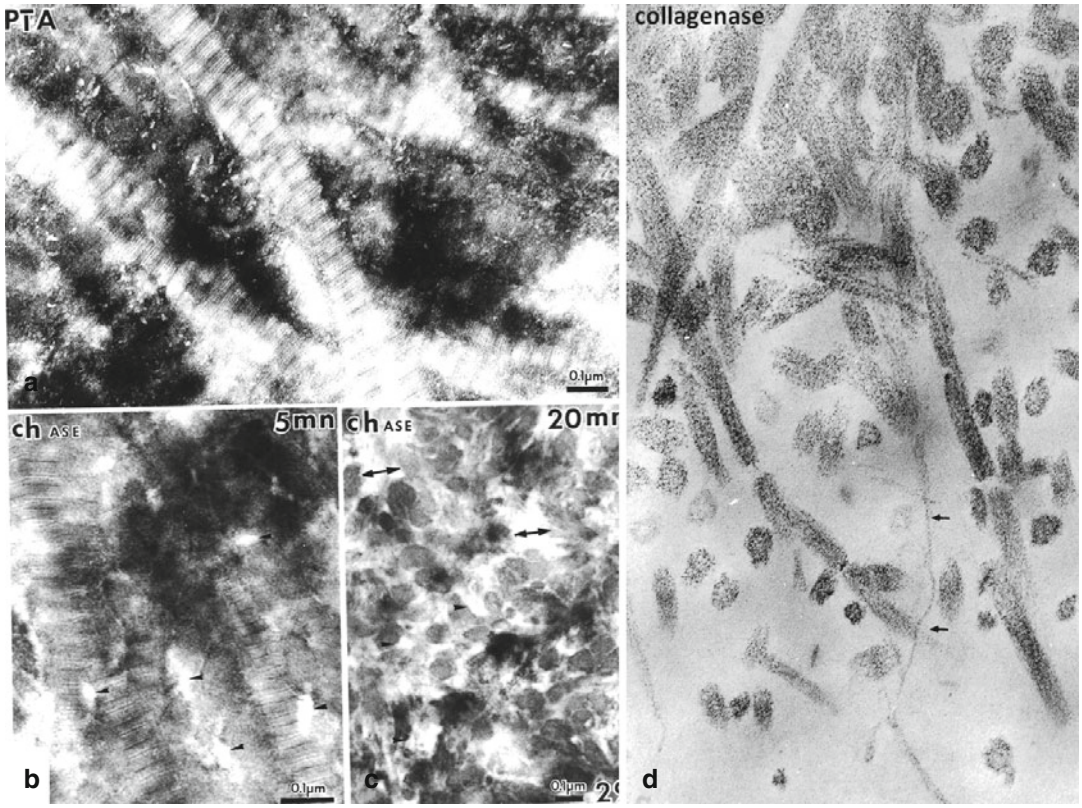


Fig. 11.5 (**a**) Dentin stained by phosphotungstic acid (PTA). The periodic banding of the collagen fibrils is clearly seen. Along the surface of collagen fibrils, and in the intercollagenic spaces, PTA positive intercollagenic spaces are densely stained. (**b**) Treatment of the section with a chondroitinase for 5 min reduce partially the PTA staining, whereas in (**c**): enzyme digestion with chase for 20 min largely suppresses the proteinaceous material located in intercollagenic spaces. Treatment of the sections with a bacterial collagenase disrupt the fibril in ¾ and ¼, (*small arrows*), and in thickness

Capillaries connect arterioles and venules. Capillaries display continuous thick or thin endothelial lining. Fenestrated endothelial cells contribute to the control and balance between the intravascular compartment and the interstitial tissue. Lymphatic capillaries have been recognized at the pulp periphery. Beneath the odontoblast compartment, the so-called Hoehl subodontoblastic compartment constitutes a second layer of cells, which may differentiate and become a second generation of odontoblasts. The renewal of odontoblasts requires newly differentiated cells, and because odontoblasts are postmitotic cells, there is a need for mesenchymal cells taking origin in the stromal pulp.

11.3 Neuropeptides in Dental Pulp

Nerve fibrils penetrate into the pulp within the apical region. They are surrounded by a myelin sheath that surrounds the axon. The role of neuropeptides, including substance P, calcitonin gene-related peptide, neurokinin A, neuropeptide Y, and vasoactive intestinal polypeptide has been discovered. Neurotransmitters or neuromodulators presumably play a variety of functions, participating in paracrine, endocrine, and neurocrine forms of communication (Caviedes-Bucheli et al. 2008).

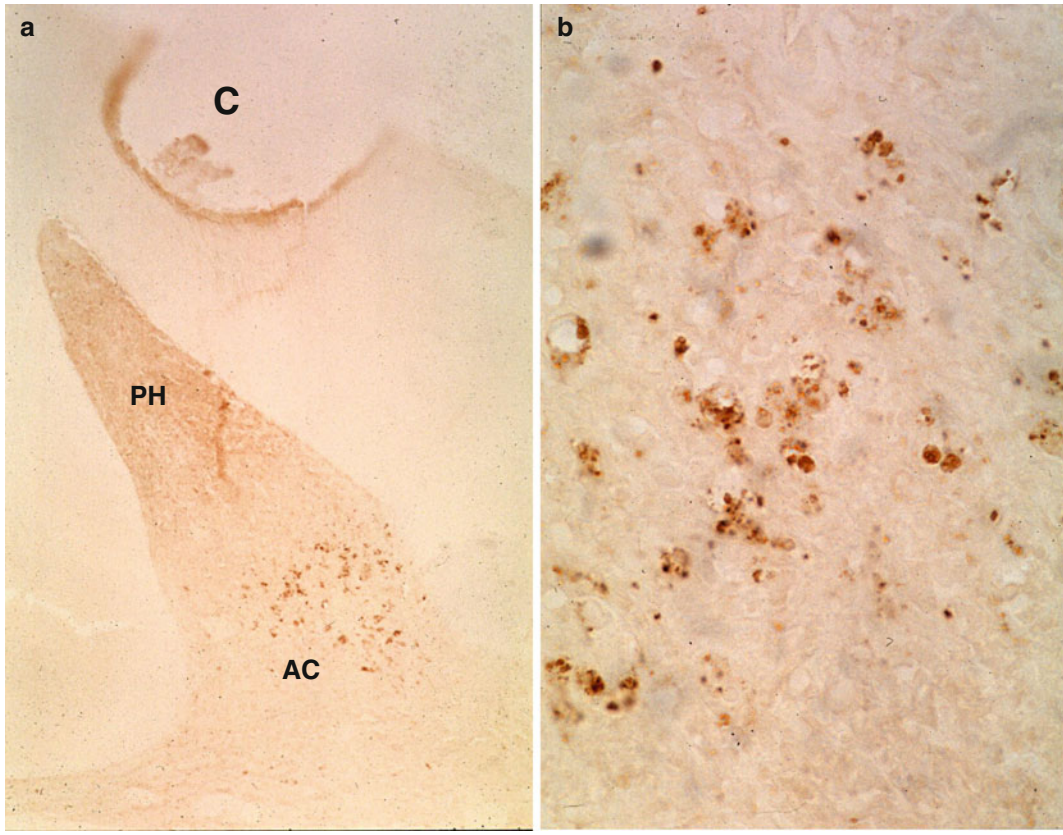


Fig. 11.6 (**a**) After the preparation of a cavity (*C*) and accumulation of cells in the pulp horn (*PH*), apoptotic cells (*AC*) are grouped in the lower part of the mesial pulp horn. (**b**) Larger magnification shows pulp cell fragmentation of apoptotic cells

Table 11.1 Composition of the pulp ECM

Component	Protein family	Specific protein
Collagen	Collagens	Type I 56 % Type III 41 % Type V 2 % Type VI 0.5 % associated with microfibrillin
Noncollagenous proteins	Phosphorylated ECM proteins	DSPP, DPP, DSP, bone sialoprotein, osteopontin, MEPE
	Non-phosphorylated proteins	Fibronectin, osteonectin
	Proteoglycans–glycosaminoglycans	Chondroitin sulfate-4 (CS-4) and CS-6 (60 %), dermatan sulfate (DS) 34 %, keratan sulfate (KS) 2 %, hyaluronic acid
	Growth factors	BMPs, type 1A and II receptors for TGFβ, activin
	Proteins taking origin from the plasma	Fibronectin
	Enzymes	Metalloproteinases (collagenases, gelatinases, stromelysine-1; tissue inhibitors of MMPs (TIMPs), alkaline and acid phosphatases, catalytic lysosomal and extracellular enzymes
	Phospholipids	Membrane and ECM phospholipids (proteolipids)

In: Goldberg (2014)

11.4 Human T-Lymphocyte Subpopulation

Pulp inflammatory cells include polymorphonuclear neutrophilic leukocytes and mast cells implicated in the defense mechanisms. Interleukin-8 expression is elevated in the irreversibly inflamed dental pulp, but lacking in the normal caries-free pulp (Huang et al. 1999).

CD45+ represented 0.94 % ± 0.65 % of cells obtained from the enzymatic digestion of the whole dental pulp cells. CD16 + CD14+ granulocyte/neutrophils (50.0 % ± 9.08 % represent the major subpopulation in CD 45+ cells, followed by CD3 T lymphocytes (32.58 ± 11 %), CD14+ monocytes (8.93 % ± 5.8 %), and HLA-DR high-Lin1+ dendritic cells (4.51 % ± 1.12 %). Minor subpopulations included CD3-CD56+ natural killer cells (2.63 % ± 1.15 %) and CD19 B lymphocytes (1.65 % ±0.89 %). In addition cells presenting a phenotype compatible with Foxp3/CD25 are seen in regulatory T lymphocytes (Gaudin et al. 2015).

Pulp cells are present for a limited period of time. Odontoblasts (Fig. 11.3a, b) are postmitotic cells that survive the initial period. Later, the so-called Hoehl's cell layer becomes apoptotic and displays a turn over more rapidly than the odontoblasts (Fig. 11.3c, d). Within the dental pulp, some pulpoblasts underwent apoptosis; their nuclei display condensed chromatin (Fig. 11.3e). Cytoplasmic inclusions contribute to the restricted aging of pulpoblasts. After the complete degradation of pulp cells, apoptotic bodies are present in the pulp extracellular matrix. They are destroyed by phagocyte or by macrophages (Fig. 11.3f). Apoptosis contributes actively to the pulp defense mechanisms.

11.5 The Carious Pulp

Carious pulps can be classified as being at the transitional stage, or displaying partial pulpitis, or total pulpitis and/or total necrosis (Di Nicolo et al. 2000).

If caries progresses slowly, there is time to form reactionary dentin or tertiary dentin (Bjorndal 2008). Pulp can be affected or infected. Bacteria are rarely seen in the unexposed pulp, whereas they are often sees in infected and necrotic pulps (Massler and Pawlak 1977). Microorganisms can reach the pulp via the dentin tubules. Often apoptotic pulpal cells at some distance from cavity preparations may display fragmentation of nuclei (Fig. 11.6a, b).

Such rapidly progressing carious lesions lead to the formation of an atubular dentin or to the complete absence of tertiary dentin. This rapidly progressing lesion leads to pulp necrosis and furthermore to periapical lesion formation. The formation of reactionary or reparative dentin is representative of the pulp response to the carious lesion. Dentin sclerosis, dead tracts, or reparative dentin are correlated with sex, age, and the type of surface location of the lesion (Stanley et al. 1983). Dentin sclerosis results from aging. The formation of translucent zone is observed in response to caries of the slow type and other mild irritations. The production of reparative dentin is directly related to the carious decay. Slowly progressing caries may become arrested caries lesions, with occlusion of the tubules by mineral deposits contributing to the formation of a "transparent zone" subjacent to the mineralized carious dentin. In this zone needle and rhombohedral crystals have been identified, together with hydroxyapatite and whitlockite crystals. They occlude the lumen of the tubules (Fig. 11.8a, b).

A gradual degradation of the dental pulp is seen. The density of the odontoblast layer slowly decreases (Fig. 11.7a), and finally, between the mineralized dentin and the pulp, an empty border is gradually inhabited.

11.6 Reactionary and Reparative Dentin Formation

Odontoblasts are postmitotic cells. After development of carious lesions, under pro-inflammatory stimuli, dental pulp cells can differentiate and produce reactionary or reparative dentin. The pro-inflammatory cytokine tumor necrosis factor α (TNFα) may be a mediator involved in dental pulp cell differentiation toward an odontoblastic phenotype. TNF-α-

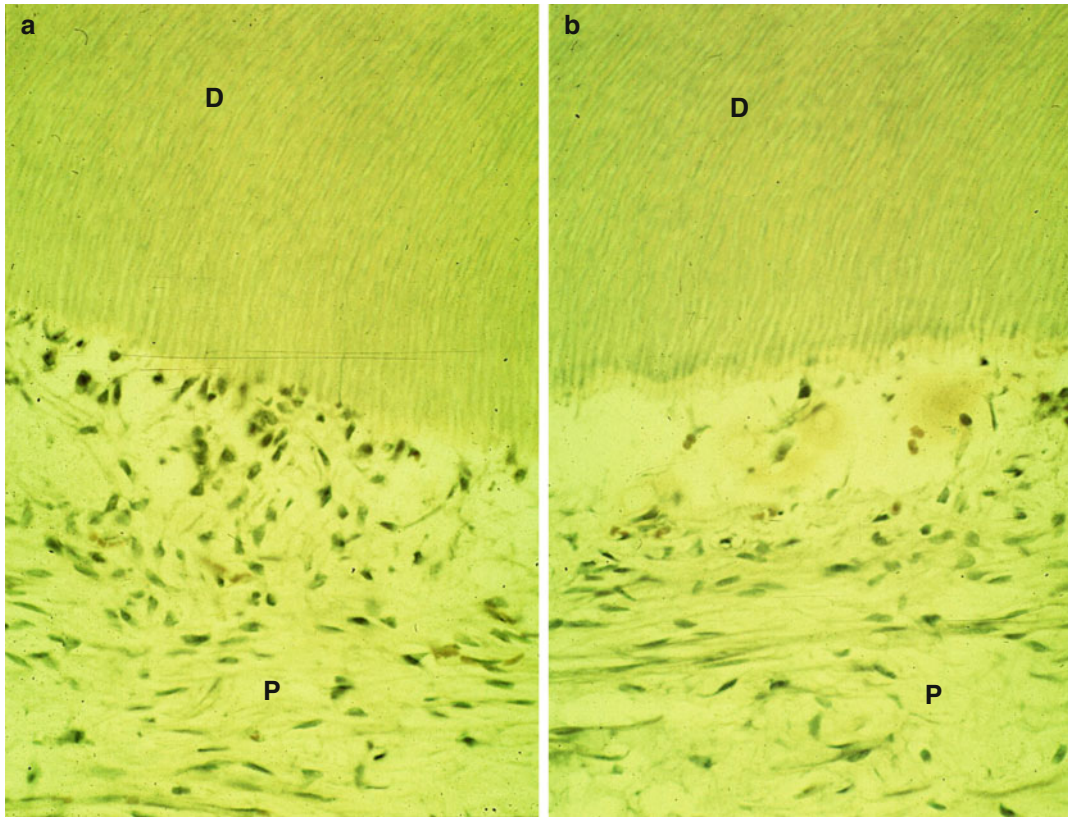


Fig. 11.7 (**a**) The preparation of a cavity induces disturbances of the odontoblast layer. *P* pulp, *D* dentin. In (**b**): the layer of odontoblast is empty. *D* dentin, *P* pulp

stimulated pulp cells display increased expression of DPP, DSP, DMP-1, and osteocalcin. The TNF-α differentiation of dental pulp cells toward an odontoblastic phenotype occurs via p38 and is negatively regulated by MMP-1 expression (Paula-Silva et al. 2009). Although there are no specific odontoblastic markers, osteocalcin, osteonectin, alkaline phosphatase, bone sialoprotein, and DSPP have been used as indicators of odontoblastic differentiation. Studies have shown that MMP-2 and MMP-9 inhibition is necessary for dentin matrices to mineralize alters dentin remodeling (Fanchon et al. 2004).

Bacterial invasion occurs in dentin that is stained bright red by 0.5 % basic fuchsine – propylene glycol solution, whereas caries-affected dentin that is bacteria-free stains pink. Collagen fibrils in infected dentin have lost their cross-banded appearance in transmission electron micrographs, indicating they are irreversibly denatured (Kuboki et al. 1977).

Reactionary dentin and reparative dentin are both strongly immunopositive for osteopontin (OPN), a phosphorylated protein of the SIBLING family, also implicated in intracellular cell signaling and inflammatory process (Aguiar and Arana-Chavez 2007). DMP-1 and collagen were associated and seem to be essential for reactionary and reparative dentin formation (Aguiar and Arana-Chavez 2010) (Figs. 11.6, 11.7, 11.8, 11.9 and 11.10).

11.6.1 Reactionary Dentin Formation

Dentin sialoprotein (DSP) may be cleaved into NH2-terminal and a COOH-terminal fragments. Using immunohistochemistry, the two DSP

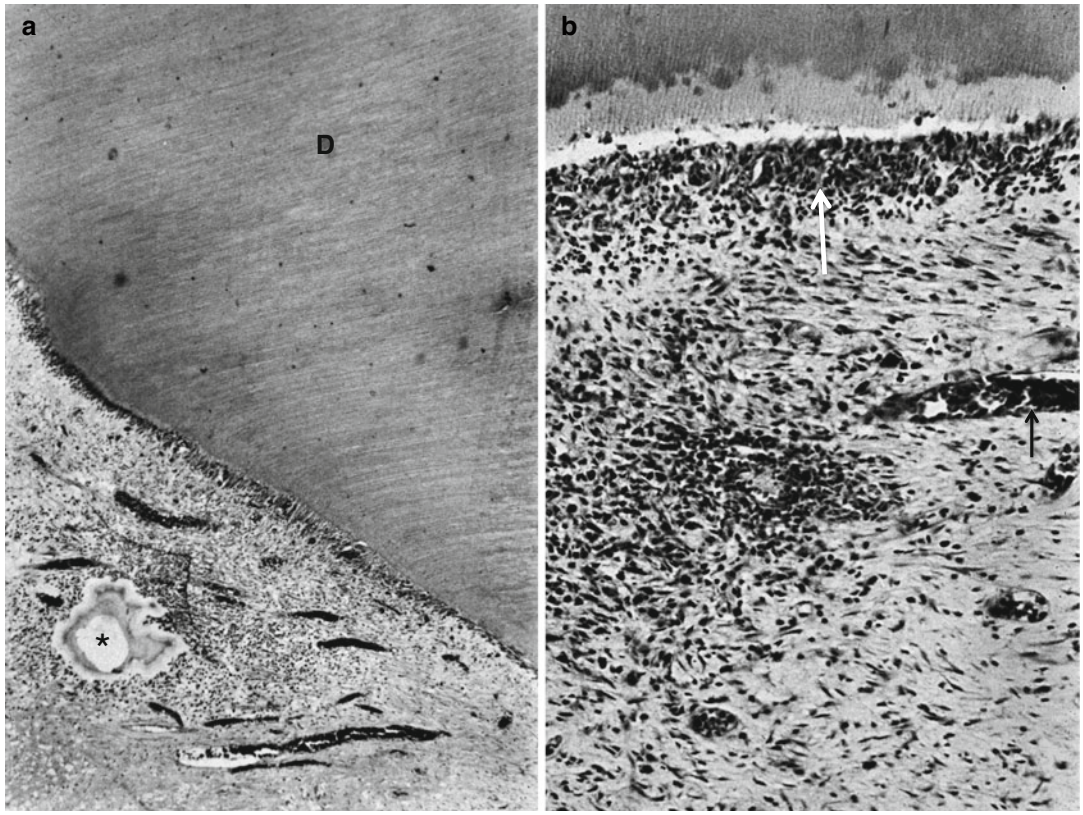


Fig. 11.8 (**a**) Accumulation of inflammatory cells and necrotic area (*asterisk*). The odontoblast layer shows continuity. *D* dentin. (**b**) Pulp cell inflammation in the subodontoblastic layer. *Red* blood accumulation is seen in vascular blood vessels (*black arrow*). Inflammation is also seen in the odontoblast layer (*white arrow*)

antibodies showed weak staining in reactionary dentin. Hence, DSP is probably less positive in reactionary dentin formation, in contrast with osteopontin, which seems to be crucial in the construction of this dentin (Yuan et al. 2012) (Figs. 11.9, 11.10, 11.11, 11.12, 11.13, 11.14, and 11.15).

11.6.2 Reparative Dentin Formation

After pulp exposure and capping with calcium hydroxide, apatite crystals were detected within *matrix vesicles* (Sela et al. 1981). Taking advantage of the A4 cell line, a multipotent stem cell derived from the molar pulp of mouse embryo, the capacity of these pulp-derived precursors to induce in vivo formation of a reparative dentin-like structure upon implantation was investigated within the pulp of a rodent incisor or a first maxillary molar after surgical exposure. One month after the pulp injury alone, a non-mineralized fibrous matrix filled the mesial part of the coronal pulp chamber. Upon A4 cell implantation, a mineralized osteodentin was formed in the implantation site without affecting the structure and vitality of the residual pulp in the central and distal parts of the pulp chamber. These results show that dental pulp stem cells can induce the formation of reparative dentin and therefore constitute a useful tool for pulp therapies (Dimitrova-Nakov et al. 2014). Differentiation of stem/progenitor cell populations of dental pulp is followed by reparative dentin formation. β-catenin was significantly upregulated during odontoblast differentiation, accompanied with reduction of Runx2 (Han et al. 2014).

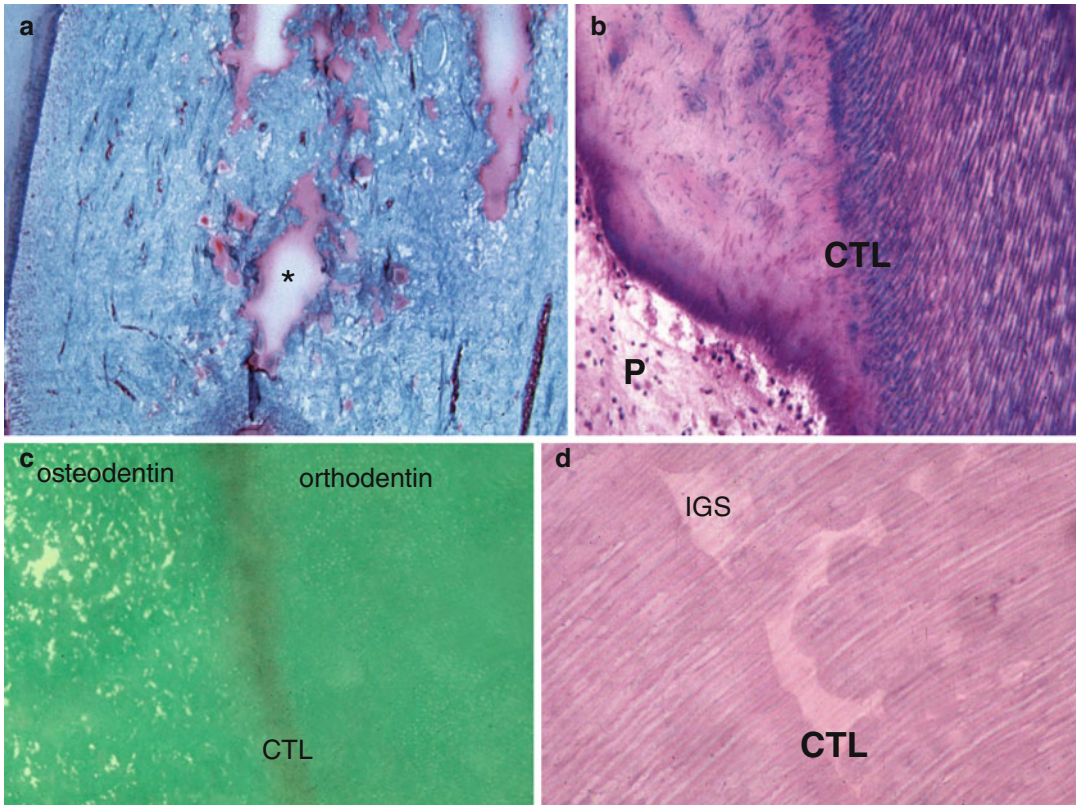


Fig. 11.9 (**a**) Necrotic empty areas (*asterisk*) where apoptotic cells melt. (**b**) Border between orthodentin (*right* part of the figure) and osteodentin (*left* part of the figure). A calciotraumatic line (*CTL*) separates the two dentin. (**c**) Stains all treated section. Near the pulp (*P*) the formation of osteodentin (*left* part of the figure) is separated from orthodentin (*right* part of the figure) by a calciotraumatic line. (**d**) Orthodentin is formed on the left. A calciotraumatic line (*CTL*) shows an interruption of dentinogenesis, revealing the presence of interglobular spaces (*IGS*)

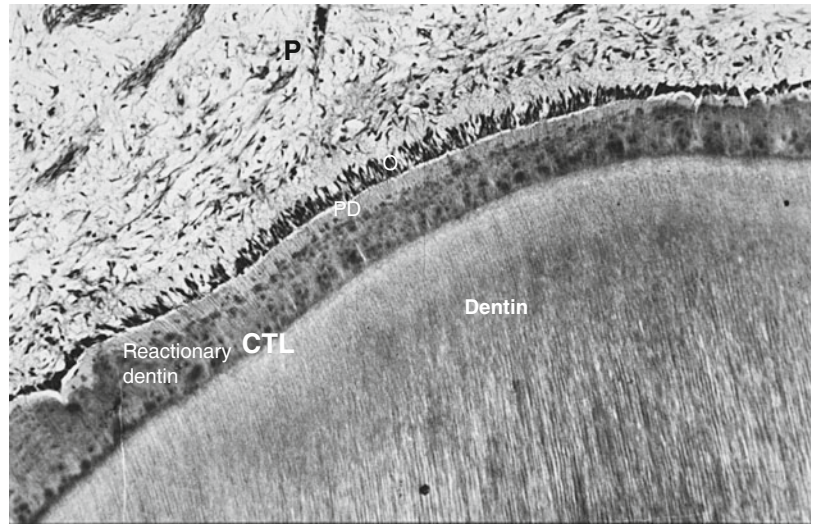


Fig. 11.10 A calciotraumatic line (*CTL*) separates the dentin from reactionary dentin. *PD* predentin, *O* odontoblasts, *P* pulp

When A4 cells were implanted in peripheral sites in dog dental pulp, elongated and polarized odontoblast-like cells were observed, whereas implanted in the center of the pulp, they produced an atubular hard tissue with lining fibroblast-like cells (Tziafas et al. 1996).

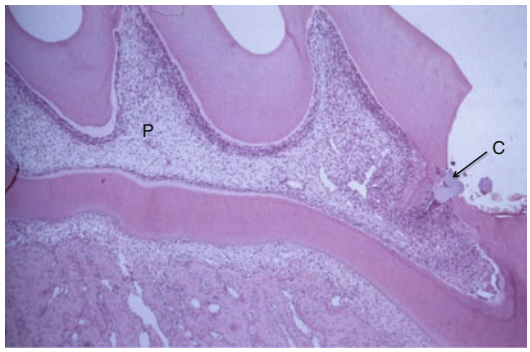


Fig. 11.11 The preparation of a cavity (*C*) provokes an inflammatory reaction in the mesial part of the pulp (*P*)

Dentin phosphophoryn (DPP) has a RGD motif and repeat sequences of aspartic acid and phosphoserine. DPP promotes cell migration in a concentration-dependent manner but has no effect on cell proliferation. Cell migration is suppressed by the addition of alpha v beta 3 integrin antibody to the culture medium (Yasuda et al. 2008).

The connective tissue growth factor/CCN family 2 (CTGF/CCN2) seems to play role in reparative dentin formation. In healthy teeth, minimal expression was evident in odontoblast subjacent to the dentin-pulp junction, whereas a strong expression was detected in the odontoblast-like cells lining the reparative dentin subjacent to dental caries. CTGH/CCN2 promoted mineralization but not proliferation (Muromachi et al. 2012).

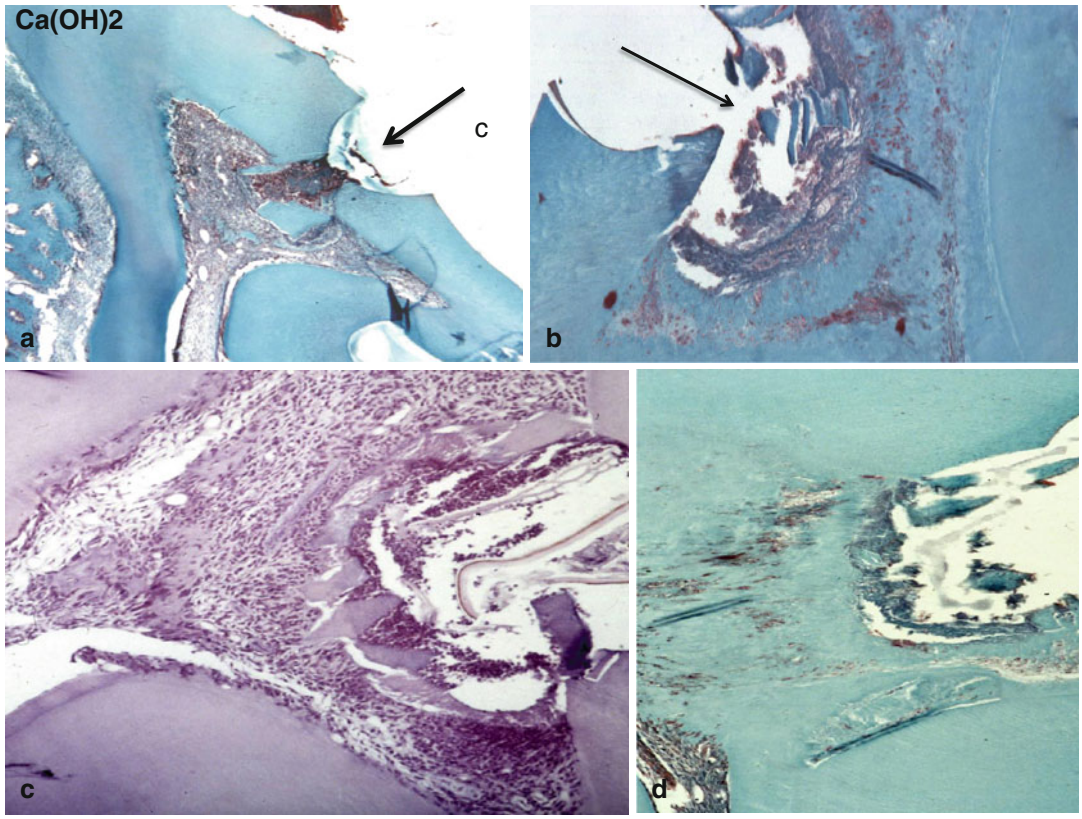


Fig. 11.12 (**a**) One week after calcium hydroxide capping (*arrow*), *c*: cavity; (**b**): a dentinal bridge starts to be formed (*arrow*). (**c**) Its thickness increases and (**d**) finally osteodentin occludes the pulp exposure

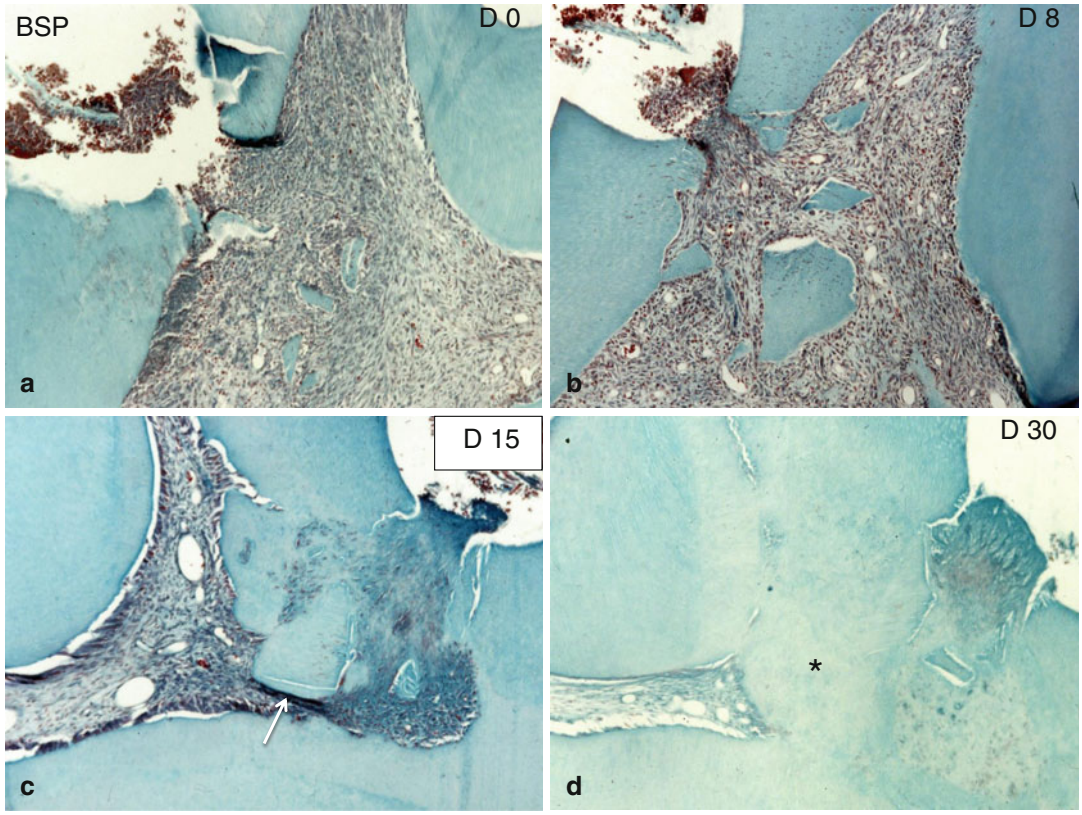


Fig. 11.13 Pulp capping with bone sialoprotein (BSP). (**a**) At day O, the pulp exposure is accompanied by the projection of dentin debris within the pulp (**b**): after 8 days, an inflammatory reaction is seen in the pulp horn. (**c**) After 15 days, dentin debris pushed in the pulp are thicker (*white arrow*) and covered with a layer of reactionary dentin. (**d**) At day 30, the pulp exposure is totally filled by reparative dentin (*asterisk*)

11.7 Pulp Capping

Using calcium hydroxide, pulp capping was introduced clinically in the year 1930 by Hermann (Schroder 1985). The chemical mechanisms of pulp capping leading to the formation of a hard tissue barrier are now better understood. The initial reaction on the dental pulp of calcium hydroxide was vascular, associated with a mild inflammation, cell migration, and proliferation (Fig. 11.11). These events were followed by cell destruction and liquefaction necrosis. The alkaline pH of calcium hydroxide demineralizes dentin matrix that solubilizes TGF-β1 and noncollagenous phosphoproteins from the matrix that recruits odontoblast-like cells to form at the site (Graham et al. 2006). Mineral trioxide aggregate (MTA) appears to have similar properties since it slowly release calcium hydroxide from the set material (Tomson et al. 2007).

Migration and proliferation of pulp cells were observed adjacent to the necrotic zone. An increased formation of the extracellular matrix, namely, *collagen*(s), concerned DNA synthesis by pulp cells. The formation of a scar involves collagen construction, with a dentin appearance. The mineralization of the barrier and cellular differentiation occurs. *Matrix vesicles* play an initial role in pulp mineralization. *Calcium carbonate* granulations initiate the mineralization of the newly formed collagen and in the differentiation of secondary odontoblasts (e.g., differentiation of the so-called Hoehl's cells and mesenchymal stem cells/pulp progenitors). One day after capping, precipitations of crystalline structures are

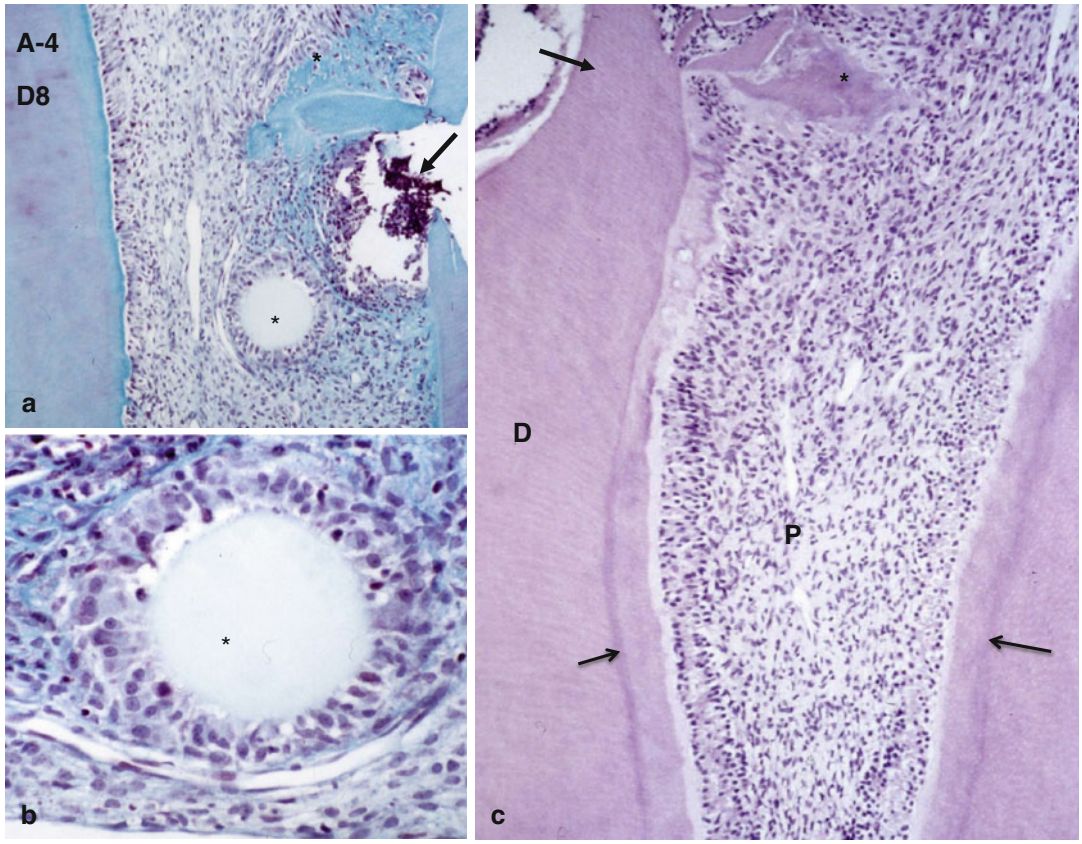


Fig. 11.14 Implantation of an amelogenin peptide (A-4) in the dental pulp. (**a**) At 8 days, implanted in the cavity (*arrow*), an inflammatory reaction is detected in the superficial area, near the agarose bead (*asterisk*). (**b**) Larger magnification of the agarose bead (see here as carrier for A-4) (*asterisk*). A ring of cells occupies the outer surface of the bead. (**c**) Pulp exposure is seen in the upper part of the root together with dentin debris (*asterisk*). No pulp inflammation (*P*) appears in the root. *Arrows* indicate the division between orthodentin (*D*) and reactionary osteodentin

observed at the interface between the superficial necrotic zone and the underlying pulp tissue. This layer is immunopositive to *fibronectin*. Odontoblast-like cells are positive at 7 to 10 days after capping. Corkscrew fiber-like fluorescent structures are visible between the cells (Yoshiba et al. 1996). Observation of adult rhesus monkeys' molars capped with Dycal or Life, $Ca(OH)_2$ calcium hydroxide implanted for 14 days, 5 weeks, and 1 and 2 years, revealed that 89 % of the dentin bridges contain tunnel defects. The tunnels fail to provide a hermetic seal (Cox et al. 1996) (Figs. 11.11, 11.12, 11.13, 11.14, 11.15, 11.16, 11.17, 11.18, and 11.19).

Capping with mineral trioxide aggregate (MTA) follows the same cascade of events reported with Ca(OH)2. After inducing a mild surface necrosis, bridging new matrix is formed and the collagenous matrix is mineralized. The cells proliferate for 3 days, appearing as *nestin*-expressing cells. They form a matrix on the fifth day. Cells displaying odontoblast-like morphology were seen by day 14. *Osteopontin* (OPN) was immunopositive just beneath the necrotic area after 1 day. OPN appears to trigger the initiation of the pulp reparative process (Kuratate et al. 2008).

MTA and Portland cements were immunostained for *dentin matrix protein-1* (DMP-1), in contrast with calcium hydroxide, which display a negative immunostaining (Neto et al. 2016). Dentin matrix protein 1 is one of the dentin noncollagenous extracellular matrix proteins implicated in regulation of mineralization. We have examined

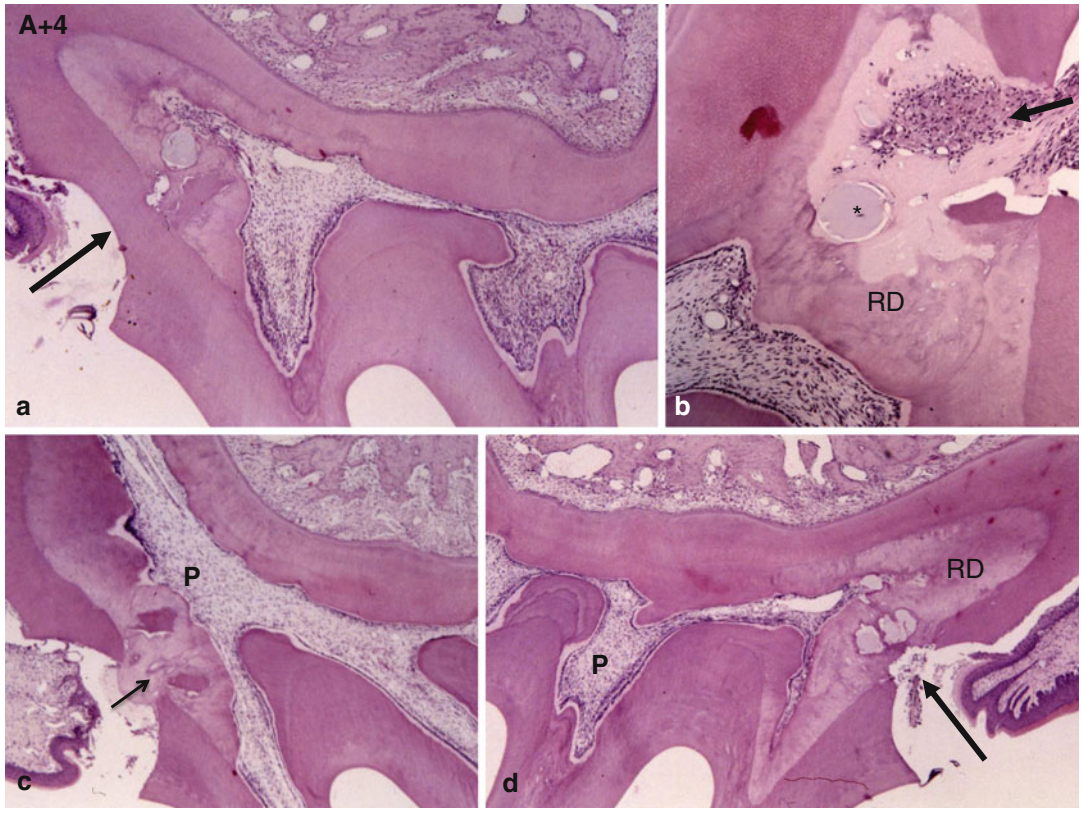


Fig. 11.15 (**a**) Reactionary dentin formation in the root, beneath a cavity (*arrow*) after implantation of A+4 amelogenin molecule. (**b**) Pulp exposure with debris pushed inside the dental pulp and an agarose bead (*asterisk*). Reparative dentin (*RD*) fills the pulp exposure. (**c**) Reparative dentin (*arrow*) fills the pulp exposure (*P*). (**d**) Connected with the mesial cavity (*arrow*), in the mesial root reparative dentin (*RD*) occludes the upper part of the root

the potential role of DMP1 in inducing cytodifferentiation of dental pulp stem cells into odontoblast-like cells and formation of reparative dentin in a rat model. Cavities were drilled and pulps exposed in maxillary first molars. Collagen matrix impregnated with recombinant DMP1 was implanted directly in group 1, while calcium hydroxide was implanted in group 2; collagen matrix that was not impregnated with rDMP1 was implanted directly in group 3, which served as control (Almushayt et al. 2006) (Figs. 11.20, 11.21, and 11.22).

When pulp exposures are capped with calcium hydroxide, positive immunostaining for *tenascin* (TN) and *fibronectin* (FN) was seen around the dentin barrier, delimitating the reparative dentin.

Bone sialoprotein (BSP) implanted in the pulp of the first maxillary molar formed a reparative dentinal bridge after 30 days. BSP stimulates the differentiation of pulp cells and contributes to the formation of a thick reparative dentinal tissue, occluding the perforation (Decup et al. 2000). After experimental pulp capping with MTA, the pulps were either capped with MTA alone or with BMP-7 followed by restoration with MTA. More complete bridge immunostaining was seen for *dentin sialoprotein* (DSP) in MTA-capped pulps, compared with BMP-7 alone, appearing as bone-like and devoid of DSP staining (Andelin et al. 2003) (Figs. 11.22, 11.23, and 11.24).

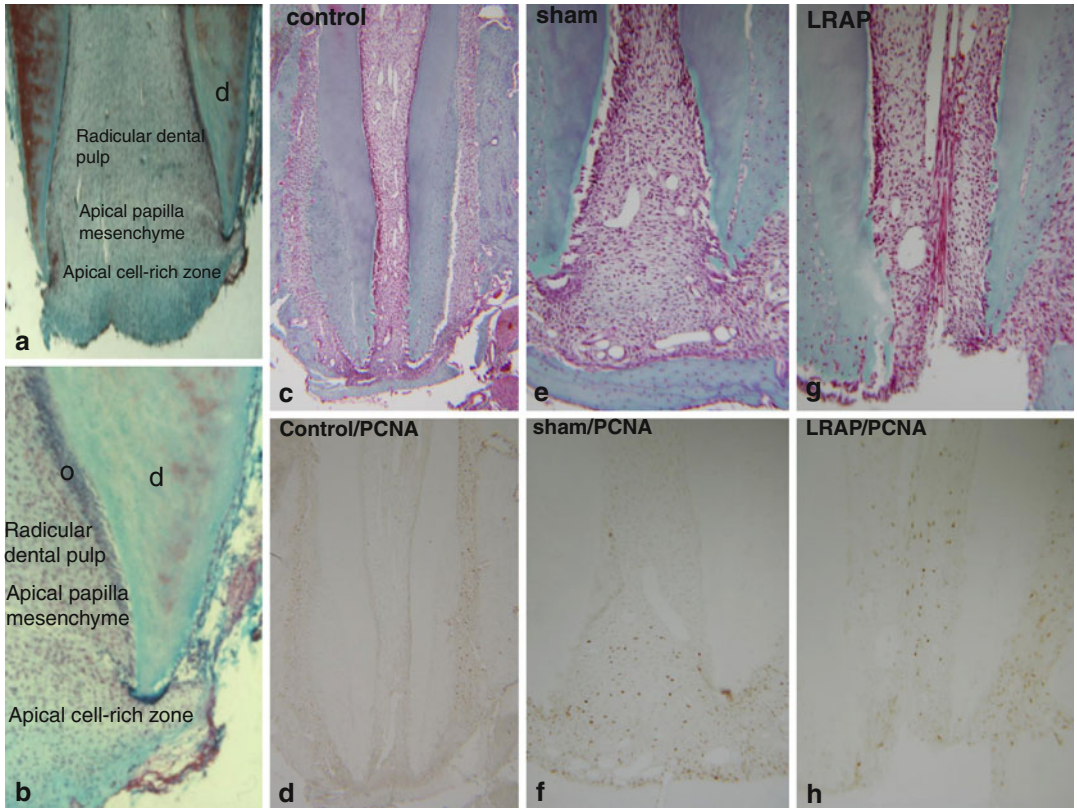


Fig. 11.16 (**a**, **b**) In the apical part of the root, three distinct zones are seen: the apical cell-rich zone, the apical papilla mesenchyme, and the radicular dental pulp. In the control teeth, (**c**, **d**) PCNA labeling is only seen in the periodontal ligament. After sham treatment, PCNA-labeled cells are detectable in the apical papilla and in the apical cell-rich zone (**e**, **f**). After amelogenin implantation in the pulp (LRAP/PCNA), proliferating labeled cells are visible in the radicular dental pulp alone (**g**, **h**)

Zone III	Apical cell-rich zone (1x1 cm^2)	Root pulp
sham	3.4	0.43
Beads implantation	2	1.33
LRAP Implantation	1	4.23

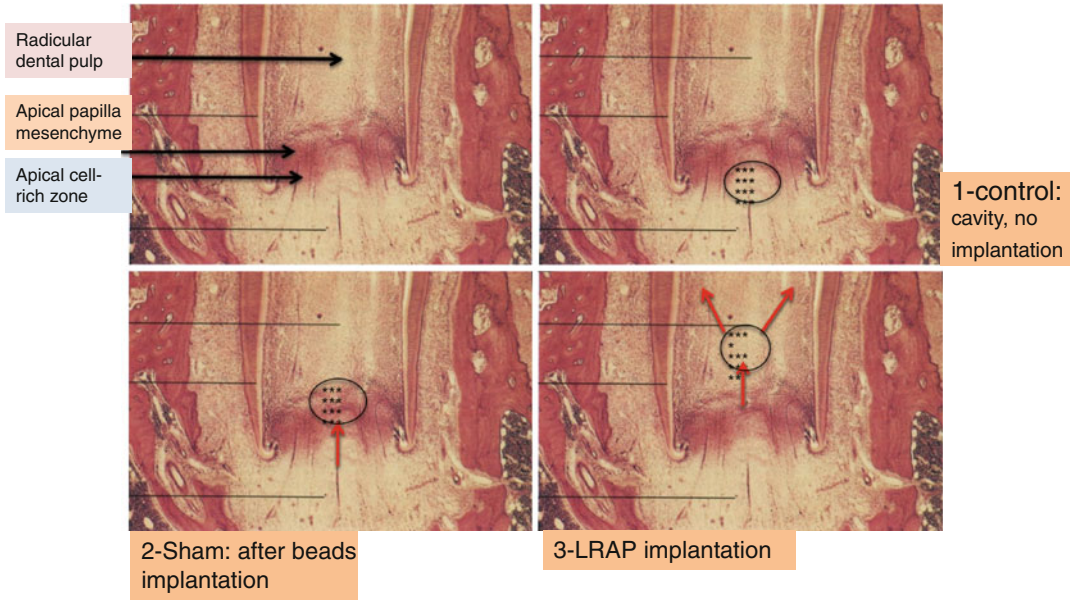


Fig. 11.17 Crude calculation of the cell density in the apical cell-rich zone and in the root pulp differs depending on the treatment (sham, bead implantation, or LRAP implantation). Labeling differs in (1) control, (2) sham, or (3) after LRAP implantation

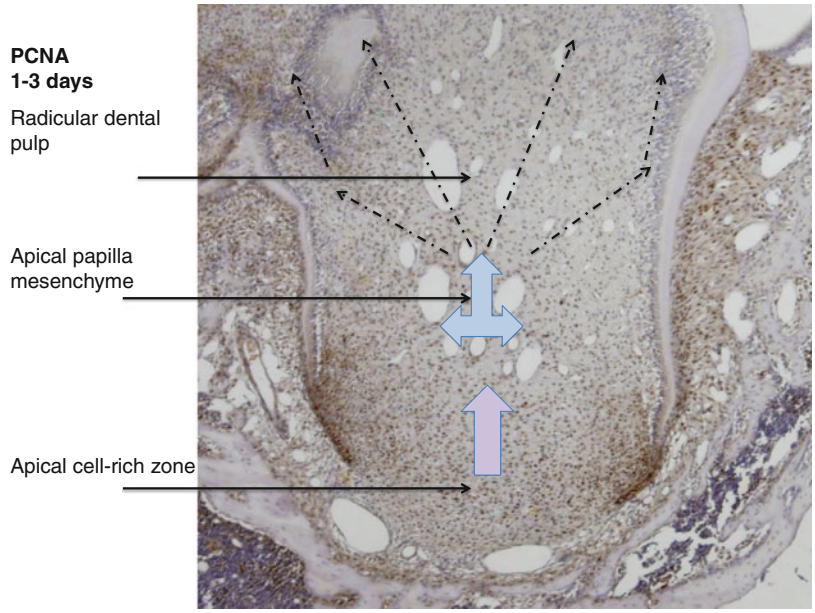


Fig. 11.18 Cells labeled in the apical cell-rich zone migrate. They move laterally in the apical papilla mesenchyme and slide in the coronal direction in the radicular dental pulp, where they become subodontoblastic and migrate in the coronal direction

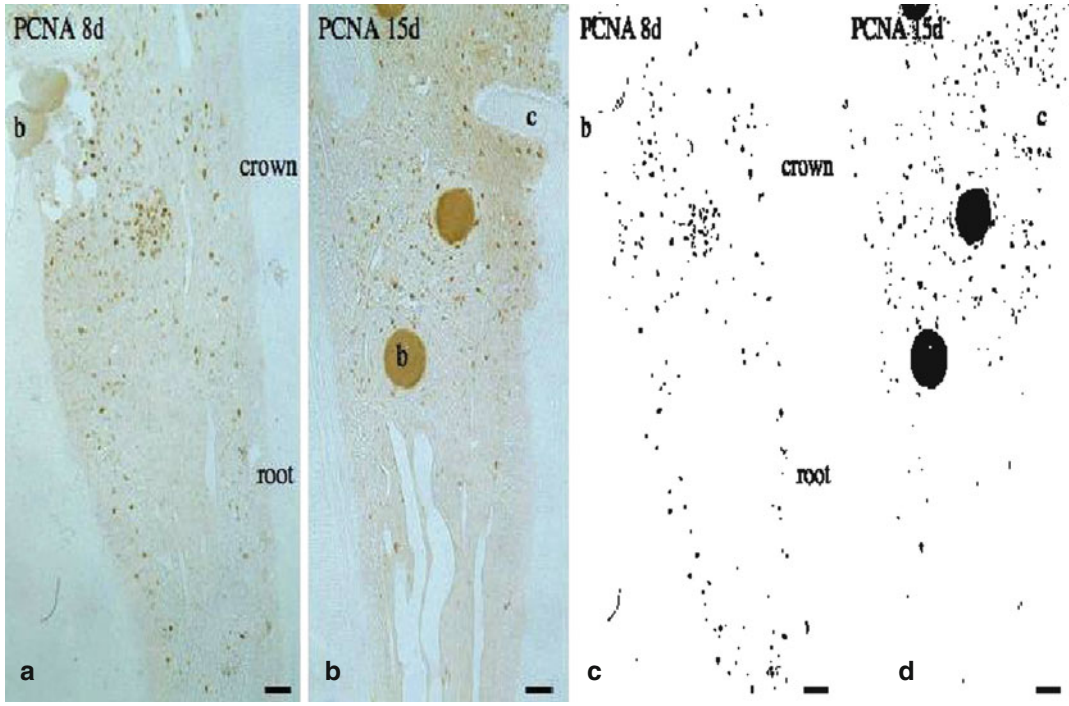
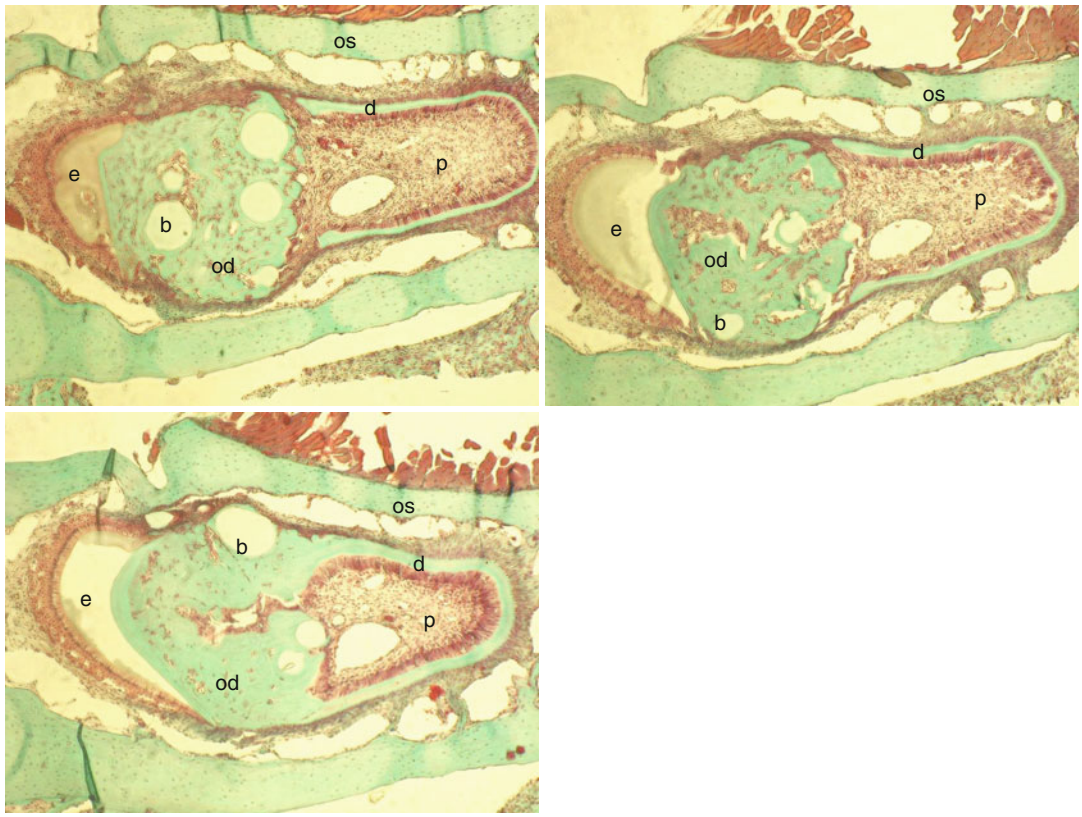


Fig. 11.19 PCNA-labeled cells are located after 8 days in the root in a subodontoblastic layer (**a**, **c**) and in the crown inside and around agarose beads. After 15 days, there is no more labeling in the root, and all the labeled cells are groups in the crown part (**b**, **d**)



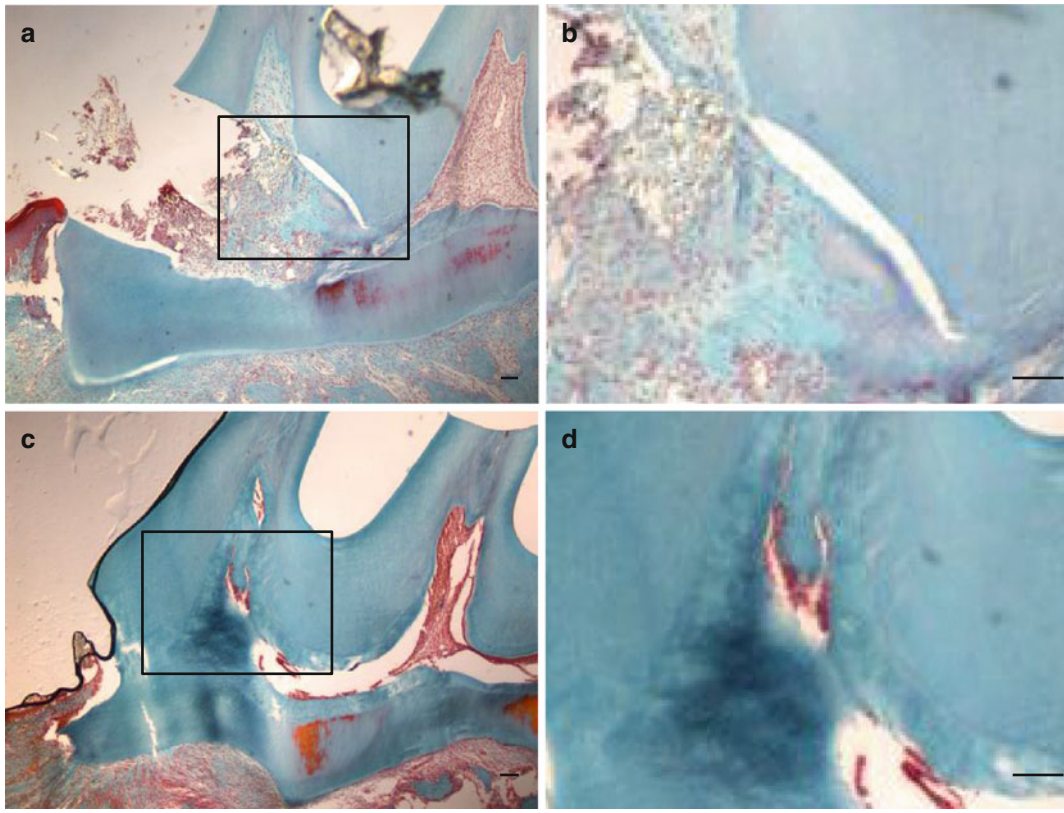


Fig. 11.21 Pulp exposure without bead implantation (control molars) contributes to the formation of a fibrous tissue. Implantation of A4 cells in the mesial pulp horn initiates 28 days after the formation of a thick reparative dentin bridge, beneath a calciotraumatic line

Fig. 11.20 Implantation of cell lines (agarose beads acting as carrier) in the dental pulp of the mouse incisor produces thick mineralized osteodentin aggregates

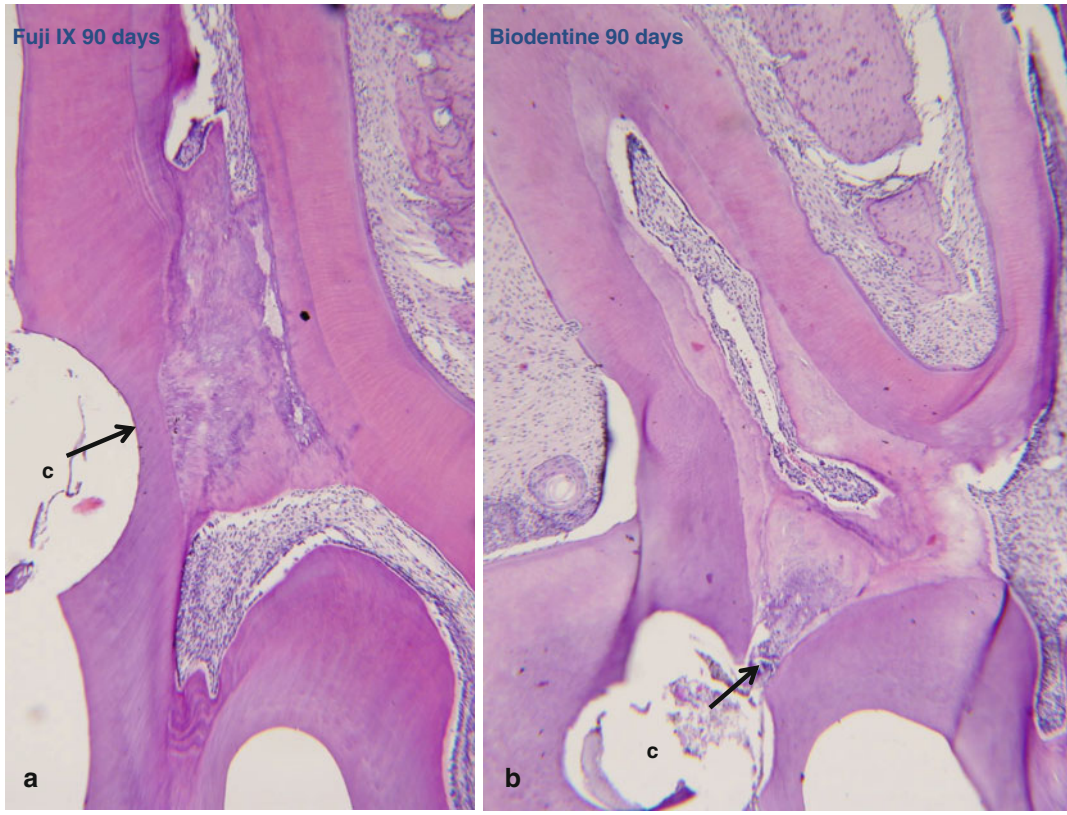


Fig. 11.22 (**a**) 90 days after filling a cervical cavity with a glass-ionomer cement (Fuji IX), reactionary dentin and a thick pulp stone obliterate the lumen of the root canal. (**b**) Direct pulp capping after 90 days with a tricalcium silicate-based cement (Biodentine) shows reparative dentin formation inside the mesial horn and root. *C* cavity. *Arrow*: pulp capping

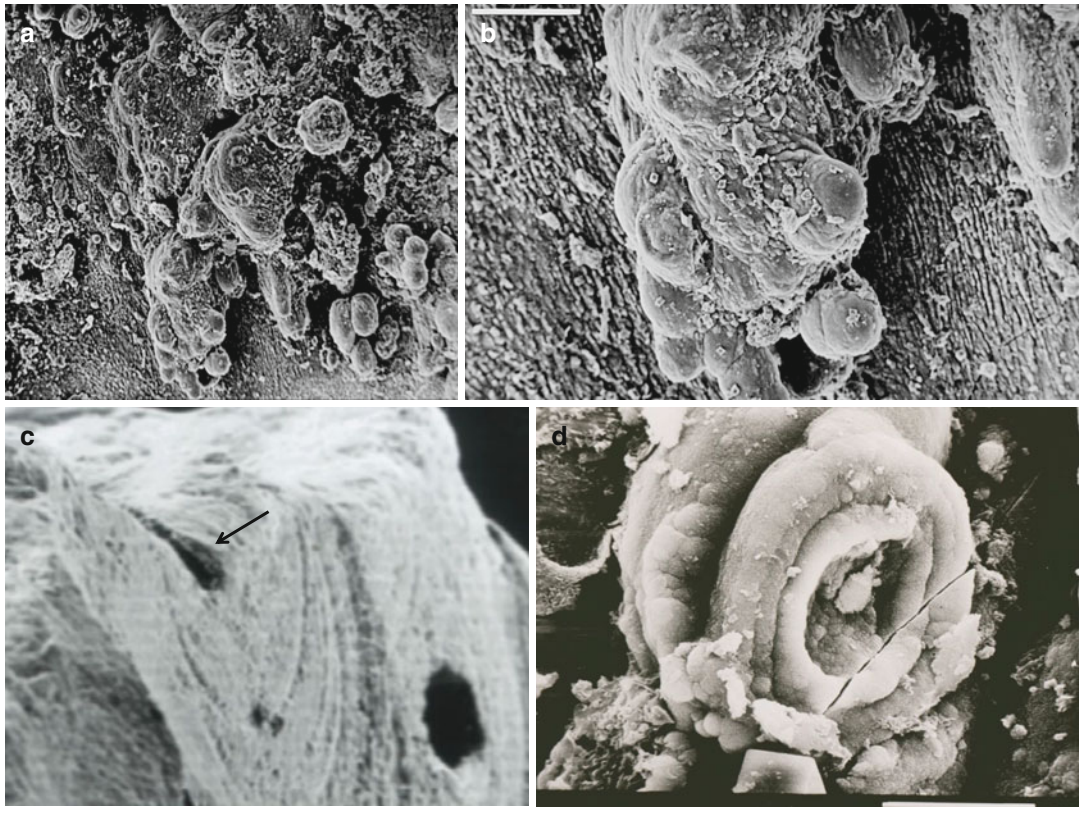


Fig. 11.23 (**a**, **b**) Reactionary dentin formed at the dentin surface. Globular structures merge with the walls of the dental pulp. (**c**) The section of a pulp stone displays the gradual increase in thickness of the pulpolithe. A clear relationship may be established between an isolated pulp stone formation and vascular initiation of the pulp mineralization. (**d**) Along the pulp surface, adherent mineralization is seen

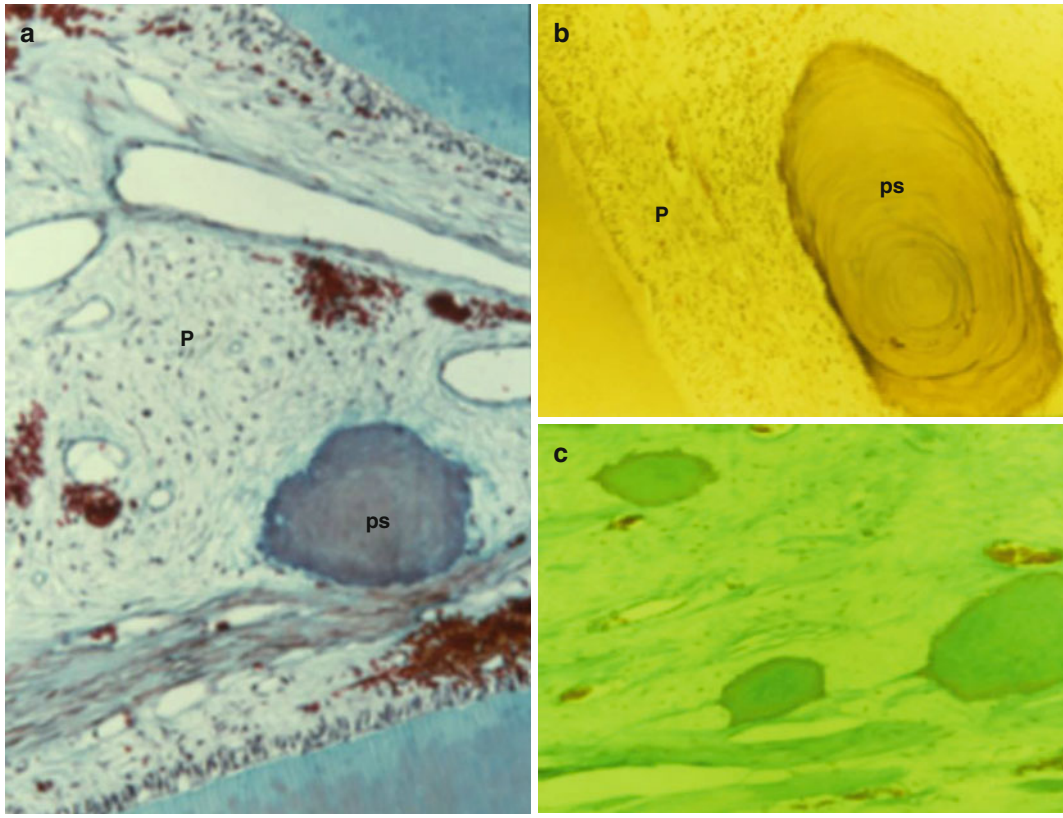


Fig. 11.24 (**a**) Isolated pulp stones (*ps*) within the dental pulp (*P*). (**b**) Lamellar growth contributes to the increase in size of the pulp stone. (**c**) Diffuse mineralization in the pulp

References

Aguiar MC, Arana-Chavez V. Ultrastructural and immunocytochemical analyses of osteopontin in reactionary and reparative dentine formed after extrusion of upper rat incisor. J Anat. 2007;210:418–27.

Aguiar MC, Arana-Chavez VE. Immunocytochemical detection of dentine matrix protein 1 in experimentally induced reactionary and reparative dentine in rat incisor. Arch Oral Biol. 2010;55:210–4.

Almushayt A, Narayanan K, Zaki AE, George A. Dentin matrix protein 1 induces cytodifferentiation of dental pulp stem cells into odontoblasts. Gene Ther. 2006;13:611–20.

Andelin WE, Shabahang S, Wright K, Torabinejad M. Identification of hard tissue after experimental pulp capping using dentin sialoprotein (DSP) as a marker. J Endod. 2003;29:646–50.

Bjorndal L. The caries process and its effect on the pulp: the science is changing and so is our understanding. J Endod. 2008;34:52–9.

Caviedes J, Munoz HR, Azueros-Holguin MM, Ulate E. Neuropeptides in dental pulp: the silent protagonists. J Endod. 2008;34:773–88.

Cox CF, Subay RK, Ostro E, Suzuki S, Suzuki SH. Tunnel defects in dentin bridges: their formation following direct pulp capping. Oper Dent. 1996;21:4–11.

Decup F, Six N, Palmier B, Buch D, Lasfargues JJ, Salih E, Goldberg M. Bone sialoprotein-induced reparative dentinogenesis in the pulp of rat's molar. Clin Oral Investig. 2000;4:110–9.

Di Nicolo R, Guedes-Pinto AC, Carvalho YR. Histopathology of the pulp of primary molars with active and arrested dentinal caries. J Clin Pediatr Dent. 2000;25:47–9.

Dimitrova-Nakov S, Baudry A, Harichane Y, Kellermann O, Goldberg M. Pulp stem cells: implication in reparative dentin formation. J Endod. 2014;40(4 Suppl): S13–8.

Fanchon S, Bourd K, Septier D, Everts V, Beertsen W, Menashi S, Goldberg M. Involvement of matrix metalloproteinases in the onset of dentin mineralization. Eur J Oral Sci. 2004;112:171–6.

Gaudin A, Renard E, Hill M, Bouchet-Delbos L, Bienvenu-Louvet G, Farges J-C, Cuturi M-C, Alliot-Licht B. Phenotypic analysis of Immunocompetent cells in healthy human dental pulp. J Endod. 2015. doi.org/10.1016/j.joen.2015.01.005.

Goldberg M. Dentin, pulp, and tooth pain. Chapter 5. In: Hand AR, Frank ME. Fundamentals of oral histology and physiology. Wiley Blackwell & Sons, Inc.; 2014. p. 85–112.

Goldberg M, Septier D, Rapoport O, Iozzo RV, Young MF, Ameye LG. Targeted disruption of two small leucine-rich proteoglycans, biglycan and decorin, excerpt divergent effects on enamel and dentin formation. Calcif Tissue Int. 2005;77:297–310.

Goldberg M, Septier D, Oldberg A, Young MF, Ameye LG. Fibromodulin-deficient mice display impaired collagen fibrillogenesis in predentin as well as altered dentin mineralization and enamel formation. J Histochem Cytochem. 2006;54:525–37.

Graham L, Cooper PR, Cassidy N, Nor JE, Sloan AJ, Smith AJ. The effect of calcium hydroxide on solubilisation of bio-active dentin matrix component. Biomaterials. 2006;27:2865–73.

Han N, Zhang Y, Li R, Li X, Zhou M, Niu Y, Zhang G. β-catenin enhances odontoblastic differentiation of dental pulp cells through activation of Runx2. PLoS One. 2014;9(2):e88890.

Huang GT-J, Potente AP, Kim J-W, Chugal N, Zhang X. Increased interleukin-8 expression in inflamed human dental pulps. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;88:214–20.

Kuboki Y, Ohgushi K, Fusayama T. Collagen biochemistry of the two layers of carious dentin. J Dent Res. 1977;56:1233–7.

Kuratate M, Yoshiba K, Shigetani Y, Yoshiba N, Ohshima H, Okiji T. Immunohistochemical analysis of nestin, osteopontin, and proliferating cells in the reparative process of exposed dental pulp capped with mineral trioxide aggregate. J Endod. 2008;34:970–4.

Linde A. The extracellular matrix of the dental pulp and dentin. J Dent Res. 1985;64 Spec issue:523–9.

Massler M, Pawlak J. The affected and infected pulp. Oral Surg Oral Med Oral Pathol. 1977;43:929–47.

Mjör IA. Dentin permeability: the basis for understanding pulp reactions and adhesive technology. Braz Dent J. 2009;20:3–16.

Muromachi K, Kamio N, Matsumoto T, Matsushima K. Role of CTGF/CCN2 in reparative dentinogenesis in human dental pulp. J Oral Sci. 2012;54:47–64.

Neto LN, Marques NC, Fernandes AP, Rodini CO, Sakai VT, Abdo RC, Machado MA, Santos CF, Oliveira TM. Immunolocalization of dentin matrix protein-1 in human primary teeth treated with different pulp capping materials. J Biomed Mater Res B Appl Biomater. 2016;104(1):165–9.

Paula-Silva FWG, Ghosh A, Silva LAB, Kapila YL. The TNF-α promotes an odontoblastic phenotype in dental pulp cells. J Dent Res. 2009;88:339–44.

Rahamtulla F. Proteoglycans of oral tissues. Crit Rev Oral Biol Med. 1992;3:135–62.

Schröder U. Effects of calcium hydroxide-containing pulp capping on pulp cell migration, proliferation, and differentiation. J Dent Res. 1985;64(Spec Iss):541–8.

Sela J, Tamari I, Hirschfeld Z, Bab I. Transmission electron microscopy of reparative dentin in rat molar pulps. Primary mineralization via extracellular matrix vesicles. Acta Anat. 1981;109:247–51.

Shibata S, Yoneda S, Yanagishihta M, Yamashita Y. Isolation of proteoglycan (versican) aggregate from rat dental pulp. Arch Oral Biol. 2000;45:563–8.

Shuttleworth CA, Ward JL, Hirschmann PN. The presence of type III collagen in the developing tooth. Biochem Biophys Acta. 1978;535:348–55.

Sone S, Nakamura M, Maruya Y, Takahashi I, Mizoguchi I, Mayanagi H, Sasano Y. Expression of versican and ADAMTS during tooth eruption. J Mol Histol. 2005;36:281–8.

Stanley HR, Pereira JC, Spiegel E, Broom C, Schultz M. The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin and dead tracts beneath various types of dental lesions according to tooth surface and age. J Oral Pathol. 1983;12:257–89.

Sulkala M, Paakkonen V, Larmas M, Salo T, Tajderhane L. Matrix metalloproteinase-13 (MMP-13) is highly expressed in human tooth pulp. Connect Tissue Res. 2004;45:231–7.

Tomson PL, Grover LM, Lumley PJ, Sloan AJ, Smith AJ, Cooper PR. Dissolution of bioactive matrix components by mineral trioxide aggregate. J Dent. 2007;35:636–42.

Tsuzaki M, Yamauchi M, Mechanic GL. Bovine dental pulp collagens: characterization of types III and V collagen. Arch Oral Biol. 1990;35:195–2000.

Tziafas D, Veis A, Alvanou A. inability of calcium hydroxide to induce reparative dentinogenesis at non-peripheral sites of dog dental pulp. Eur J Oral Sci. 1996;104:623–6.

Wisithphrom K, Windsor J. The effects of Tumor Necrosis Factor α, Interleukin-1β, Interleukin-6, and Transforming Growth Factor-β1 on pulp fibroblast mediated collagen degradation. J Endod. 2006;32:853–61.

Yamamoto R, Oida S, Yamakoshi Y. Dentin sialophosphoprotein-derived proteins in the dental pulp. J Dent Res. 2015;94(8):1120–7.

Yasuda Y, Izumikawa M, Okamoto K, Tsukuba T, Saito T. Dentin phosphophoryn promotes cellular migration of human dental pulp cells. J Endod. 2008;34:575–8.

Yoshiba K, Yoshiba N, Nakamura H, Iwaku M, Ozawa H. Immunolocalization of fibronectin during reparative dentinogenesis in human teeth after pulp capping with calcium hydroxide. J Dent Res. 1996;75:1590–7.

Yuan G, Yang G, Song G, Chen Z, Chen S. Immunohistochemical localization of the NH2-terminal and COOH-terminal fragments of dentin sialoprotein in mouse teeth. Cell Tissue Res. 2012;349:805–14.