Reactionary and Reparative Dentin-Like Structures

10

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10.1 Introduction

In reaction to a slow carious decay, continuous abrasion or noxious effects of monomers released by resin composites, odontoblasts may be wounded but are still alive (Figs. 10.1, 10.2, and 10.3a–c). They synthesize and secrete a true extracellular matrix which contributes to the formation of a mineralized *reactionary tubular dentin-like or a bone-like structure*. A "calciotraumatic" or a "reverse" line separates the newly formed reactionary dentin from the primary or secondary dentin already formed before any development of the traumatic decay (Figs. 10.4a, b and 10.5a–d). It happens that odontoblasts wounded by the release of carious toxins are unable to survive. During dentinogenesis, odontoblast cell bodies constitute a thick layer at the pulp periphery, and four rows of cells may be scored during early dentin formation. Aging influences the thickness of this layer, which is gradually reduced. Although there is no direct evidence that the cells from the Hoehl's layer may be reactivated, differentiated, and become secondary odontoblasts, this is probably what happens. Even if some differences are well identified between a true orthodentin and reactionary dentin-like structure, odontoblasts and/or Hoehl's cells are responsible for the formation of reactionary dentin.

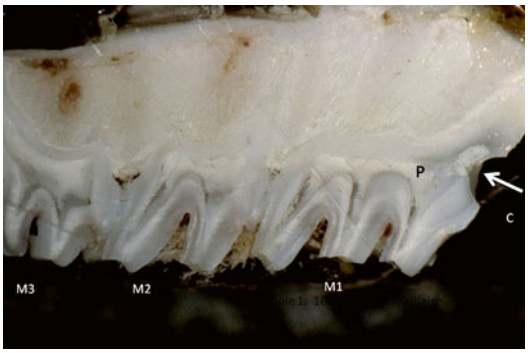

Fig. 10.1 Longitudinal section of the three mandibular molars (M1, M2, and M3). A cavity was prepared in the mesial part of M1. *P* pulp, *C* cavity

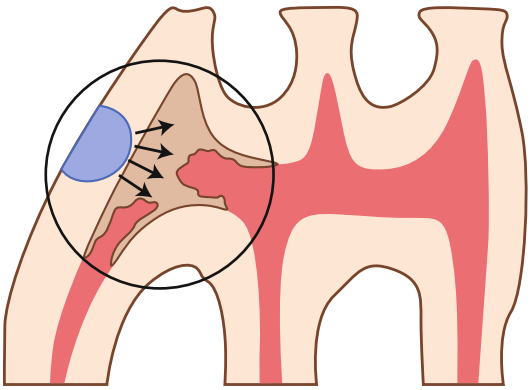

Fig. 10.2 Schematic drawing showing the formation of reactionary dentin beneath a cavity prepared in the mesial aspect of the first molar

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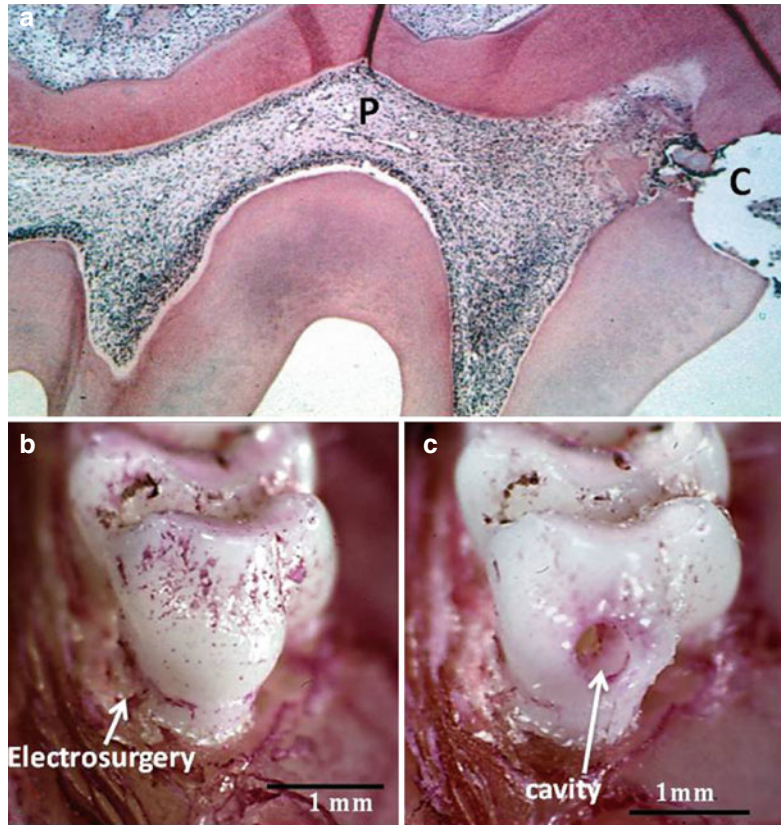

Fig. 10.3 (**a**) Histological section of the demineralized first mandibular molar. After the preparation of a cavity in the mesial aspect (*c*), a slight reaction occurs in the pulp (*P*). In (**b**), the tooth is seen after a cervical electrosurgery, and in (**c**), a cavity is drilled in the mesial aspect

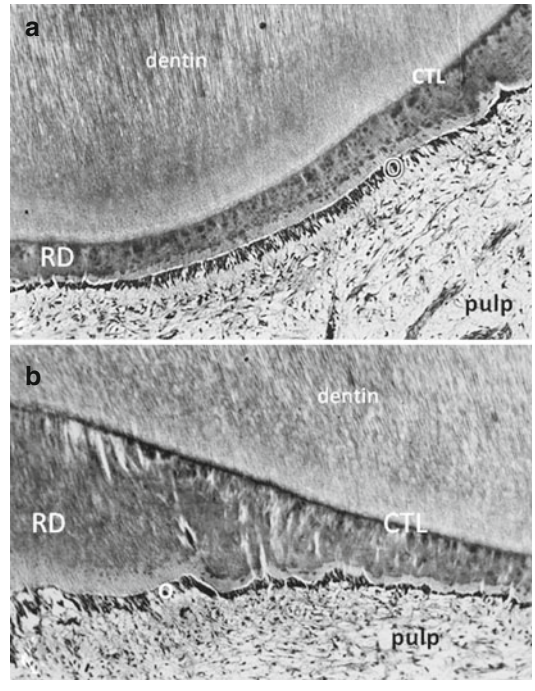

Fig. 10.4 Atubular reactionary dentin (*RD*) is formed by the odontoblasts and Hoehl's cells, beneath a calciotraumatic line (*CTL*) at the junction between a tubular dentin and odontoblast layer (*O*). The dental pulp is fibrous but still alive. (**a**) Thin reactionary dentin formation; (**b**) thicker reparative dentin formation, produced in the same experimental conditions

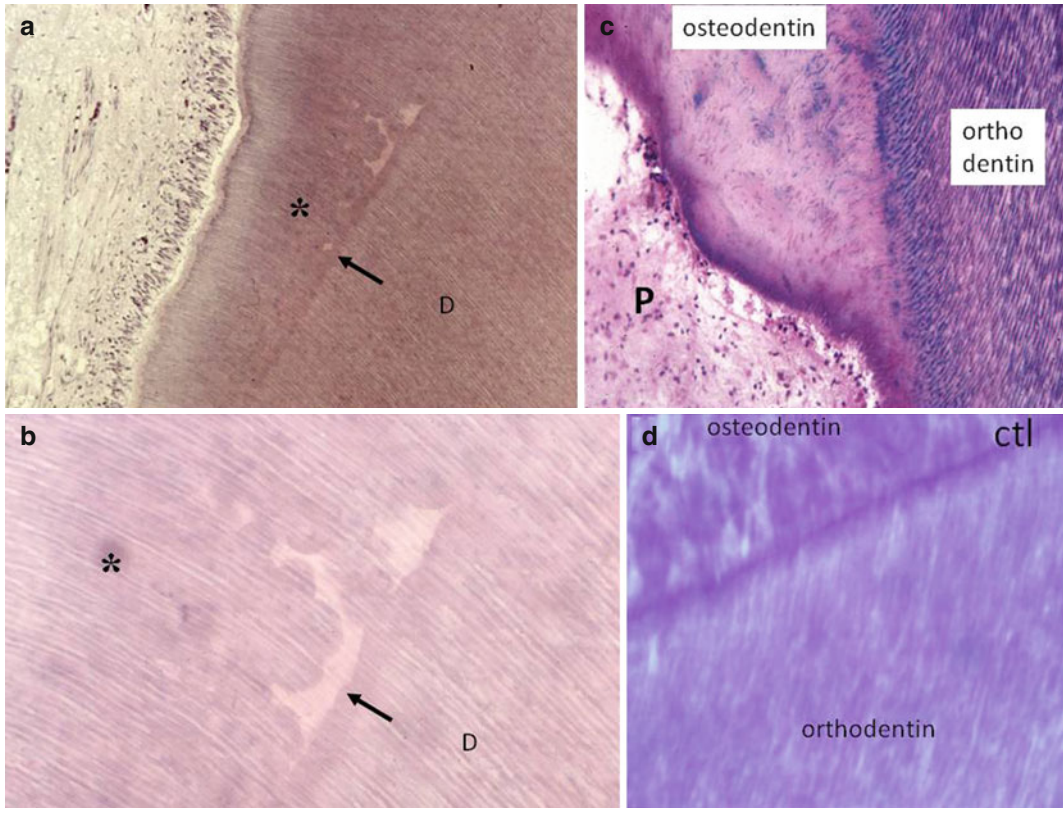

Fig. 10.5 (**a**, **b**) The preparation of a cavity induces the interruption of dentinogenesis. Beneath the tubular dentin formation, the *dark arrows* show interrupted physiological dentin deposit. The effects of the trauma lead to calcospherite formation (globular structures). The newly formed dentin (*asterisks*) comprises globular structures and interglobular spaces. Odontoblasts and pulp are "normal." (**c**) After a more severe decay, tubular orthodentin (stained in purple by the "stains all" method) is interrupted; and the new osteodentin formed in reaction to the trauma, is atubular, and contains a few osteoblast lacunas. The dental pulp is apparently sound. (**d**) Reactionary osteodentin is separated from orthodentin by a calciotraumatic line (*CTL*)

If the carious lesion is progressing quickly, the lesion may destroy the residual dentin and penetrate the dental pulp. The pulp exposure favors the diffusion of bacteria from the contaminated dentin within the dental pulp. Pulp inflammatory cells control the infection and immune cells tend to slow down the progression of the lesion.

The *reparative dentin-like* structure that is formed constitutes an attempt to close the pulp exposure. In this case, odontoblasts and Hoehl's cells are irreversibly wounded. Pulp progenitors or stem cells are implicated in the formation of a reparative dentin bridge or in a "bone-like" structure, also named osteodentin (Fig. 10.6a–d). Pulp cells are embedded in a bone-like structure similar to osteocyte-like cell being. These cells are located inside a lacuna. Reparative dentin-like fills partially or totally the mesial part of the pulp chamber of a rat's molar. The dentin bridge seen at early stages of pulp capping expands and seals the mesial pulp chamber. The mineralization process expands up to the isthmus between the mesial and central parts. In humans, the reparative process is initially located beneath the pulp exposure. Then an osteodentin bridge gradually loads the pulp chamber. The mineralized structure is either in continuity with the newly formed reactionary dentin or appears as free pulp stone (calcospherites), developing around endothelial cells of capillaries.

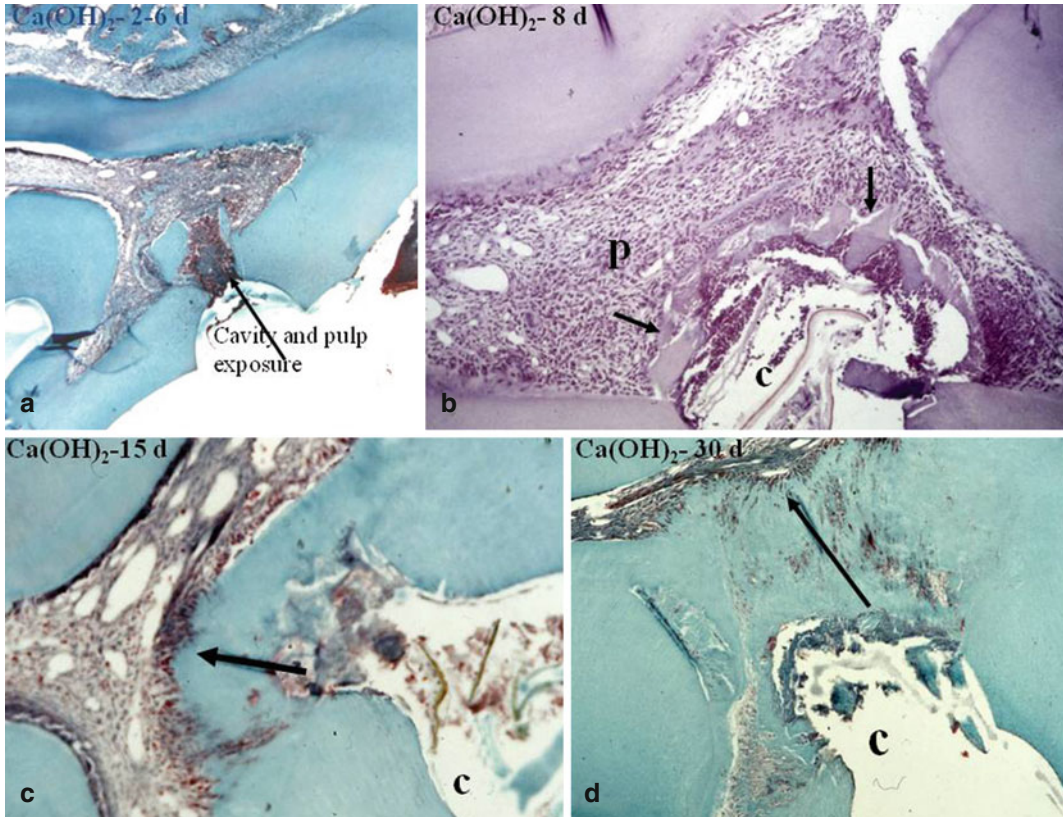

Fig. 10.6 In (**a**), 2–6 days after pulp capping a pulp exposure with $Ca(OH)_2$, some dentin debris are pushed within the pulp. A pulp inflammatory process is limited to the mesial part of the pulp chamber. In (**b**), after 8 days, the calcium hydroxide induces the early formation of a dentin bridge, in close association with dentin debris (*arrows*). In (**c**), after 15 days, the dentin bridge is thicker and more homogeneous. (**d**) After 30 days, the dentin bridge occludes totally the pulp exposure. However, some tunnellike structures or cell remnants persist, allowing bacterial penetration toward the dental pulp. *P* pulp, *C* cavity

In many publications, some confusion occurs between reactionary and reparative dentin-like structures. To be precise, a clear-cut difference should be made between a tubular or atubular reactionary dentin, formed under the control of odontoblasts and Hoehl's cells, and reparative dentin, appearing mostly as osteodentin and produced by pulp stem cells or progenitors.

A series of questions result from the available valid data. Wound healing results from repair or regeneration processes. Many major questions arise, including can we restore the original architecture and the biological function of the injured pulp tissue? Complete regeneration occurs during the fetal period, within the 24 weeks of gestation. However, in a clinical setting, we are treating young patients with already erupted teeth and/or adult patients. This implies that the postnatal wound healing combines a cascade of events leading to pulp repair and/or regeneration. The recruitment and differentiation of potential progenitors/stem cells are prerequisites. This is followed by pulp reconstruction.

10.2 In Vivo Approach

10.2.1 Preparation of a Cavity Without Pulp Exposure

In response to the preparation of a cavity in the mesial aspect of the first maxillary molar, odontoblasts, Hoehl's cells, and pulp tissue reflect the reaction to the preparation of a cavity. Drilling a cavity

caused the displacement of some odontoblasts and their penetration into dentin tubules. Inflammatory exudation was seen soon after drilling. The endothelium of capillaries showed an increase of pinocytic vesicles, an event associated with the formation of an exudative lesion. After 1 day, many damaged odontoblasts degenerate. Cells with a high nucleus/cytoplasm ratio and prominent nucleoli accumulate around the subodontoblastic capillaries. Newly differentiating odontoblasts received nutritional supply from the capillaries. After 3 days, differentiating odontoblasts increased in number. They start to produce reactionary dentin by 5 days after cavity preparation. Differences were seen between the original dentin layer formed before the drilling of the cavity and reactionary dentin-like formation [1].

10.2.2 Preparation of a Cavity Followed by a Pulp Exposure

Different repair responses were recorded between the coronal and radicular areas after the implantation of bone morphogenetic protein-7 (BMP-7) in a pulp exposure. Used as a capping agent, BMP-7 induces after 8 days the formation of a reparative osteodentin bridge, formed by globular mineralized areas at the exposure site and leaving unmineralized interglobular areas containing pulp remnants. A heterogeneous mineralization was seen in the coronal part of the pulp. Complete filling of the radicular pulp by a homogeneous mineralization was seen in the root, behind a calciotraumatic line. These results emphasize the biological differences between the coronal and radicular parts of the pulp [2]. Indeed, the crown is formed under the control of enamel organ, whereas the extracellular matrix (ECM) secreted by the epithelial Hertwig's root sheath influences the root construction.

10.3 Reactionary Dentin (Tertiary Dentin)

Reviewing data on reactionary dentinogenesis, Smith et al. [3] differentiate between tertiary dentin and reparative dentinogenesis (Figs. 10.6a–d and 10.7a–d). It is indeed difficult to discriminate between the dentin secreted by postmitotic odontoblasts and a mineralized structure formed by a new population of pulp-derived, odontoblast-like cells. The tertiary dentin beneath a carious decay may comprise both reactionary and reparative dentins. Hence, a clear-cut definition for each of the tissues is needed and is difficult to obtain.

Reactionary dentin is formed in response to a carious decay, to excessive abrasion, or to the cytotoxic effects of monomers released by a restorative material. This is how the pulp limits undesirable noxious effects. Trans-dentin stimulation is mediated by bioactive extracellular matrix components during a cavity preparation, the dental pulp being nonexposed. As an example, OP-1, used as a cavity liner, stimulates the formation of reactionary dentin [4]. Odontoblasts respond to the stimulating presence of ECM. Diffusion of ECM proteins, as determined by the residual dentin thickness (RDT) after preparation of a cavity, influences the signaling process [5]. They synthesize and secrete the ECM of a tubular or atubular dentin-like structure beneath a calciotraumatic line [6]. This line is similar to the reverse line seen in the bone. Sometimes this dentin-like structure is globular, but very often appears as lamellar or amorphous. Reactionary dentin also named tertiary dentin differs from the orthodentin formed prior to the lesion. The matrix of reactionary dentin displays reduced hardness and lowers elastic modulus. The residual dentin thickness (RDT) interferes with the pulp activity. The maximal dentin deposition of reactionary dentin appears beneath cavities with an RDT varying between 0.5 and 0.25 mm. Odontoblasts are reducing in number in cavities closer to the indirectly injured pulp. The restoration material influences also the odontoblast activities [7].

10.3.1 Molecules Expressed or Influencing Reactionary Dentin Formation

Differences were observed between reactionary dentin (RD) and primary or secondary orthodentin with respect to the distribution of *five SIBLINGs* (*BSP*, *OPN*, *DMP-1*, *DSPP*, and *DSP* (a fragment of DSPP)). BSP and OPN were

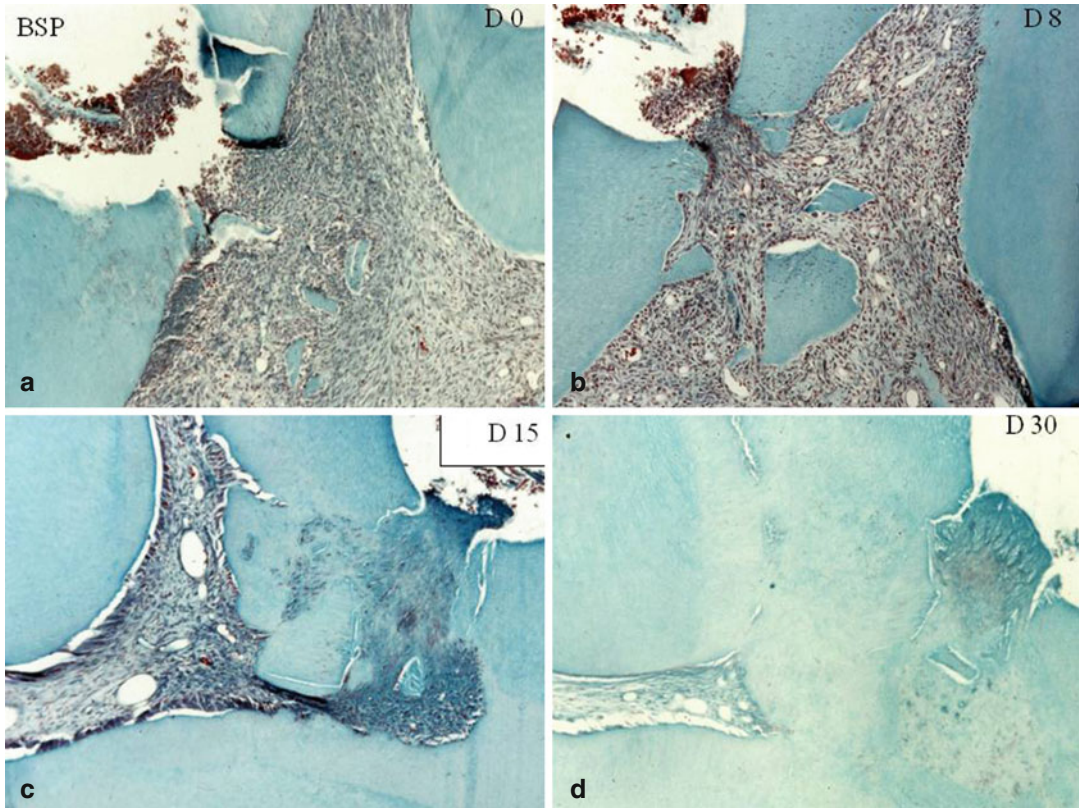

Fig. 10.7 Capping effects of bone sialoprotein (*BSP*). At day 0 (**a**) and at day 8 (**b**). Dentin debris are pushed within the pulp. (**c**) At day 15, beginning of the formation of a reparative dentin bridge. (**d**) 30 days after pulp capping with BSP, a thick and homogeneous dentin bridge closes totally the pulp exposure

observed in RD but not in predentin, whereas the expression of DMP-1 and DSP was lower in RD compared with predentin [8]. DSP was increased in density and spatial resolution. This molecule contributes to putative HAp nucleation on collagen scaffold. DSP antibodies showed weak staining in RD, whereas osteopontin (OPN) was extensively positive in RD [9]. OPN was not detected in physiological and reactionary dentin, but seen to be strongly immunoreactive in reparative dentin alone [10] (Fig. 10.7a–d).

10.3.2 Other ECM Molecules

- *Metalloproteinase-2* (*MMP-2*) is increased. Parallel upregulation occurs for TIMP-2 and for the membrane type 1-matrix metalloproteinase (MT1-MMP). Furthermore, the genes encoding components of the TGF-β signaling pathway, namely, *SMAD-2* and *SMAD-4*, may explain the increased synthesis of collagen [11, 12].
- *Odontogenic ameloblast-associated protein* (*ODAM*) is expressed by ameloblasts and odontoblasts. The molecule plays a role in enamel mineralization, possibly through the regulation of MMP-20. Experimental results show that rODAM accelerates reactionary dentin formation near the pulp exposure area, preserving normal odontoblasts in the remaining pulp [13].
- Finally, the expression of *Toll-like receptors* was altered in response to caries.

10.4 Reparative Dentin-Like: From the Initial Formation of a dentin Bridge to Pulp Mineralization

10.4.1 Cells Implicated in Reparative Dentin-Like Formation

When odontoblasts are injured and destroyed, replacement cells express types I and III collagen gene-specific riboprobes. The cells forming reparative dentin synthesize type I collagen but not type III. Antibodies raised against DSP positively stain the cells. Therefore, they are odontoblast-like cells [14].

Adult human dental pulp stem cells (hDPSCs) have been isolated from the pulp as precursor cells. They differentiate into cells implicated in the formation of reparative dentin. Lysyl oxidase-like 2 (LOXL2) was downregulated when hDPSCs differentiate into odontoblast-like cells. Therefore, LOXL2 has negative effect on the differentiation of pulp cells into odontoblasts [15]. In contrast, the other LOX family members including LOX, LOXL1, LOXL3, and LOXL4 are increased.

Cells emerging from dental pulp explant were studied to elucidate the origin of precursor cells implicated in the formation of reparative dentin. Early outgrowing cells emerging from cultured explant were round or elongated, with thin spinous processes. They were highly mobile and contain numerous lipid vesicles. Radioautography suggested that these lipids resulted from micropinocytosis. After 10–20 days, the cells started to be converted into fibroblast-like cells, less mobile and lacking lipid vesicles. It was concluded that they might be mononuclear phagocytic/histiocytic mesenchymal cells [16].

In vivo, 10 days after $Ca(OH)^2$ pulp capping, focal calcifications were seen within the collagen-rich matrix. Numerous extracellular matrix vesicles display electron-dense material composed of hydroxyapatite crystals [17]. At this early stage, similarities were detected between chondrocyte and the mineralization of cartilage septa and the early formation of reparative dentin.

Frozoni et al. [18] have used the 3.6-green fluorescent protein (GFP) transgenic mice to in vivo the biological sequence of events during pulp healing and reparative dentinogenesis. After pulp exposure and capping, followed by chemical fixation and processing for histological and epifluorescence analysis, immediately after pulp exposure no 3.6-GFP-labeled odontoblast was detected at the injury site. Four weeks after the surgery, reparative dentin started to be formed, and this was even more obvious at 8 weeks. The cells expressing 3.6-GFP lined an atubular dentin bridge. A few fluorescent cells were embedded within the atubular matrix, hence appeared as a bone-like structure (Figs. 10.8a–c and 10.9a–d).

Dentin regeneration may be obtained by using porcine deciduous pulp stem cells mixed with β-tricalcium phosphate. Four months after transplantation, regeneration of a dentin-like structure was completely completed, closing the roof defects [19] (Fig. 10.10a, b).

Canonical Wnt/β-catenin regulates odontoblast-like differentiation, improves moderately the expression of type I collagen, and enhances strongly the expression of osteopontin. Wnt-1 inhibited alkaline phosphatase and the formation of mineralized nodules. Scheller et al. [20] concluded that the canonical Wnt signaling regulates negatively the differentiation and mineralization of dental pulp stem cells.

10.4.2 Cell Mediators Implicated in Reparative Dentin-Like Formation

Bone morphogenetic proteins (BMPs) are multifunctional proteins structurally related to the *transforming growth factor-beta* (TGF-beta) and *activin*, which can induce cartilage and bone growth in vivo. BMPs are members of the TGF-beta superfamily. Their effects on pulp cells and reparative dentin formation have been reported.

The *TGF-beta1* superfamily influences the expression of BSP, DSP, TGF-beta1 receptor I, and Smad 2/3 proteins during reparative dentinogenesis. In vitro, TGF-beta2 had minimal effect on cultured tissues, whereas TGF-beta1 and

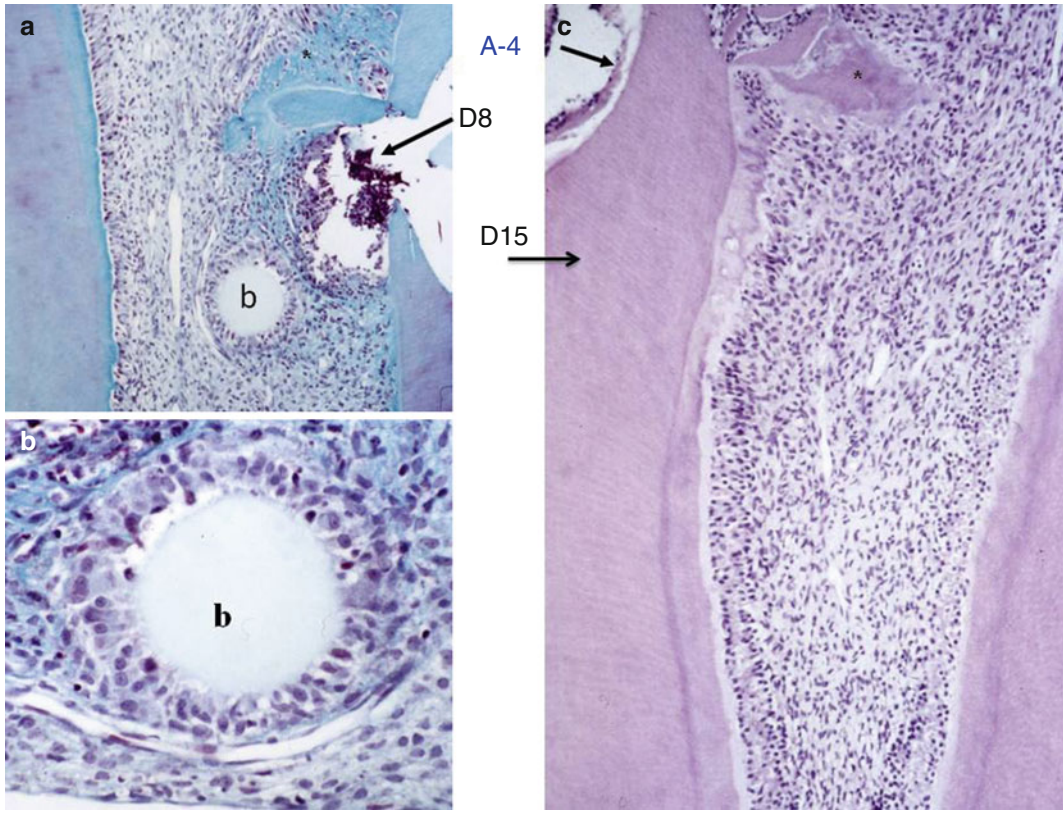

Fig. 10.8 (**a**) Implantation within the dental pulp of beads loaded with an amelogenin gene splice product A-4 after 8 days. (**b**) Committed cells are recruited and form a ring around the agarose bead (*b*). (**c**) At 15 days, reactionary dentin is formed uniformly along the root canal, behind a calciotraumatic line. In the crown, a mineralized area starts to be formed (*asterisk*)

TGF-beta3 stimulate the secretion of ECM by odontoblasts. They are mitogenic for pulp cells and might be important during reparative processes [21]. The level of BSP is increased, but DSPP is decreased. Smad 2/3 level was higher in the reparative dentin than in the normal dentin. Hwang et al. [22] concluded that both dentinogenetic and osteogenetic characteristics are mediated by TGF-beta1.

10.4.2.1 Bone Morphogenetic Proteins

The nuclear proto-oncogenes c-jun and jun-B were induced by growth factors identified as *bone morphogenetic proteins* (*BMPs*). The gene products enhance the expression of osteocalcin and collagen types. In tooth germs, c-jun and jun-B were co-expressed in the odontoblast lineage. In rat adult molars, c-jun was expressed in the odontoblast layer, in contrast with jun-B, which was absent from the cells. After cavity preparation, c-jun and jun-B were expressed only in the pulp cells lining the irregular surface of the thick reparative dentin. The limited expression of jun-B suggests that this gene is involved in active formation of reparative dentin, but is not a major actor [23].

Reparative dentin was formed by recombinant *human osteogenic protein-1* (*OP-1 or BMP-7*) in a time and dose dependences [24]. hOP-1 implanted in vivo in extra- and intra-skeletal sites induces cartilage and bone. The reparative dentin was not completely mineralized after 6 weeks of healing. Radicular pulp vitality was maintained and reparative dentin formed, and mineralization

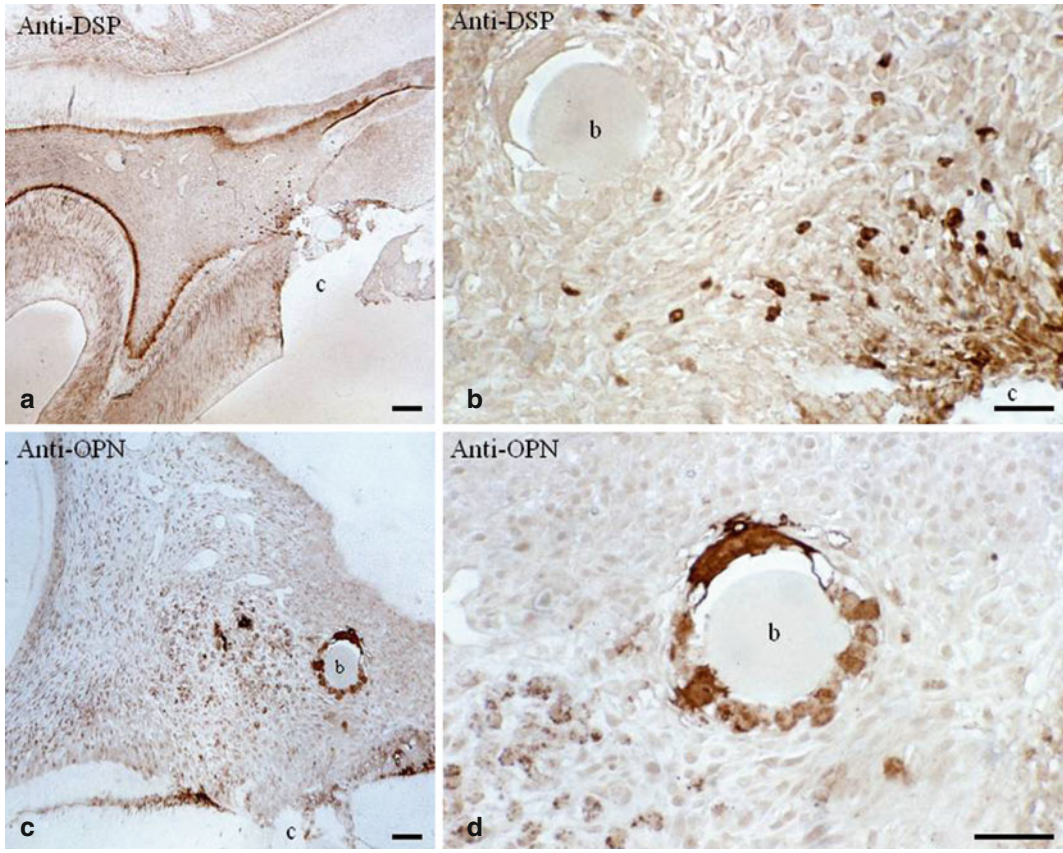

Fig. 10.9 (**a**) After a pulp exposure and dentin sialoprotein (*DSP*) immunolabeling, an odontoblast layer is densely stained. (**b**) No staining is detected around the beads (*b*), but near the pulp exposure (*c* cavity), odontoblast-like cells are well stained by the antibody. (**c**) In contrast, osteopontin immunolabeling is dense around the carrier bead (*b*) and in close proximity. (**d**) The cells located around the bead are densely immunostained by the anti-osteopontin. The two immunostainings reveal that the nature of cells nearby the pulp exposure (*DSP* dentin ECM protein) is very different from the cells implicated in pulp inflammation and bone formation (osteopontin)

was nearly 75 % complete after 1 month and more than 95 % after 4 months [25]. However, implantation of BMP-7 in inflamed dental pulp did not produced reparative dentin in LPS-treated pulp [26]. Indeed, BMP-7 gene induces reparative dentin formation, except when the dental pulp is inflamed due to a bacterial infection [27].

Pellets of *bone morphogenetic protein-2* (*BMP-2*) implanted into amputated pulps stimulate direct progenitor/stem cell differentiation into odontoblasts and reparative dentin formation [28]. The BMP signal is probably mediated by interaction of types I and II BMP receptors. RT-PCR suggests that resident pulp cells are able to respond to BMPs to initiate tissue formation [29].

10.4.2.2 Other Growth Factors

- The *lymphoid enhancer-binding factor 1* (*Lef1*) is a transcription factor that mediates Wnt signaling and regulates DSPP expression [30].
- The in vivo transfer of *growth/differentiation factor 11* (*Gdf11*) by electroporation stimulates the reparative dentin formation [31].
- The proinflammatory cytokine *tumor necrosis factor-α* (*TNF-α*) may be a mediator involved in the differentiation of pulp cells toward the odontoblast phenotype. TNF-α stimulates the differentiation of dental pulp cells toward an odontoblast phenotype via p38, while negatively regulating MMP-1 expression. Extracellular DPP and DSP were detected

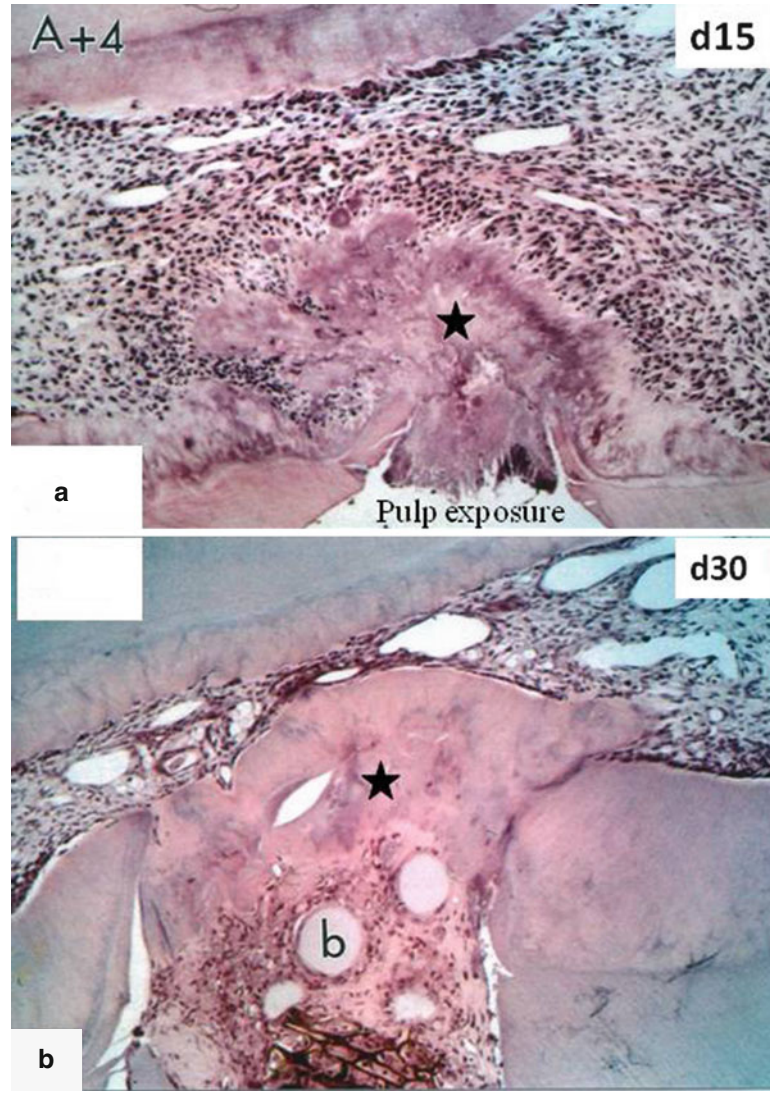

Fig. 10.10 In (**a**), A+4 was implanted in the dental pulp 15 days earlier. A forming homogeneous reparative dentin bridge (*asterisk*) is closing the pulp exposure. (**b**) After 30 days, the reparative dentin bridge is much thicker (*asterisk*) and many beads (*b*) are embedded within dentin

in higher amount in conditioned media from TNF-α-treated pulp cells [32].

- The *connective tissue growth factor/CCN family 2* (*CTGF/CCN2*) is involved in reparative dentinogenesis through the formation of mineralized tissues in human carious teeth [33].
- Recombinant human *insulin-like growth factor-1* (*rhIGF-1*) used for direct pulp capping was seen to induce after 28 days a whole dentin bridge where tubular dentin formation was observed [34].
- *Glypican-1* (*GPC-1*), a cell surface heparin sulfate proteoglycan, is acting as a coreceptor for heparin-binding growth factor and member of the TGF-β. It is related to reparative dentin formation. Downregulation of expression resulted in a 3.9-fold increase of *TGF-β1* expression in the pulp cells and 0.3-fold decrease in *DSPP* expression compared with control. The two molecules are necessary at the onset of differentiation, but should be downregulated before other molecules could be in formation [35].
- *Fibroblast growth factor 23* (*FGF23*) increased the predentin volume and expression of biglycan in dentin. FGF23 overexpression plays a negative role on dentinogenesis [36].

10.4.2.3 ECM

Collagens

Absent in normal dentin, type III collagen is present in reparative dentin. Type III collagen is more frequently observed in the root than in the crown. They are mostly located in the peritubular dentin. Collagen fibrils showed a clear cross-banding and unusual collagen aggregations, segment, and fibrous long-spacing-like structures, intensely stained for type I collagen, but weakly for type III collagen. The type III collagen-positive fibers often extended toward the pulp beyond the odontoblast layer. They may be produced at least partially by the pulp cells [37].

10.4.2.4 SIBLINGs

- *Dentin phosphophoryn* (*DPP*), *either phosphorylated or dephosphorylated*, promotes cell migration in a concentration-dependent manner, but has no effect on cell proliferation. The addition of αvβ3 integrin antibody to the medium suppressed the cell migration. Porcine DPP-derived RGD peptide significantly promotes the cell migration of human dental pulp cells [38].
- *Bone sialoprotein* (*BSP*) implanted in the pulp of the first maxillary molar induced a slight inflammation 8 days after implantation. After 15 days, a dentin bridge starts to be formed. After 30 days osteodentin formation filled the mesial part of the pulp chamber, which occluded totally the pulp exposure [39].
- BSP induced a homogeneous and well-mineralized reparative dentin. BMP-7 gave reparative dentin of the osteodentin type in the coronal part of the tooth and generated the formation of a homogeneous mineralized structure in the root canal [40].
- *Dentin matrix protein-1* (*DMP-1*) induces cytodifferentiation of dental pulp stem cells into odontoblasts [41]. After pulp exposure and implantation of collagen matrix impregnated with DMP-1, it was seen that DMP-1 acts as a morphogen on undifferentiated cells that have the capacity to regenerate a collagen-rich dentin-like tissue.
- *Dentonin*, a peptide derived from the phosphorylated extracellular matrix protein (*MEPE*), promotes the proliferation of pulp cells at day 8 after implantation in the pulp exposure. Osteopontin, weakly labeled at day 8, was increased at 15 days. DSP was undetectable at any time. Dentonin affects primarily the initial cascade of events leading to pulp healing [42].
- The layer of *fibronectin* is denser in fibrodentin after 3 days after MTA pulp capping, more than after Ca(OH)2. Fibronectin is involved in the initial stages of odontoblast differentiation. Dystrophic calcifications in association with cell debris and irregular fibrous matrix were fibronectin positive, supporting that fibronectin plays a mediating role during reparative dentinogenesis [43].
- *Ameloblastin* (also named amelin or sheathlin) influences reparative dentin formation [44], whereas the *amelogenin* gene splice products A+4 and A-4 determine the reorientation of CD45-positive cells to an osteochondrogenic lineage as shown by the positive markers RP59, Sox9, and BSP [45]. A peptide including two amelogenin exons (exons 8 and 9) enhances leucine-rich amelogenin peptide (LRAP)-mediated dental pulp repair [46].
- *MMP-3 and MMP-9* are upregulated at 24 h and 12 h, respectively, after the pulp wound, whereas MMP-2 and MMP-14 are unchanged. MMP-3 induced angiogenesis, pulp healing, and reparative dentin formation [47].

10.5 Summary

In response to a dentin lesion (caries or abrasion) or to the diffusion of resin monomers or to the release of bacterial toxins, odontoblasts and/or Hoehl's cells produce a reactionary dentin-like layer that increases the residual dentin thickness. This leads to the formation of a *reactionary dentin-like* layer (Fig. 10.11a, b). In such a case, dentin adsorbs the noxious molecules and contributes to the closure of dentin lumens by mineral precipitation, and the protection of the pulp is increased.

After a pulp exposure, pulp cells are recruited, differentiate into odontoblast-like cells, and contribute to the formation of a *dentin bridge*.

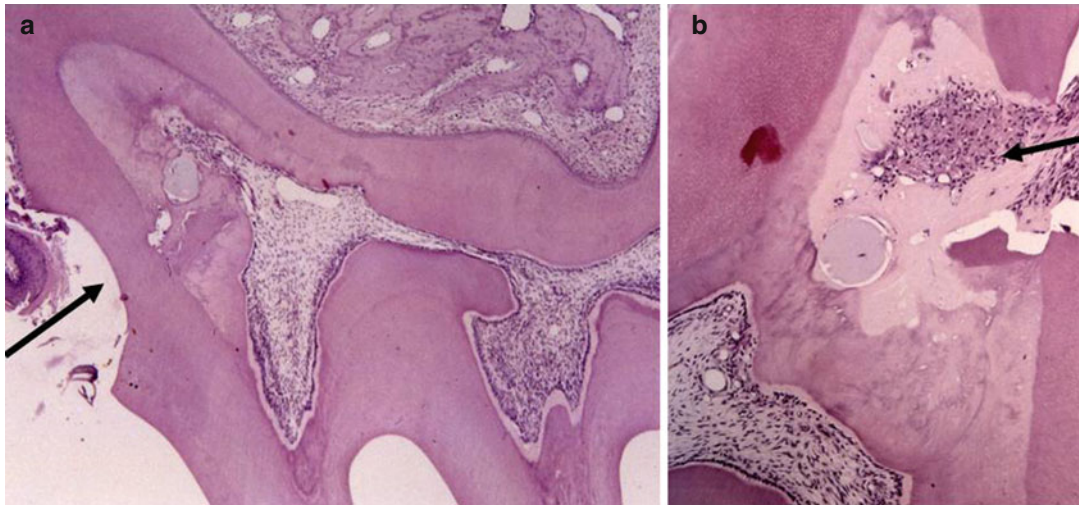

Fig. 10.11 Ninety days after A-4 implantation, reactionary dentin is formed in the pulp chamber and in the root canal (**a**). Reparative dentin fills the pulp exposure (**b**). Here an agarose bead and cell remnants are seen, embedded in the dentin bridge

For a long time, pulp capping has been a therapy, but defects in the dentin bridge lead to failures of the capping method. New therapies arise from the implantation of ECM molecules and the massive formation of *reparative dentin-like* structures. Either as appended dentin layers or as diffuse mineralization within the pulp, the remaining vital tissue is alive.

Comprehensive studies on the formation of the two different dentin-like layers will lead to a better understanding of the mechanisms that are involved. They may be used in the framework of new regenerative therapies.

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