

Basic mechanisms of cytodifferentiation and dentinogenesis during dental pulp repair

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ABSTRACT Based on recent literature, the specific potential of mature pulp cells to differentiate into polarized cells able to elaborate reparative dentin is described. These odontoblast-like cells are distinguished, by morphological criteria, from the other matrix-formative cells involved in non-specific defensive mechanisms of dental pulp. The suitable tissue conditions and the normal cascade of reparative events, allowing initiation of dentinogenesis in sites of amputated pulp, are presented. This is followed by a review of current observations on specific dentinogenic events, induced in various culture systems or in intrapulpal sites of mature teeth by artificial bio-molecules or bio-matrices. Data from these experiments are focused on the role of extracellular matrix molecules and growth factors in acquisition of the odontoblast-like cell phenotype and initiation of reparative dentinogenesis.

KEY WORDS: *dentin, dental pulp, repair, odontoblast-differentiation, dentinogenesis*

Introduction

It has been demonstrated that dentinogenesis can be initiated in the absence of dental epithelium and basement membrane, as an intrinsic potential of mature dental pulp to repair or after interaction of pulp cells with specific inductive influences. Although the phenomenon of post-developmental dentinogenesis has been under investigation for 3 decades, definitive data concerning the mechanisms of reparative dentinogenesis and the related cytodifferentiation of odontoblast-like cells are not yet available. The aim of the present article is to present the entire sequence of events leading to expression of odontoblast-like cell phenotype, during the healing process of amputated dental pulp or in the presence of specific inductive influences and also to suggest common molecular aspects in these events.

Healing potential of the dental pulp

Dental pulp pathology represents a network of inflammatory reactions of pulp cells, microcirculation and nerves whenever dentin and pulp is affected by caries or mechanical, chemical or physical trauma. The initial vascular reactions during pulp inflammation take place in the rigid enclosed pulp chamber, creating conditions of increased hydrostatic tissue pressure and compression of venules. Despite the fact that the increase of intrapulpal pressure is a local phenomenon (Van Hassel, 1971) and feedback mechanisms control changes in hydrostatic pressure within the pulp chamber (Heyeraas, 1990), exacerbation of an initial trauma leads very often to general tissue necrosis. Dental pulp repair depends on dynamic interactions between type of trauma, reactions of the neurovascular system within the pulp environment and structural/functional state of the pulp tissue.

Whenever in the present article the conditions within dental pulp environment are favorable for tissue healing, allowing expression of the intrinsic potential of pulp cells, the dental pulp is characterized as appropriate environment.

Cells and matrices in dental pulp healing

Since a broad spectrum of matrices are elaborated in various pathological conditions of the pulp, a variety of terms have been used to describe the deposited matrices and the related formative cells (for review see Baume, 1980). For the understanding of the dentinogenic phenomenon, it is important to distinguish reparative dentinogenic events from secondary dentinogenesis, as well as from the defensive matrix formation.

Three types of dentinogenesis can be described in matured teeth.

The post-developmental secretory function of primary odontoblasts is characterized as *secondary dentinogenesis*. Normal secondary dentin is elaborated along the circumpulpal dentin walls during the life span of odontoblasts, while irregular secondary dentin (reactionary dentin) is synthesized by odontoblasts in response to external stimuli. Current data concerning the phenotype of odontoblasts and reactionary dentinogenesis are extensively discussed by the contributing scientific papers in this volume.

Abbreviations used in this paper: BMP, bone morphogenetic protein; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; EGF, epidermal growth factor; ECM, extracellular matrix; FGF, fibroblast growth factor; GuHCl, guanidine hydrochloride; IGF, insulin-like growth factor; PBS, phosphate buffered saline; PDGF, platelet-derived growth factor; RGD, arginine-glycine-aspartic; TGFB, transforming growth factor beta.

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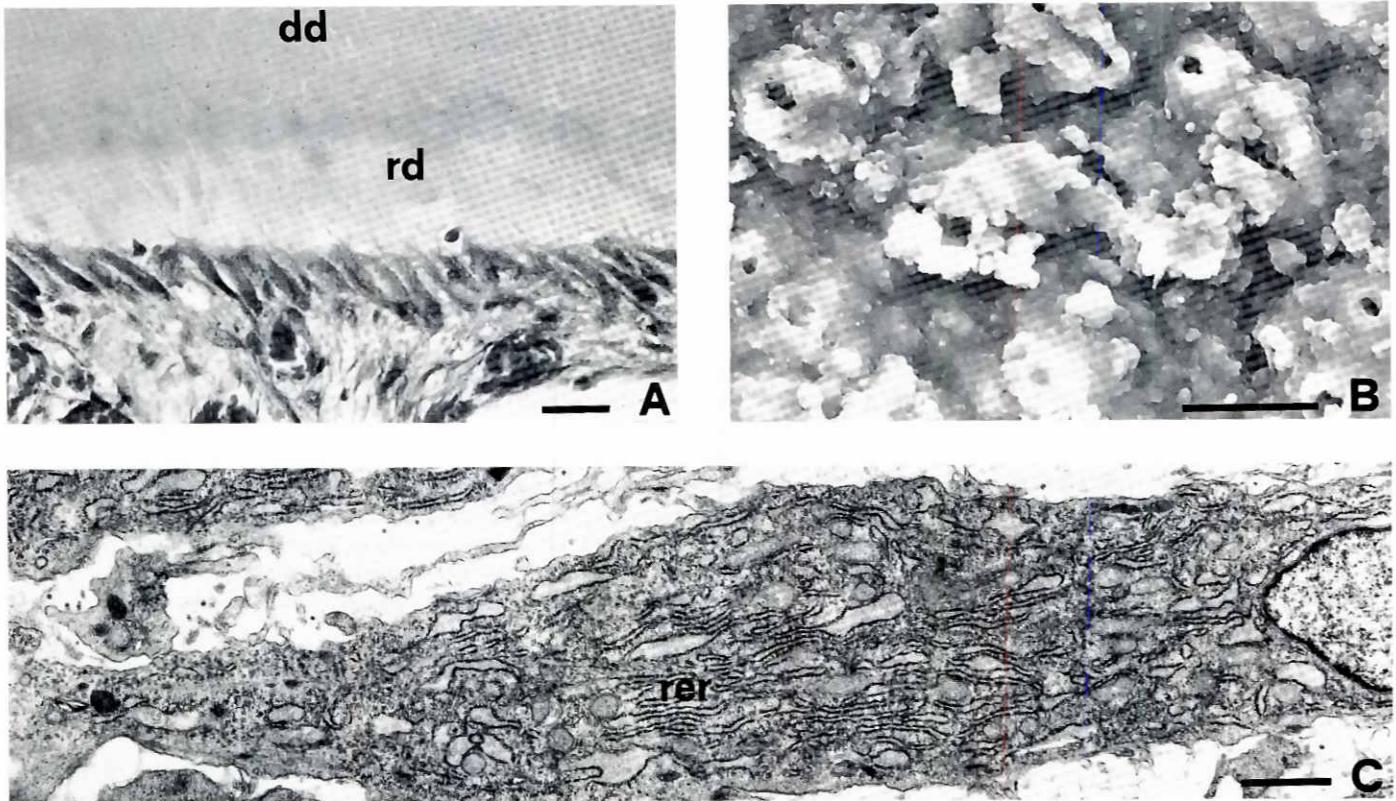


Fig. 1. Reparative dentinogenesis after implantation of demineralized dentin (dd) in healthy dog dental pulps. (A) Histological micrograph of pulp tissue fixed in Bouin's fixative and stained with Mayer's hematoxylin-eosin. Tubular matrix (rd) deposited by newly differentiated odontoblast-like cells. Scale bar, 10 μ m. (B) Scanning electron micrograph of the newly mineralized matrix; the unmineralized zone of secreted matrix has been removed by treatment in sodium hypochloride. Tubular surface structure typical of reparative dentin. Scale bar, 10 μ m. (C) Transmission electron micrograph of the supranuclear zone of an odontoblast-like cell; tissue was fixed for 3 h in 3% glutaraldehyde buffered with 0.1 cacodylate, post-fixed in 1% osmium-tetroxide in the same buffer and stained with uranyl acetate and lead citrate. The rough endoplasmic reticulum (rer), became parallel to the long axis of the cell. Scale bar, 1 μ m.

The secretion of tubular matrix in a polar predentin-like pattern by polarized odontoblast-like cells, which have been differentiated in the absence of dental epithelium and basement membrane, is characterized as *reparative dentinogenesis*. This type of dentin has been also named tertiary, replacement, neodentin, etc. (Fig. 1A,B).

The differentiated cells which secrete reparative dentin morphologically resemble primary odontoblasts (Fig. 1C). They are elongated cells with clear nuclear, cytoplasmic and secretory polarity. The biochemical specificity of their phenotype has not been yet elucidated and they are therefore called odontoblast-like cells, but other terms such as new odontoblasts, dentinoblasts or second generation odontoblasts have also been used. Criteria for assessment of differentiated odontoblast-like cells are not well defined in the literature. Cells associated with polar deposition of tubular matrix have often been identified as odontoblast-like cells, despite the absence of cell polarization and/or cell elongation. Only specific biochemical markers for odontoblastic activity and dentin ECM-specific proteins (phosphoproteins, 53 kDa dentin sialoprotein and osteocalcin) would recognize the odontoblastic specificity of odontoblast-like cells (Veis *et al.*, 1984; Butler *et al.*, 1992). In just such an attempt, positive staining reaction of odontoblast-like cells (induced by implantation of autogenous dentin in intrapulpal sites) to anti-rat dentin osteocalcin anti-serum was occasionally detected (Tziafas, 1994a).

The elaboration of atubular matrix by cuboidal or spindle-shaped pulp cells (pulpoblasts or fibrodentinoblasts) during reparative process in a non-appropriate pulp environment (Figs. 2A,B, 3A,B) is characterized as *fibrodentinogenesis*. The osteotypic form of fibrodentin is named osteodentin or trabecular dentin, elaborated by osteodentinoblasts and osteodentinocytes (Fig. 2A,B,C). The cells related to the fibrodentin synthesis showed ultrastructurally minor differences from the undifferentiated pulp cells (a few organelles and numerous lipid droplets), while their product exhibited numerous foci of highly mineralized ground substance with strong GAG reaction and dispersed collagen fibres (Baume, 1980).

Only dental pulp cells possess the capacity to form dentin matrix. It is generally accepted that no other population of mesenchymal cells is able to differentiate into odontoblast or odontoblast-like cells. This specific ability seems to originate in neural crest and be acquired by morphogenetic influences given during tooth development. Interactions between the oral ectoderm and the adjacent ectomesenchymal cell population, during the initial events of tooth formation, may determine the odontoblastic potential of dental papilla/pulp cells (Thesleff and Vaahtokari, 1992).

Origin of odontoblast-like cells

Odontoblast-like cells might originate from the undifferentiated progenitor pulp cells and/or from the pool of pulp fibroblasts.

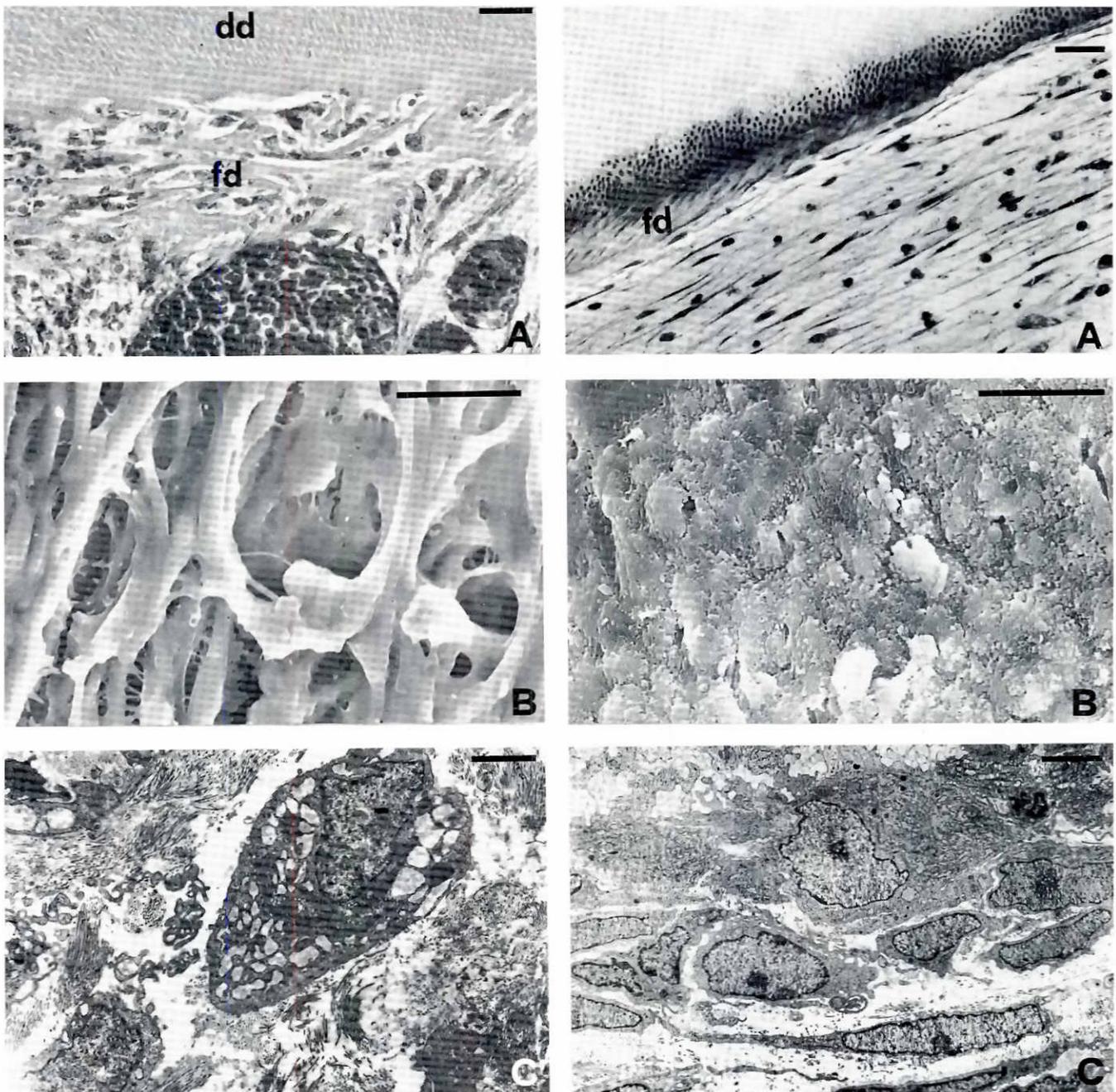


Fig. 2 (left). Fibrodentinogenesis (osteodentinogenesis) after implantation of demineralized dentin (dd) in inflamed dog dental pulps. (A) Histological micrograph of pulp tissue processed as mentioned in Fig. 1A. Fibrodentin matrix (fd) deposited in an osteotypic pattern by osteodentinoblasts. Scale bar, 10 μ m. **(B)** Scanning electron micrograph of the newly secreted matrix. Osteotypic surface structure. Scale bar, 4 μ m. **(C)** Transmission electron micrograph of osteodentinoblasts and their surrounding matrix; tissue was processed as mentioned in Fig. 1C. High biosynthetic activity of the osteodentinoblasts associated with elaboration of collagen fibres. Scale bar, 3 μ m.

Fig. 3 (right). Fibrodentinogenesis along the circumpulpal dentin after severe pulpal trauma. (A) Histological micrograph of pulp tissue processed as mentioned in Fig. 1A. Fibrodentin matrix (fd) deposited in a polar pattern by fibrodentinoblasts. Scale bar, 10 μ m. **(B)** Scanning electron micrograph of the newly mineralized matrix; the tissue was processed as mentioned in Fig. 1B. Atubular surface structure. Scale bar, 10 μ m. **(C)** Transmission electron micrograph of fibrodentinoblasts; the tissue was processed as mentioned in Fig. 1C. Scale bar, 3 μ m.

Progenitor pulp cells, residing in the sub-odontoblastic cell-rich zone (Hohl, 1896), have been suggested to be involved in the cytodifferentiation process, when they are stimulated by specific molecular signals without any replication of their DNA (Cotton,

1968; Takuma and Nagai, 1971; Slavkin, 1974; Torneck and Wagner, 1980). These cells may be derived from the dividing preodontoblasts before their terminal differentiation into odontoblasts. On the other hand, fibroblasts, vascular cells, or

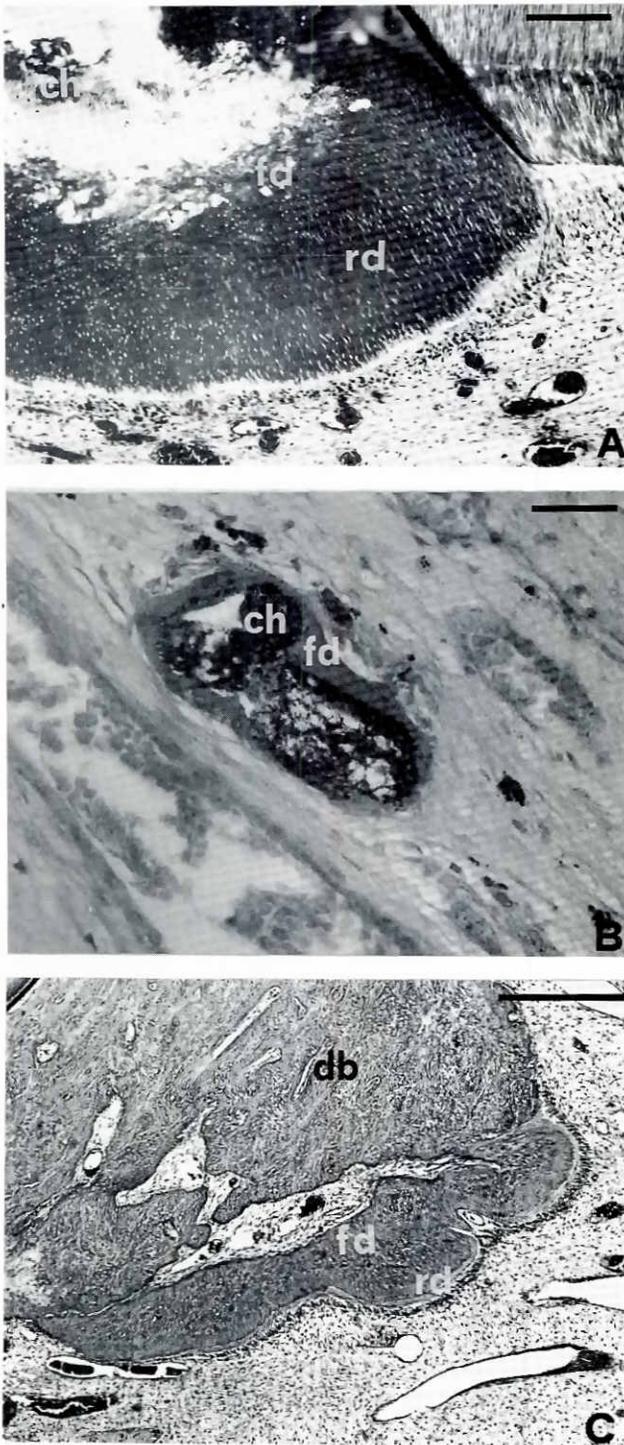


Fig. 4. Histological micrographs of pulp tissue responses to i) chemical irritation induced by calcium hydroxide (ch) in site of amputated pulp for 29 days (A), ii) chemical irritation induced by calcium hydroxide in intrapulpal site for 6 weeks (B), iii) bio-active stimulation induced by autogenous demineralized bone (db) in site of amputated pulp for 3 weeks (C). Zones of fibrodentin matrix (fd) can be seen, followed by reparative dentin formation (rd) and a layer of odontoblast-like cells in site of wound healing (A) or in the presence of bio-matrix (C). The tissues were fixed in buffered formalin solution, demineralized in trichloroacetic acid and the sections were stained with hematoxylin-eosin. Scale bar, 80 μ m.

even undifferentiated cells of the central pulp can replicate their DNA during their migration toward the pulp region where reparative dentinogenesis is initiated (Sveen and Hawes, 1968; Feit *et al.*, 1970; Fitzgerald, 1979; Yamamura *et al.*, 1980; Yamamura, 1985). Fitzgerald *et al.* (1990) showed that at least two replications of DNA are required after pulp amputation and capping treatment, taking place before cell migration and at the site of expression of the new phenotype. It remains an open question whether the cells undergoing differentiation into odontoblast-like cells derive only from the group of original ectomesenchymal dental papilla cells, or from both ectomesenchymal cells and other mesenchymal cells that constitute the mature pulp cell population.

Initiation of dentinogenesis as a part of wound healing process

Clinical data and experimental observations on animal teeth have repeatedly demonstrated that mature dental pulp responds naturally to external low-grade irritations by producing reparative dentin (for review see Baume, 1980). This phenomenon takes place stereotypically, as an intrinsic defensive mechanism, in an appropriate pulp environment.

- a) After surgical pulp amputation in germ-free rats (Kakehashi *et al.*, 1965; Tsuji *et al.*, 1987; Inoue and Shimono, 1992), a zone of osteotypic matrix was found in the wound surface, while under limited masticatory forces, odontoblast-like cells were detected along the fibrodentin matrix from the 5th post-operative day. Cytodifferentiation of odontoblast-like cells and tubular dentin formation was never found after extensive removal of the crown dentin, despite the presence of osteodentinal bridge (Tsuji *et al.*, 1987; Inoue and Shimono, 1992).
- b) When an appropriate material is placed in contact with the amputated pulp, aiming to restrict bacterial contamination of exposed dental pulp, a chemical irritation of the underlying tissue was seen, stimulating formation of dentinal bridge around the wound site (Fig. 4A). Calcium hydroxide represented the more favorable capping material. Schroder (1985) reviewed the reparative process following capping of pulp-tomized human teeth with calcium hydroxide. Initially the cells under the wound surface proliferate, migrate and elaborate new collagen in contact with the firm necrotic zone of amputated pulp, or the material interface. Then the necrotic zone, as well as the new collagen layer, attracts mineral salts forming calcified matrices of fibrodentin nature. Finally, a layer of odontoblast-like cells is formed in association with the primitive matrix and a tubular matrix is secreted in a polar predentine-like pattern. Calcium hydroxide has not shown any specific effect for the induction of dentinogenesis (Cvek *et al.*, 1987); the beneficial effect of this material has been attributed to the initial chemical irritation, due to release of hydroxyl ions (Stanley and Lundy, 1972; Heys *et al.*, 1980; Schroder, 1985; Cox and Bergenholtz, 1986; Cvek *et al.*, 1987; Mjor *et al.*, 1991). Implantation of a calcium hydroxide-containing material into the central pulp of dog molars (Fig. 4B) never induced odontoblast-like cell differentiation (Tziafas, unpublished data). Lesot *et al.* (1993) speculated a chemical effect of calcium hydroxide on the circum-pulpal dentin, which can result in dissolution of growth factors. On the other hand, many other materials able to cause a low-grade irritation allowed formation of hard tissue by similar mechanisms (Heys *et al.*, 1981; Granath, 1982; Cox *et al.*, 1987; Cvek *et al.*, 1987). The

surface interfacial properties of capping materials also seem to be important for the temporo-spatial pattern of dentinal bridge formation (Fagnudes and Cox, 1994).

- c) Biologically active molecules, such as the BMP (Nakashima, 1990b; Lianjia *et al.*, 1993; Nakashima, 1994), the osteogenic protein (Rutherford *et al.*, 1993), or biomatrices, such as the demineralized dentin (Nakashima, 1989; Robson and Katz, 1992), were placed in contact with amputated pulp in capping situations of non-human teeth. Enhancement of the normal sequence of morphogenetic events in the repairing dental pulp, i.e. fibrodentin matrix formation followed by reparative dentinogenesis (Fig. 4C), was always found.
- d) Replacement of destroyed primary odontoblasts and reparative dentin formation has also been described, following local injuries caused by rapidly advancing carious lesions, tooth grinding, filling materials, prolonged air-blast and coronal tooth fractures, ischemic injuries produced during transplantation or replantation of tooth germs and immature or apicoectomized teeth and general tooth injuries following local irradiation, systemic injection of antimetabolic agents, or nerve resection. These dentinogenic events must be distinguished from reactionary dentinogenesis, described in another paper of the present issue by A. Smith. After destruction of the odontoblast layer, increased cell mitoses occur in the underlying pulp, cells of spindle shape migrate towards the region of necrosis and differentiate into elongated polarized cells which then elaborate reparative dentin (Sayegh, 1967; Cotton, 1968; Sveen and Hawes, 1968; Harris and Griffin, 1969; Stanley, 1981; Heritier *et al.*, 1990). Early elaboration of fibrodentin matrix very often precedes the appearance of odontoblast-like cells (Atkinson, 1976; Senzaki, 1980).

These data clearly indicate that during the wound healing process pulp cells normally express their dentinogenic potential in the presence of a fibrodentin substrate (Fig. 4A,C). Interaction of pulp cells with this primitive biomatrix might trigger the acquisition of the odontoblast-like cell phenotype, as during basement membrane-preodontoblast interactions (Ruch, 1985; Veis, 1985). The growth factors present in the circumpulpal dentin (Finkelman *et al.*, 1990), the specific pulpal ECM molecules and the newly synthesized growth factors and ECM molecules by the repairing pulp cells may regulate cell kinetics and differentiation. It is known that fibrodentin matrix constitutes a fibronectin-containing substratum (Magloire *et al.*, 1988; Tziafas *et al.*, 1995), but data concerning the expression of growth factors are not as yet available. Pulp environment plays an important role for the expression of the dentinogenic potential during wound healing process. Takei *et al.* (1988) found that transplanted pulp cells were able to produce bone, cartilage or dentin, depending on the environment. It has been repeatedly demonstrated that transplantation of dissociated pulp tissue never gives rise to odontoblast-like cell differentiation but exhibits osteotypic fibrodentinogenesis (Zussman, 1966; Hoffman, 1967; Yamamura *et al.*, 1980; Ishizeki *et al.*, 1990). Pulp cells differentiated into odontoblast-like cells only after implantation of tissue within the pulp chamber and in fibrous environment with sufficient vascularity (Takei *et al.*, 1988).

Induction of dentinogenesis *in vitro*

Two *in vitro* models have been used to approach the molecular basis of odontoblast-like cell differentiation:

- a) Culture of isolated dental papillae, containing dividing and post-mitotic preodontoblasts, on Millipore filters coated with collagen

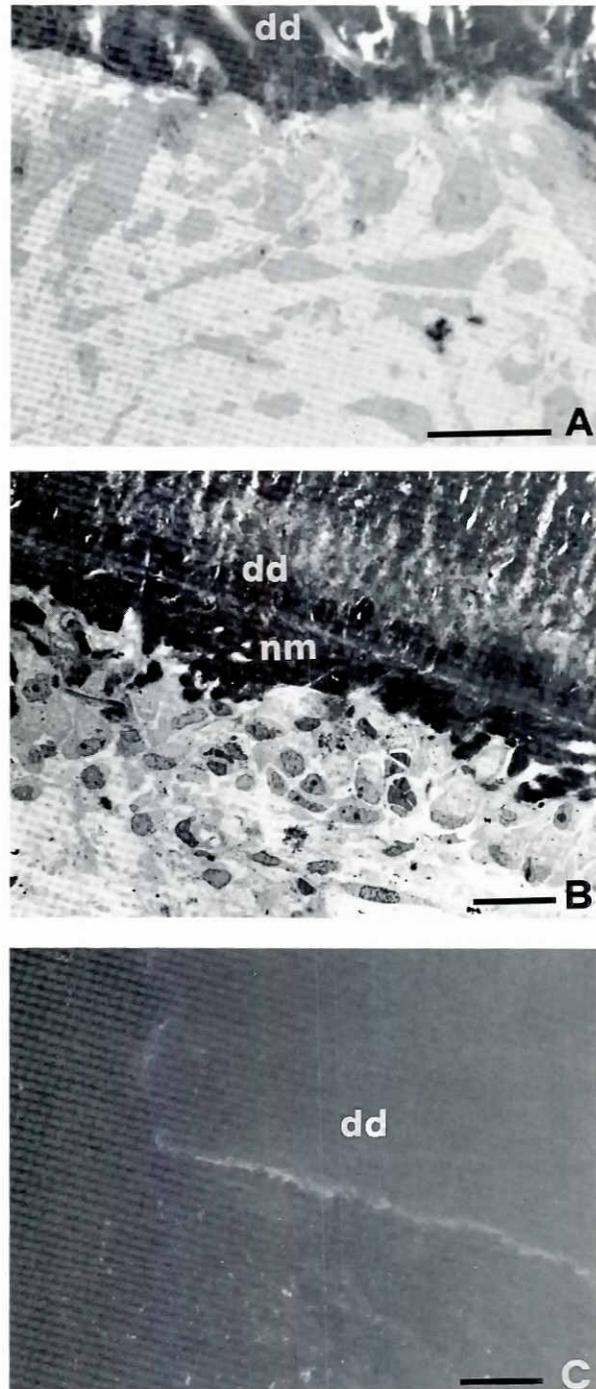


Fig. 5. Histological micrographs of pulp tissue responses to implantation of demineralized dentin (dd) for 3 (A) or 7 (B) or 6 (C) days in intrapulpal sites of dog teeth. Scattered columnar cells showing nuclear polarization in close proximity to the implant (A) and a layer of odontoblast-like cells arranged along the newly secreted matrix (nm) in a polar pattern (B). The tissues in A and B were processed as mentioned in fig. 1C and the semithin sections were stained with toluidine blue. (C) A band of fibronectin positive reaction at the pulp-implant interface. The tissue was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer solution. The section was treated with non-immune rabbit serum, goat polyclonal antibodies against human fibronectin and fluorescein-isothiocyanate conjugated rabbit anti-goat IgG. Scale bars, 16 μ m (A, B) and 30 μ m (C).

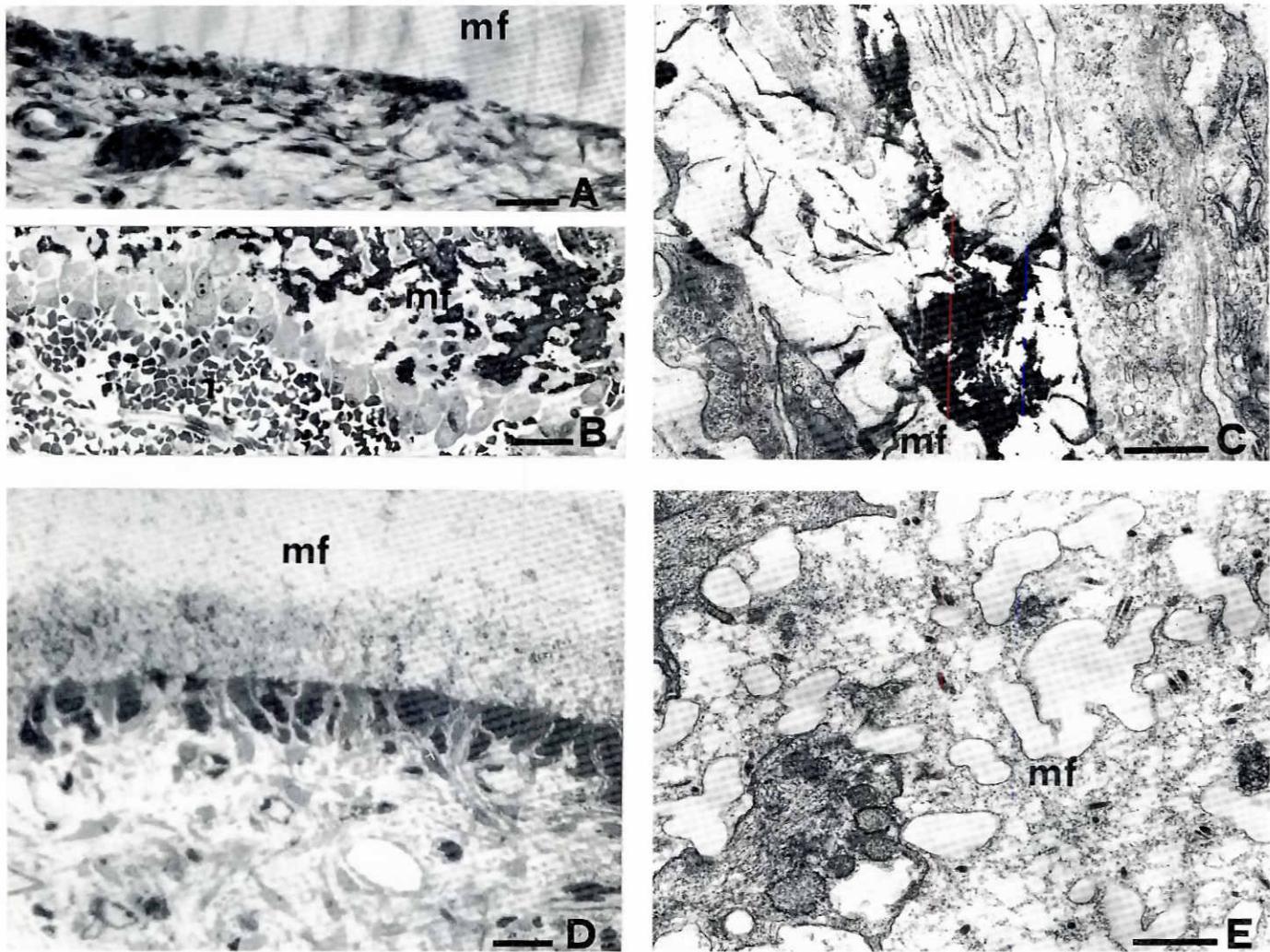


Fig. 6. Histological (A,B,D) and transmission electron (C,D) micrographs of pulp tissue responses to implantation of Millipore filters (mf) coated with phosphate buffer saline solution containing 1 mg/ml of dog albumin (A) or 1 mg/ml of bovine fibronectin (B,C) or 300 µg/ml of rabbit EDTA-soluble dentin matrix constituents (D,E) in intrapulpal sites of dog teeth for one week. Layers of elongated polarized odontoblast-like cells in direct contact with the Millipore filters coated with fibronectin (B) and dentin constituents (D). No evidence of cell organization in response to control implant (A). Initial events of new matrix deposition can be seen (C,E): electron-dense material apically to the polarized cells in response to fibronectin-containing implant (C); matrix vesicles in the more distal part of the cytoplasmic processes and extracellular collagen fibres within the filters coated with the dentin constituents (E). The tissues were fixed in buffered formalin and the section was stained with hematoxylin-eosin (A), or as mentioned in Fig. 1C (C,E), and Fig. 5A (B,D). Scale bars, 10 µm (A,B,D) and 1 µm (C,E).

type I and/or fibronectin, did not show any cell polarization (Thesleff and Hurmerinta, 1981; Lesot *et al.*, 1985). Maintenance of the cytological state of polarized cells was found when isolated dental papillae, containing post-mitotic preodontoblasts, incubated in the presence of dental pulp biomatrix (Cam *et al.*, 1986), or some fractions of dentin matrix (Lesot *et al.*, 1986), or hyaluronic acid and/or chondroitin sulfate (Tziafas *et al.*, 1988). In recent experiments, dental papillae, containing only dividing preodontoblasts, has been grown *in vitro* in semi-solid medium supplemented by TGFβ1 or BMP2 combined with heparin or fibronectin, or EDTA-soluble fractions of dentin (Bègue-Kirn *et al.*, 1992; Lesot *et al.*, 1993). The dentin proteins, as well as the combination of TGFβ1 or BMP2 with matrix molecules, stimulated polarization and functional differentiation of odontoblast-like cells, at the periphery of the explants. The inductive effect of

dentin proteins was lost when TGFβ activity had been previously blocked by monoclonal antibodies (Bègue-Kirn *et al.*, 1992). These experiments showed that the critical role of the basement membrane can be replaced *in vitro* by artificial growth factors and matrix molecules. A synergistic action between various growth factors, such as the combination of IGF-I and TGFβ1, has been suggested to control elongation, polarization and polar matrix secretion, during reparative dentinogenesis (Lesot *et al.*, 1994).

b) Growth of human pulp cells on specific substrates, such as type I collagen-chondroitin sulfate sponge (Bouvier *et al.*, 1990), fibronectin-coated glass (Veron *et al.*, 1990), or calcium carbonate microcrystals, which had been produced by the reaction of calcium hydroxide with culture medium (Seux *et al.*, 1991), exhibited some cytological features of the odontoblastic

phenotype. Further, in primary cultures of adult bovine pulp cells i) BMP-2 and BMP-4 increased alkaline phosphatase activity and osteocalcin synthesis in a dose-dependent manner (Nakashima, 1992a; Nakashima *et al.*, 1994) and ii) growth factors, involved in the normal cascade of reparative events (PDGF, TGF β , acidic and basic FGF and EGF), stimulated DNA synthesis, most of them (except the EGF) inhibited alkaline phosphatase activity, while their effects in sulfated proteoglycan synthesis depended on the degree of differentiation of the cells (Nakashima, 1992b).

Induction of dentinogenesis in intrapulpal sites

Implantation of biologically active molecules or bio-matrices in intrapulpal sites, via mechanical exposures of dog teeth, represented a suitable model to approach the specific molecular conformation required for induction of reparative dentin formation. It is evident that the normal sequence of reparative events do not take place in this model. However, these data allow us to evaluate the role of ECM molecules and/or diffusible morphogenetic polypeptides in reparative dentinogenesis.

Implantation of dentin matrix

Autogenous dentin, which was obtained from the crowns of primary molars and demineralized in 0.5 N acetic acid, implanted in pulp sites of young dogs. The implanted matrix induced polarization and functional differentiation of odontoblast-like cells (Fig. 5A,B) within 2 post-operative weeks (Tziafas and Kolokuris, 1990). The appearance of polarized cells around the demineralized dentin implants were found after an initial matrix synthesis by non-polarized cells of spindle or polygonal shape (Fig. 5B), while only scattered polarizing cells were also observed (Fig. 5A) in direct contact with the demineralized dentin (Tziafas *et al.*, 1992b). After a short-term implantation of native predentin for 3 and 7 days, odontoblast-like cells were consistently found in close proximity to the implant (Tziafas *et al.*, 1992b). The ability of demineralized dentin to induce bone formation, via a chondrogenic pathway, was observed repeatedly in muscle, skin, bone marrow and periodontal ligament sites (Bang and Urist, 1967; Yeomans and Urist, 1967; Urist, 1971; Inoue *et al.*, 1986; Somerman *et al.*, 1987). The osteogenic factors responsible (Urist and Strates, 1971; Butler *et al.*, 1977; Conover and Urist 1979), named bone morphogenetic proteins (BMPs), have not been completely characterized, but are considered as members of the family of TGF β (Wozney *et al.*, 1988) and have exhibited a mitogenic activity in cultures of fibroblasts (Canalis *et al.*, 1985; Veis *et al.*, 1989). Furthermore, Amar *et al.* (1991) isolated and partially characterized a rat dentin matrix polypeptide, different from the TGF β and BMP, exhibiting chondrogenic activity *in vitro*. The inductive effect of dentin matrix on the dental ectomesenchymal cells was first suggested in capping experiments, where foci of reparative dentin had been detected around native dentin chips. Further studies with demineralized dentin in capping situations (Anneroth and Bang, 1972; Nakashima, 1990a) confirmed its dentin-inductive activity.

Complementary experiments in intrapulpal sites have shown that the EDTA- and/or GuHCl-insoluble dentin induced a dentinogenic response similar to the undemineralized dentin matrix (Tziafas, 1994b). The EDTA-soluble fraction of proteins present in dentin matrix (molecules belonging to the TGF β superfamily), have shown inductive effects on preodontoblasts *in vitro* (Bègue-Kirn *et al.*, 1992) and pulp cells *in vivo* (Smith *et al.*, 1990; Tziafas *et al.*,

unpublished data). It seems that either biologically active molecule(s) remain(s) in the EDTA-insoluble dentin matrix, or multiple molecular pathways can initiate dentin-induced dentinogenesis in intrapulpal sites. It has been suggested that dentin matrix might provide appropriate mechanical support for polar secretion of specific ECM molecules and growth factors, triggering differentiation of pulp cells (Thesleff and Vaahtokari, 1992). Accumulation of fibronectin in the interfacial surface of implanted matrix (Fig. 5C) has been recently shown as a short term response after intrapulpal implantation of demineralized dentin (Tziafas *et al.*, 1995). Fibronectin might operate the matrix-cytoskeleton interactions, leading to the cytological modifications of polarization as in normal odontoblast differentiation (Lesot *et al.*, 1992).

Differential activity has been demonstrated after implantation of autogenous demineralized and native dentin into mature pulp of old dogs; the native but not the demineralized dentin induced reparative dentin formation (Tziafas *et al.*, 1993; Tziafas, 1994b). Different responses have also been reported after placement of dentin proteins on the dental pulp of young and old ferrets (Smith *et al.*, 1990). It seems that initiation of dentinogenic events in the non-appropriate pulp environment of old animals (where number of cells and concentration of morphogenetic factors have been dramatically reduced, as a result of the aging process), requires exogenous growth factors.

Implantation of allogenic plasma fibronectin

Elongated polarized cells, were found in direct contact with pieces of Millipore filters, which had been coated with 1 mg/ml of allogenic plasma fibronectin solution (Fig. 6B,C), when they were implanted in intrapulpal sites for a short-term period. Furthermore, a thick layer of dentin was found around these implants after a 4-week exposure (Tziafas *et al.*, 1992a). Neither control filters coated with PBS or dog albumin, nor those treated with low concentration of fibronectin solution (0.2 mg/ml), exhibited any particular effect (Fig. 6A). Complementary implant experiments in mature pulp of old animals did not confirm the dentinogenic activity of fibronectin (Tziafas, 1994b).

These experimental data support the statement of Veis (1985) that pulp cells immobilizing on a fibronectin-rich substrate can be differentiated into odontoblast-like cells. Veron *et al.* (1990) suggested that the arrangement of pulp cells on a fibronectin substrate may inhibit the synthesis of this glycoprotein, possibly via a feed-back mechanism, and this regulation could be one step in the expression of their odontoblastic potential. Interactions between the exogenous fibronectin and the pulp cells are not RGD-dependent: Millipore filters coated with a synthetic polypeptide, incorporating multiple copies of the RGD sequence, did not induce any morphogenetic activity (Tziafas, 1994b). Endogenous growth factors might intervene in the fibronectin-induced dentinogenic events, anchoring to the implanted substrate, due to the ability of fibronectin to bind TGF β (Fava and McLure, 1987; Mooradian *et al.*, 1989), or possibly related molecules, while TGF β activity contained in the exogenous fibronectin solution (Fava and McLure, 1987) could not be excluded (Lesot *et al.*, 1993).

Dentinogenic events induced by BMP or related molecules

As mentioned above, when Millipore filters coated with the EDTA-soluble dentin matrix constituents were implanted in intrapulpal sites of dog molars, a specific dentinogenic activity was observed (Tziafas *et al.*, unpublished data). Polarized cells, with increased metabolic activity and numerous cytoplasmic processes

invading the pores of the filters were seen (Fig. 6D,E). A thick layer of tubular matrix was found around the implanted filter after 4 weeks. The control filters had no particular effect.

These EDTA-soluble dentin proteins also exhibited a clear morphogenetic activity when placed in mechanical pulp exposures of young ferrets (Smith *et al.*, 1990). Capping experiments of pulp exposures with BMP or osteogenic protein resulted in stimulation of rapid osteodentin matrix formation, while polarized odontoblast-like cells were only found in association to this primitive osteotypic response (Nakashima, 1990b, 1994; Lianjia *et al.*, 1993; Rutherford *et al.*, 1993, 1994).

Conclusions

The present review has drawn attention to the events initiating cytodifferentiation of odontoblast-like cells and new tubular matrix secretion during wound healing or in response to specific inductive influences. Appropriate environmental conditions within the pulp chamber (absence of inflammation and sufficient oxygen supply) are strongly required for the expression of the specific dentinogenic ability of dental pulp cells (Inoue *et al.*, 1981). Matrix molecules including fibronectin (Magloire *et al.*, 1988, 1992), growth factors including molecules belonging to the TGF β superfamily and competent pulp cells [a specificity implying the ability of dental pulp cells to express matrix molecules with affinity for growth factors or with potentializing activities, or membrane receptors (Lesot *et al.*, 1994)] might be interacted to initiate dentinogenesis. The presence of an insoluble substrate providing mechanical support seems to be of critical importance.

Information supporting our knowledge on reparative dentinogenesis could be summarized as followed:

1. Stimulated pulp cells during the wound healing process synthesize fibrodentin, which may provide mechanical support for accumulation of specific ECM molecules and growth factors. Interactions between this dynamic substrate and competent pulp cells might further control polarization and functional differentiation of odontoblast-like cells.
2. Native dentin contains specific information that is capable of eliciting the modulation of cell activity and/or extracellular matrix behavior in an appropriate pulp environment. The EDTA-soluble dentin components, as well as the EDTA-insoluble dentin matrix, have been shown to induce dentinogenic events. Synergistic interactions between exogenous growth factors present in the implanted dentin matrix and endogenous growth factors or ECM molecules, accumulated on the implant, might trigger the acquisition of odontoblast-like cell phenotype.
3. Substrates coated with plasma fibronectin, the whole molecule and not multiple copies of the RGD sequence, are able to induce polarization and functional differentiation of odontoblast-like cells. The strong affinity of fibronectin to binding endogenous growth factors of the TGF β superfamily might be related to its dentinogenic activity in intrapulpal sites.

It is important to realize that knowledge of the molecular events taking place during cytodifferentiation of odontoblast-like cells is still strictly limited. It is absolutely necessary to identify the phenotype of these cells and the nature of fibrodentin matrix by specific markers. The temporal and spatial patterns of expression of growth factors and specific ECM molecules might also be completely characterized. Understanding of the molecular basis of the mechanisms controlling cytodifferentiation and

dentinogenesis during the wound healing process could direct scientists to devise realistic treatment modalities of the amputated pulp.

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