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Ultrastructural and immunocytochemical analyses of osteopontin in reactionary and reparative dentine formed after extrusion of upper rat incisors

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Abstract

Reactionary dentine and reparative dentine are two strategies used by the dentine–pulp complex to respond to injury. The reactionary dentine is secreted by original odontoblasts, while the reparative dentine is formed by odontoblast-like cells. Osteopontin (OPN) is a non-collagenous protein usually present in the repair of mineralized tissues. It is likely to be present in newly formed dentine but there are no studies attempting to detect it in reactionary and reparative dentine. The aim of the present study was to examine the ultrastructural characteristics, as well as the presence and distribution of OPN in reactionary and reparative dentine by provoking extrusion of the rat incisor. The right upper incisors of 3-month-old male rats were extruded 3 mm and then repositioned into their original sockets. At 3, 7, 10, 15, 20, 30 and 60 days after surgery, the incisors were fixed in glutaraldehyde–formaldehyde and then processed for scanning and transmission electron microscopy and for immunocytochemistry for OPN. After extrusive trauma, the dentine–pulp interface showed the presence of reactionary and reparative dentine, which varied in aspect, thickness and related cells. OPN was not detected in the physiological and reactionary dentine, while it was strongly immunoreactive in the matrix that surrounded the entrapped cells of reparative dentine. In addition, original odontoblasts subjacent to the physiological dentine contained OPN in their Golgi region. The present findings showed that reparative dentine shares some structural characteristics with primary bone, especially in relation to its OPN content. The odontoblast-like cells resemble osteoblasts rather than odontoblasts.

Key words dental pulp; immunocytochemistry; odontoblast; osteopontin; reactionary dentine; reparative dentine; tertiary dentine.

Introduction

Pulp repair is a phenomenon by which the human dentine–pulp complex responds to injury through the deposition of tertiary dentine. Three types of dentine can be present in the mature human tooth. The dentine formed by odontoblasts up to the end of the tooth development is called primary dentine. As odontoblasts continue slowly to secrete dentine matrix after the apical foramen is formed, a secondary type of dentine is laid down throughout life. Thus, as both primary and secondary dentine are secreted by the same odontoblasts, they have a similar tubular structure. In addition, a tertiary dentine may be laid down in specific regions of the dentine–pulp interface. There are two subtypes of tertiary dentine, that secreted by the original odontoblasts in response to an appropriate stimulus, called reactionary dentine, and the reparative dentine formed by odontoblast-like cells, which arise from cells present in the dental pulp after the death of the original odontoblasts (Arana-Chavez & Massa, 2004). Whereas reactionary dentine is tubular and therefore

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shares similar aspects with primary and secondary dentine, reparative dentine has an dystrophic, atubular matrix with cells entrapped in its mineralized matrix (Smith et al. 1995; Tziafas, 1995). In this context, some animal teeth, such as the rat incisor, which continuously erupts, do not close their apical end; therefore, no primary or secondary dentine can be distinguished, only a physiological dentine. However, reactionary dentine or reparative dentine can be formed in these teeth, although they cannot be considered as tertiary dentine.

As occurs in other mineralized tissues, the mechanism and control of the reparative dentine formation involves cell differentiation and initial organic matrix deposition and mineralization, which may be regulated by a number of factors and by non-collagenous matrix proteins. Among the non-collagenous proteins postulated to be present in these processes is osteopontin (OPN), a phosphorylated glycoprotein composed of approximately 300 amino acid residues that has been linked to several physiological and pathological events (Denhardt et al. 2001; Mazzali et al. 2002; Qin et al. 2004). It is present in many mineralized and soft tissues including bone, cementum, calcified cartilage, kidney stones, tumours, body fluids and brain (Sodek et al. 2000). The idea that OPN is involved in regulation of matrix mineralization and control of cell adhesion arose on the basis of its tissue distribution, particularly within the interfibrilar regions and within the interfacial structures as cement lines and laminae limitans in bone, and the fact that is contains cell-matrix binding motifs in its structure (Nanci, 1999; Giachelli & Steitz, 2000). In addition, OPN exhibits a high affinity for calcium and calcium phosphate crystals, probably due to the quantity of phosphorylation sites (Denhardt et al. 2001; Qin et al. 2004) and the presence of a polyaspartic acid motif in its molecule (Giachelli & Steitz, 2000). Furthermore, OPN contains the arg-gly-asp (RGD) sequence that might mediate cell–matrix interactions (Butler, 1989; Giachelli & Steitz, 2000; Denhardt et al. 2001; Gravallese, 2003; Gericke et al. 2005; for a detailed review, see Sodek et al. 2000).

In reparative dentine, OPN could be involved in the initial stages of the formation and mineralization of this tissue as well as in inducing some of the events required for the appearance of a new generation of odontoblasts. These events include cell division, chemotaxis, cell migration, cell adhesion, cytodifferentiation (Smith et al. 1995) and intracellular signalling (Sodek et al. 2000).

Despite the importance of the reparative dentine for pulp repair and the high concentration of OPN in some mineralized tissues, there are no studies confirming the presence and distribution of OPN in this tissue as well as its relation to the appearance of odontoblast-like cells (Butler et al. 2003). The exact relationship between OPN of dentine matrix secreted after trauma and its related cells is important in understanding tertiary dentinogenesis as well as the role of OPN in mineralized tissue formation.

Thus, the purpose of the present investigation was to examine the effect of extrusive trauma in the formation of reactionary and reparative dentine deposition as well as the presence and distribution of OPN in these tissues by using post-embedding colloidal gold immunocytochemistry.

Materials and methods

Surgical procedure, fixation and decalcification

All animal procedures were in accordance with principles of laboratory animal care (NIH publication 85-23, 1985) and were authorized by the Ethical Committee for Animal Research of the University of São Paulo, Brazil (Protocol no. 008/03).

Thirty-five male Wistar rats weighing between 180 and 250 g were randomly divided into two groups. The first (experimental) group comprised 28 animals whose upper right incisor was submitted to extrusion. The second (control) group comprised seven animals whose incisors were not extruded.

The animals were anaesthetized intramuscularly with a combination of ketamine hydrochloride (Ketalar®, Parke Davis, Brazil) and xylazine (Rompun®, Bayer SA, Brazil) at concentrations of 6 and 0.7 mg per 100 g body weight, respectively. After a sepsis with an alcoholic solution of iodine, 2 mm of the incisal edge was cut with a diamond cylindrical bur. A sindesmotomy was made then around the right upper incisors with a modified Hollemback 3S instrument (Golgran, SP, Brazil) followed by the extrusion of 3 mm with an adapted #151 paediatric dental forceps (Golgran). These teeth were then gently put back to their original positions. The length of the removed incisal edge and amount of displacement of the teeth during extrusion were measured with an endodontic millimetre ruler (Fig. 1). The animals exhibited normal appearance throughout the experiment. Animals from each group were

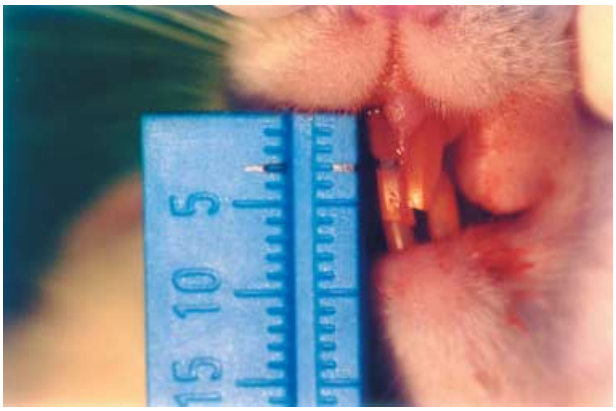

Fig. 1 Three-millimetre extrusion of the right upper rat incisor.

anaesthetized as described previously and killed at 3, 7, 10, 15, 20, 30 and 60 days after surgery.

The animals were killed via high doses of anaesthetics, following which the hemimaxillae containing the right maxillary incisors were removed and placed in fixative containing 0.1% glutaraldehyde plus 4% formaldehyde at pH 7.2 with 0.1 M sodium cacodylate. After removal of all soft tissues, the right hemimaxillae were immersed in a beaker containing 40 mL of fixative at room temperature, which was subsequently placed in a larger glass recipient full of ice and immediately taken inside a Pelco 3400 laboratory microwave (MW) oven (Ted Pella, Redding, CA, USA). The temperature probe of the oven was submerged in the fixative and the specimens were exposed to MW irradiation at a 100% setting for three periods of 5 min with the temperature programmed to a maximum of 36 °C (Massa & Arana-Chavez, 2000). After MW irradiation, specimens were transferred into fresh fixative and left there overnight at 4 °C. They were then washed in 0.1 M sodium cacodylate buffer, pH 7.2, for 90 min. Apart from the samples for scanning electron microscopy (SEM), all hemimaxillae were decalcified in an aqueous solution of 4.13% ethylenediaminetetraacetic acid (EDTA) under MW irradiation for 15 h in the same oven. Specimens were placed in a beaker containing 25 mL of the decalcifying solution, which was placed in a larger glass recipient filled with ice. The temperature probe was submerged in the EDTA solution, and the specimens were immediately exposed to MW irradiation at medium setting for periods of 30 min, with the temperature programmed to a maximum of 37 °C. The decalcifying solution was changed every 2 h over a total period of 15 h. After decalcification, the hemimaxillae were washed extensively in 0.1 M sodium cacodylate buffer, pH 7.2.

Processing for SEM

After careful dissection of incisors from alveolar bone, one undecalcified tooth of each group was fractured parallel to its long axis. Each piece was then treated with 2% sodium hypochlorite for 30 min in an ultrasonic apparatus (Branson 1210, Danbury, CT, USA) to remove all the soft tissues covering bone and teeth. The sections were subsequently washed with distilled water, transferred to 30% ethanol and dehydrated in increasing concentrations of ethanol. To avoid shrinkage of specimens during air-drying, sections were immersed in 100% hexamethyldisizilane (Electron Microscopy Sciences, Fort Washington, PA, USA) for 10 min and left under a fume hood equipped with an exhaust system for complete evaporation of HMDS. Specimens were placed on aluminium stubs using a colloidal silver adhesive and sputter-coated with gold in a Bal-Tec SCD 050 apparatus (Bal-Tec SCD 050, Liechtenstein). The specimens were examined in a Jeol 6100 scanning electron microscope (Jeol Ltd, Tokyo, Japan), operated at 10–15 kV.

Processing for transmission electron microscopy

After decalcification and washing, some specimens were post-fixed in cacodylate-buffered 1% osmium tetroxide for 2 h and then dehydrated in graded concentrations of ethanol and embedded in hard grade LR White resin (London Resin Company, London, UK). Toluidine blue-stained 1-μm-thick sections were examined with a light microscope. Regions containing the portion located between the fourth and the seventh millimetres from the apical end of the incisor were trimmed for ultrathin sectioning. Sections, 80 nm thick, were cut with a diamond knife on a Reichert Ultracut E ultramicrotome and collected on parlodion-coated 200-mesh nickel grids.

Post-embedding colloidal gold immunocytochemistry

Grids containing unosmicated sections were incubated with OPN chicken egg yolk primary antibody (Nanci et al. 1996) diluted 1 : 150 for 5 h. They were then incubated with a rabbit anti-chicken IgG secondary antibody (Sigma Chemical Co.) diluted 1 : 2000 for 1 h.

The sites of antibody–antigen binding were then revealed by incubating the grids on a drop of protein A–gold complex for 30 min. Grids were washed with PBS between incubation steps and sections blocked by placing the grids on a drop of PBS–1% ovalbumin for 20 min. After the protein A–gold, the grids were jet-washed with PBS followed by distilled water. All steps were carried out at room temperature (Arana-Chavez & Nanci, 2001). Sections were then stained with uranyl acetate and lead citrate and examined in a Jeol 1010 transmission electron microscope (Jeol Ltd) operated at 80 kV. Controls for the specificity of the labelling consisted of incubating the sections with the secondary antibody followed by protein A–gold, or with protein A–gold alone.

Results

As the rat incisor continuously erupts and the cells at its apical end proliferate and differentiate throughout life, there is no primary and secondary dentine in the rat incisor, but only a physiological dentine. As a consequence, physiological, reactionary and reparative dentine are the terms used in the present study for the types of dentine observed in the rat incisor in order to compare them with their corresponding types in human teeth.

Rat incisors injured by extrusive luxation exhibited extensive areas of both reactionary and reparative dentine. Although dilated blood vessels could be observed, no areas with necrosis or infection were detected in the central region of dental pulp. The characteristics of the extracellular dentine matrix and cells related to the dentine–pulp interface throughout the several periods after trauma are described below.

Morphology

Although 3 days after trauma the specimens showed dentine with a generally normal aspect, several features were noted in the dentine–pulp interface. In some regions the odontoblastic layer showed signs of disarrangement and degeneration of its cells, while in others odontoblasts were reduced in number or even absent (Fig. 2A). When denuded dentine areas were observed, clusters of fusiform cells appeared in close relation to the dentine surface.

Seven days after extrusion, the regions of denuded dentine were coated by an irregular layer of cubic cells, which often exhibited their nuclei located near the dentine matrix. In the dentine–pulp interface in close relation to remaining original odontoblasts, the start of the formation of a tubular dentine matrix (reactionary dentine) was observed. The zone of reactionary dentine was clearly distinguishable from physiological dentine, because the former had a decreased number of tubules and there was a slight change in tubular orientation in relation to the physiological dentine. An intensely stained line was discerned between both types of dentine in many teeth.

Ten days after extrusion, the cubic or shorter columnar cells coated areas of atubular dentine matrix that corresponded to reparative dentine. Adjacent areas of tubular reactionary dentine were coated with original odontoblasts (Fig. 2B). In addition, some sections showed a reparative dentine matrix that had changed dramatically, sometimes exhibiting entrapped cells. Cubic cells appeared in the dentine–pulp interface (Fig. 2C). They showed several cisternae of rough endoplasmic reticulum, well-developed Golgi complex and many mitochondria. In general, the thickness of the reactionary dentine matrix was greater than that observed in the reparative dentine. Specimens 15 and 20 days after extrusion exhibited similar characteristics.

Thirty days after luxation, areas of reactionary and reparative dentine as well as the same arrangement of odontoblasts and cubic cells were observed, thus resembling the earlier periods. After these periods, some dentine matrix areas displayed a simultaneous presence of physiological, reactionary and reparative dentine. The reparative dentine was always the deepest, i.e. near the dentine–pulp interface (Fig. 3A). The closest reparative dentine to the physiological dentine was more irregular and contained more entrapped cells than reparative dentine related to the dentine–pulp interface. At higher magnifications, the areas of reparative dentine showed a typical primary bone-like appearance (Fig. 3B).

Sixty days after the operation, the amounts of tertiary dentine appeared higher than that at earlier periods. As a consequence, the pulpal chamber was almost completely obliterated.

Immunocytochemistry

Immunolabelling for OPN was visible in the original odontoblasts in contact with physiological dentine in the first periods after extrusion. The intracellular

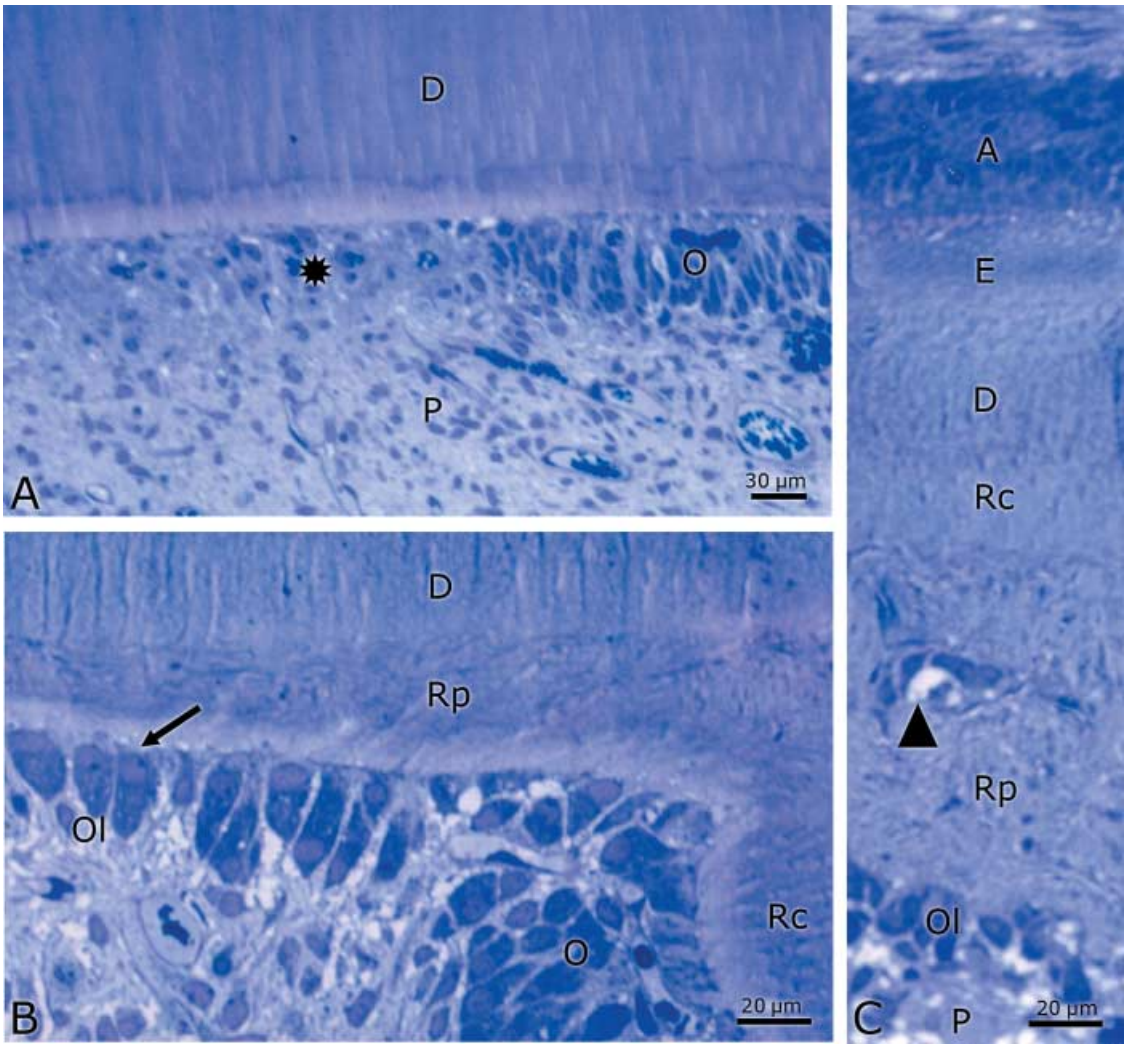

Fig. 2 Light micrographs showing several regions of the dentine–pulp interface of rat incisors after extrusion. (A) Region without odontoblasts (asterisk) can be seen at the left side of the micrograph 3 days after extrusion, while the dentine–pulp interface at the right side exhibits a normal appearance. (B) Both areas of reactionary and reparative dentine are observed under the physiological dentine in a rat incisor 10 days after extrusion. Note that cubic cells, some of them exhibiting their nuclei (arrows) located near the dentine matrix, are subjacent to reparative dentine, while reactionary dentine is coated by tall columnar odontoblasts. (C) Region from a rat incisor 10 days after extrusion in which the three types of dentine observed in the present study can be discerned: physiological, reactionary and reparative dentine. Note that the regular tubular appearance of physiological dentine remains through the reactionary dentine. That appearance, however, changes dramatically in reparative dentine in which some entrapped cells (arrowheads) are observed. Cubic cells appear in the dentine–pulp interface. A, ameloblast; E, enamel; D, physiological dentine; Rc, reactionary dentine; O, original odontoblasts; Rp, reparative dentine; Ol, cubic odontoblast-like cells; P, dental pulp. Toluidine blue staining.

pattern of staining appeared within the perinuclear area of odontoblasts (Fig. 4A,B). OPN was never immunodetected in the adjacent physiological dentine matrix.

In subsequent periods, a reparative dentine matrix strongly immunoreactive for OPN containing several entrapped cells was noted (Fig. 5A,B). Although the particles of OPN were distributed in all reparative dentine matrix, they were abundant in the regions near spaces, some of which contained cellular debris (Fig. 5C), while others appeared empty (Fig. 5D) as a result of partial or full degeneration of the entrapped cells. The short columnar cells adjacent to reparative matrix, which exhibited an osteoblast-like aspect, did not contain gold particles (Fig. 5E). The pattern of localization and distribution of the gold particles for

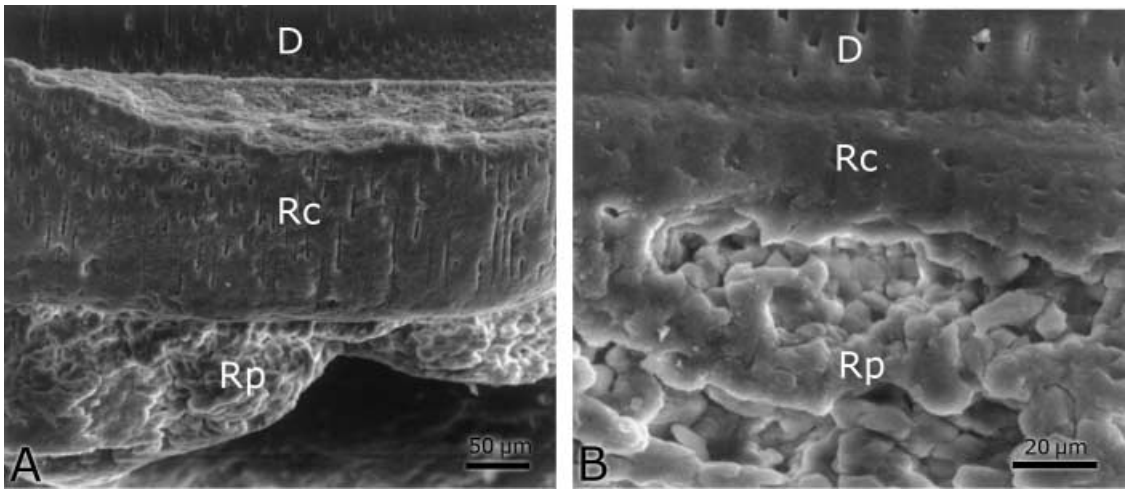

Fig. 3 Scanning electron micrographs. (A) Region of a rat incisor 30 days after extrusion in which physiological, reactionary and reparative dentine are observed, and (B) a higher magnification view of all types of dentine. Note several spaces in reparative dentine that correspond, *in vivo*, to the lacunae into which the entrapped cells are located. D, physiological dentine; Rc, reactionary dentine; Rp, reparative dentine.

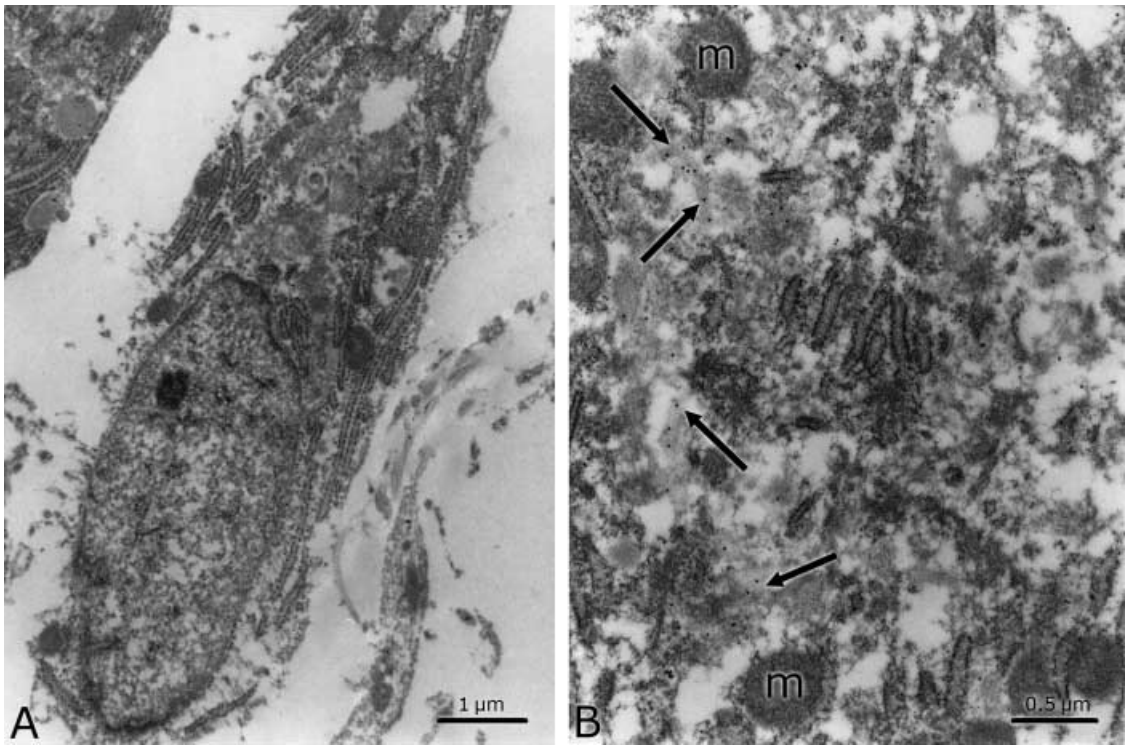

Fig. 4 Transmission electron micrographs. (A) Original odontoblast subjacent to the physiological dentine from a rat incisor 3 days after extrusion. It appears with a typical columnar shape, the nucleus in its basal pole and abundant rough endoplasmic reticulum and Golgi apparatus. (B) Higher magnification view of the odontoblast cytoplasm shows labelling for OPN in the Golgi region (arrows). Note that mitochondria (m) are free of gold particles, revealing the specificity of immunoreaction. Unosmicated.

OPN on reparative dentine matrix was seen in all periods after 3 days.

Control incubations resulted in the absence of gold particles in the cells of the dentine–pulp interface and entrapped cells as well as the physiological and tertiary dentine matrix. Ultrathin sections showing immunoreactivity for OPN in the reparative dentine did not show gold particles in the enamel or physiological dentine (Fig. 5F).

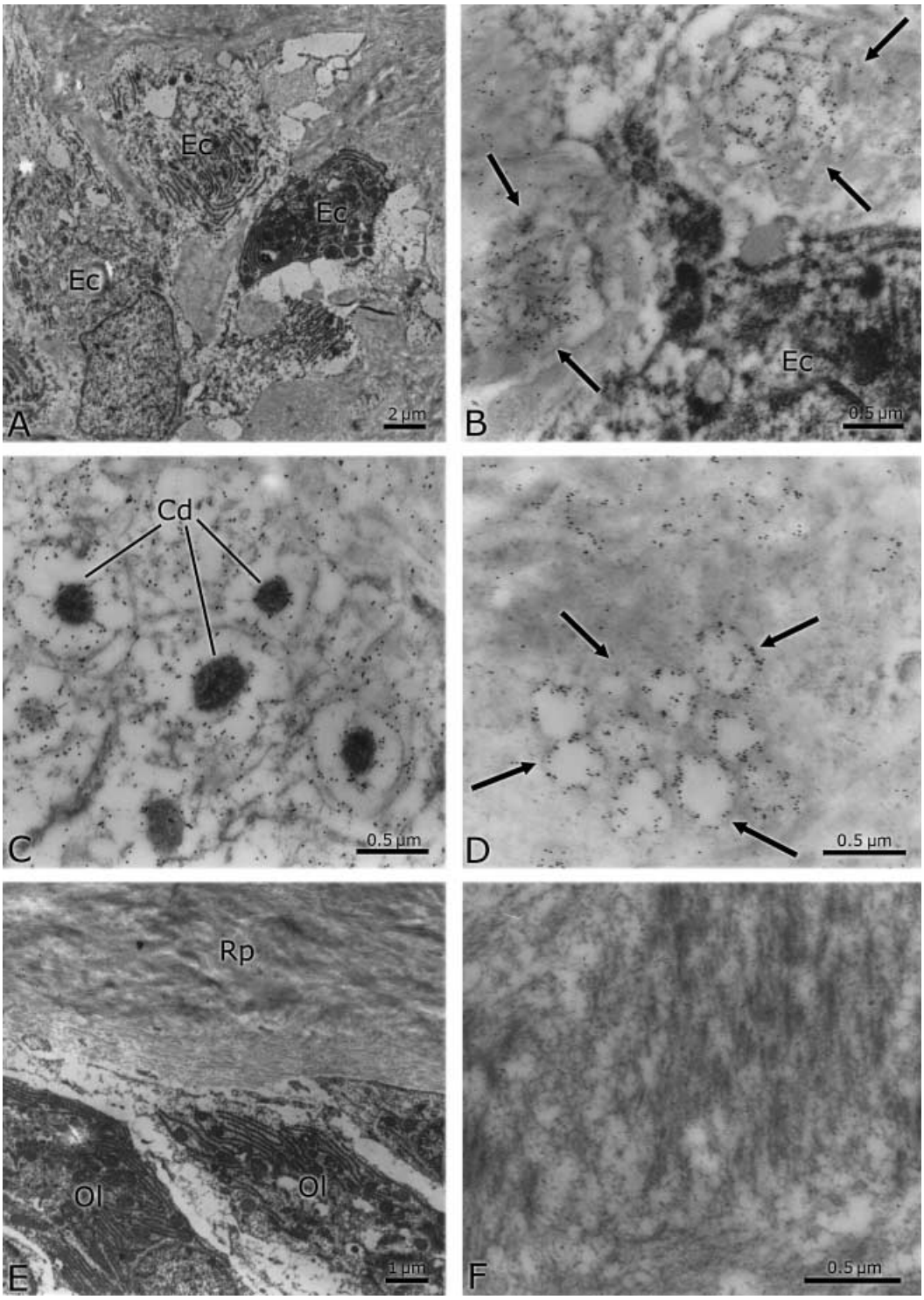

Fig. 5 Transmission electron micrographs showing several regions of extruded rat incisors processed for post-embedding colloidal gold immunocytochemistry. (A) Area of reparative dentine secreted after dentine–pulp injury displaying several entrapped cells (Ec). In the higher magnification view of the reparative dentine (B), gold particles immunoreactive for OPN form patches (between arrows) in the dentine matrix in close relation to entrapped cells (Ec). (C) Many patches of gold particles are found in the reparative dentine matrix, especially in the regions near to cellular debris (Cd). (D) Intense immunoreactivity for OPN in the dentine matrix closely related to several empty spaces (between arrows). (E) Interface between reparative dentine (Rp) and short columnar cells (Ol). Note that these cells exhibit numerous cisternae of rough endoplasmic reticulum, well-developed Golgi apparatus and several mitochondria. (F) Enamel of an extruded rat incisor that was incubated in the same section as a negative control for the immunocytochemistry for OPN appears free of gold particles. Unosmicated.

Discussion

The present study shows that extrusive trauma on the upper rat incisor affects the dental pulp and the odontoblast layer, triggering the formation of significant amounts of reactionary and reparative dentine. Only the reparative dentine was strongly immunoreactive for OPN, a non-collagenous protein abundant in bone matrix but not usually immunodetected in primary and secondary dentine.

Formation of tertiary dentine has been studied mainly by performing cavity preparations with rotatory instruments in order to expose the dental pulp followed by placing capping agents and restorative materials into the cavities (Fitzgerald et al. 1990; Smith et al. 1994; Goldberg et al. 2001). In the present study the 3-mm extrusion of upper rat incisor disturbed the dental pulp, including the dentine–pulp interface, mainly due to the vascular damage that occurred during extrusion rather than by external agents such as bacterial contamination. As the rat incisor tip usually contains large amounts of tertiary dentine, the areas evaluated were restricted to the portion located between the fourth and the seventh millimetres from the apical end, i.e. rather than to areas in which dentine deposition had already ceased and in which the dentine–pulp interface is in a resting stage. The first 3 mm, which correspond to the apical end, were not examined because it is an odontogenic area and the tissue subjacent to the forming dentine is more similar to a dental papilla rather than to a dental pulp itself.

An apparently normal odontoblast layer covered some areas of reactionary dentine, while in others predentine was absent and the odontoblast layer disrupted. In addition, several areas displayed the simultaneous presence of physiological, reactionary and reparative dentine. It is possible that the extrusive trauma has not been sufficient to destroy the original odontoblasts immediately, which secreted a thin layer of reactionary dentine. Subsequently, however, odontoblasts disappeared and pulpal cells differentiated into odontoblast-like cells for secreting a layer of reparative dentine over the pre-existing reactionary dentine. The presence of both types of tertiary dentine in the same region is a rare occurrence in studies using the pulp-capping model because dental pulp is often exposed and the original odontoblasts are quickly destroyed.

Regions of odontoblast-like cells subjacent to reparative dentine were found close to areas with original odontoblasts subjacent to reactionary dentine. Comparing the thickness of both types of dentine, the reactionary dentine was always thicker. Indeed, reactionary dentine matrix formation only requires the up-regulation of secretory activity of original odontoblasts (Smith et al. 1995; Smith, 2003), while secretion of reparative dentine requires a cascade of events involving cell division, chemotaxis, cell migration, cell adhesion and differentiation into the odontoblast-like cells (Tziafas, 1994; Spahr et al. 2002).

The type of tertiary dentine formed after trauma was related to the type of cells subjacent to the dentinal surface. The cells subjacent to reactionary dentine were original odontoblasts. Interestingly, in the first time points after extrusion, the original odontoblasts subjacent to the physiological dentine contained OPN in their Golgi region. As original odontoblasts usually do not contain intracellular OPN at the resting stage, the intracellular presence of this protein may be required for signalling events in order to trigger the reactivation of odontoblasts for secreting the reactionary dentine. It has been reported that odontoblasts contain OPN in their Golgi region when they are differentiating, i.e. when their activity is starting, although this protein is not immunodetected in the adjacent physiological dentine (Arana-Chavez & Nanci, 2001). In addition, OPN may mediate signals for blocking a possible apoptosis of odontoblast in response to trauma perhaps by autocrine mechanisms, as has been postulated in endothelial and kidney epithelial cells (Denhardt et al. 1995; Scatena et al. 1998; Mazzali et al. 2002). On the other hand, the odontoblast-like cells adjacent to the reparative dentine resembled osteoblasts rather than odontoblasts. Some of these cells displayed their nucleus near the dentine matrix, resembling osteoblasts that are entrapping by bone matrix (Nefussi et al. 1991; Franz-Odendaal et al. 2006). In addition to the entrapped cells, reparative dentine was devoid of dentinal tubules, thus resembling primary bone.

OPN was present in the matrix that surrounded the entrapped cells of reparative dentine, while physiological and reactionary dentine showed no immunoreactivity for this non-collagenous protein. Although biochemical studies usually detect small amounts of OPN in physiological dentine (Fujisawa et al. 1993), immunocytochemical studies have failed to find this protein, possibly due to its presence in amounts below the threshold of immunodetection, or because it may be rapidly processed or even masked by other dentine

matrix components (Arana-Chavez & Nanci, 2001). The distribution of OPN in the reparative dentine matrix among the collagen fibrils or forming patches found throughout the matrix resembles the pattern of immunolabelling observed in primary bone (McKee & Nanci, 1996). This implies that the production of OPN was up- and down-regulated during reparative dentine secretion, thus contributing to the local variations observed at certain sites of matrix. The amount and distribution of OPN in the reparative dentine could at least partially depend on its fast rate of formation, similarly to the development of primary bone (Nanci, 1999). However, although OPN is usually a major constituent of the interfaces between mineralized tissues (McKee & Nanci, 1996), the interfaces between physiological, reactionary and reparative dentine showed no immunoreaction for OPN. It is possible that other non-collagenous components may be participating in the adhesion between physiological and tertiary dentine.

In summary, the present findings showed that reparative dentine shares some structural characteristics with primary bone, especially in relation to its OPN content. Correspondingly, the odontoblast-like cells resemble osteoblasts rather than odontoblasts themselves.

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References

Arana-Chavez VE, Nanci A (2001) High-resolution immunocytochemistry of noncollagenous matrix proteins in rat mandibles processed with microwave irradiation. *J Histochem Cytochem* **49**, 1099–1109.

Arana-Chavez VE, Massa LF (2004) Odontoblasts: the cells forming and maintaining dentine. *Int J Biochem Cell Biol* **36**, 1367–1373.

Butler WT (1989) The nature and significance of osteopontin. *Connect Tissue Res* **23**, 123–136.

Butler WT, Brunn JC, Qin C (2003) Dentin extracellular matrix (ECM) proteins: comparison to bone ECM and contribution to dynamics of dentinogenesis. *Connective Tissue Res* **44**, 171–178.

Denhardt DT, Lopez CA, Rollo EE, Hwang SM, An XR, Walther SE (1995) Osteopontin-induced modifications of cellular functions. *Ann NY Acad Sci* **760**, 127–142.

Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS (2001) Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. *J Clin Invest* **107**, 1055–1061.

Fitzgerald M, Chiego DJ, Heys DR (1990) Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. *Arch Oral Biol* **35**, 707–715.

Franz-Odendaal TA, Hall BK, Witten PE (2006) Buried alive: how osteoblasts become osteocytes. *Dev Dyn* **235**, 176–190.

Fujisawa R, Butler WT, Brunn JC, Zhou HY, Kuboki Y (1993) Differences in composition of cell-attachment sialoproteins between dentine and bone. *J Dent Res* **72**, 1222–1226.

Gericke A, Qin C, Spevak L, et al. (2005) Importance of phosphorylation for osteopontin regulation of biomineralization. *Calcif Tissue Int* **77**, 45–54.

Giachelli CM, Steitz S (2000) Osteopontin: a versatile regulator of inflammation and biomineralization. *Matrix Biol* **19**, 615–622.

Goldberg M, Six N, Decup F, et al. (2001) Application of bioactive molecules in pulp-capping situations. *Adv Dent Res* **15**, 91–95.

Gravallese EM (2003) Osteopontin: a bridge between bone and the immune system. *J Clin Invest* **112**, 147–149.

Massa LF, Arana-Chavez VE (2000) Ultrastructural preservation of rat embryonic dental tissues after rapid fixation and dehydration under microwave irradiation. *Eur J Oral Sci* **108**, 74–77.

Mazzali M, Kipari T, Ophascharoensuk V, Wesson JA, Johnson R, Hughes J (2002) Osteopontin – a molecule for all seasons. *Q J Med* **95**, 3–13.

McKee MD, Nanci A (1996) Osteopontin at mineralized tissue interfaces in bone, teeth and osseointegrated implants: ultrastructural distribution and implications for mineralized tissue formation, turnover and repair. *Microsc Res Tech* **33**, 141–164.

Nanci A, Zalzal S, Gotoh Y, McKee MD (1996) Ultrastructural characterization and immunolocalization of osteopontin in rat calvarial osteoblast primary cultures. *Microsc Res Tech* **33**, 214–231.

Nanci A (1999) Content and distribution of noncollagenous matrix proteins in bone and cementum: relationship to speed of formation and collagen packing density. *J Struct Biol* **126**, 256–269.

Nefussi JR, Sautier JM, Nicolas V, Forest N (1991) How osteoblasts become osteocytes: a decreasing matrix forming process. *J Biol Bucalle* **19**, 75–82.

Qin C, Baba O, Butler WT (2004) Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. *Crit Rev Oral Biol Medical* **15**, 126–136.

Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli CM (1998) NF-kappaB mediates alphaybeta3 integrin-induced endothelial cell survival. *J Cell Biol* **141**, 1083–1093.

Smith AJ, Tobias RS, Cassidy N, et al. (1994) Odontoblast stimulation in ferrets by dentine matrix components. *Arch Oral Biol* **39**, 13–22.

Smith AJ, Cassidy N, Perry H, Bégue-Kirn C, Ruch J-V, Lesot H (1995) Reactionary dentinogenesis. *Int J Dev Biol* **39**, 273–280.

Smith AJ (2003) Vitality of the dentin-pulp complex in health and disease: growth factors as key mediators. *J Dent Educ* **67**, 678–689.

Sodek J, Ganss B, McKee MD (2000) Osteopontin. *Crit Rev Oral Biol Med* **11**, 279–303.

Spahr A, Lyngstadaas SP, Slaby I, et al. (2002) Expression of amelin and trauma-induced dentin formation. *Clin Oral Invest* **6**, 51–57.

Tziafas D (1994) Mechanisms controlling secondary initiation of dentinogenesis: a review. *Int Endod J* **27**, 61–74.

Tziafas D (1995) Basic mechanisms of cytodifferentiation and dentinogenesis during dental pulp repair. *Int J Dev Biol* **39**, 281–290.