Observation of the Internal Configuration of Rat Incisor Odontoblasts by Scanning Electron Microscopy Using the AODO Method

By

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—Received for Publication, October 4, 1995—

Key Words: Internal structures, Rat incisor odontoblasts, Scanning electron microscopy (SEM) using the aldehyde prefixation – osmium tetroxide postfixation – dimethyl sulfoxide (DMSO) freeze fracture – osmium tetroxide maceration (AODO) method

Summary: The internal configuration of rat incisor odontoblasts was studied mainly by scanning electron microscopy (SEM) using the AODO method (low concentration aldehyde prefixation, osmium tetroxide postfixation, dimethyl sulfoxide (DMSO) freeze-fracture, osmium tetroxide maceration). The present SEM findings were compared with the results obtained by conventional transmission electron microscopy (TEM) of epon-embedded specimens. The following results were obtained: 1) Functioning odontoblasts were characterized by a concentric, laminar rough endoplasmic reticulum (rER) with many long mitochondria interposed. 2) A network of tubular smooth endoplasmic reticulum (sER) was observed in the odontoblast process and distal portion of both functioning and resting odontoblasts. 3) The tubulo-vesicular elements which have been found to present a modified Golgi-GERL organelle with secretory and absorptive functions were demonstrated in both the functioning and resting odontoblasts. Structurally they consist of the sER network and strings of granules and vesicles. 4) Various types of cytoplasmic bodies, e.g., lysosomes, cytosomes and multivesicular bodies, related to the sER were also noted in both functioning and resting odontoblasts. 5) Microapocrine secretion of membranous vesicles of various sizes into the predentin and along the lateral branchings of odontoblast processes in the circumpulpal dentin was observed during the matrix apposition stage of the odontoblasts. The present morphological study revealed the three-dimensional configuration of the intra- and extra-cellular structures related to dentinogenesis by odontoblasts.

There have been many transmission electron microscopy (TEM) studies on the morphological changes and the fine structure related to secretion and absorption by odontoblasts (OBs) at different developmental stages (Reith, 1968; Tsuboi, 1968; Takuma and Nagai, 1971; Weinstock and Leblond, 1974; Gartner et al., 1979; Goldberg and Septier, 1982; Takuma and Nagai, 1971; Suzuki, 1985; Rule, 1986; Avery, 1987; Nagai, 1970a; Nagai, 1970b; rule, 1989; Romagnoli et al., 1990; Linde and Goldberg, 1993). The occurrence of various kinds of cytoplasmic bodies closely related to the Golgi complex and the smooth (agranular) endoplasmic reticulum (sER) has been reported during early and late dentinogenesis, respectively, in some of these studies (Tsuboi, 1968; Takuma and Nagai, 1971; Romagnoli et al., 1990), while others have suggested that the Golgi elements and some cytosomes (lysosomes) are organelles concerned with autodigestion and regulatory functions of the OBs at different developmental stages (Nagai, 1970a; Romagnoli et al., 1990). Additionally, the occurrence of coated vesicles at the cell membrane, the resorption of proteoglycans and fibrillar components and the formation of a lysosomal-vacuolar apparatus have been elucidated by TEM examination of freeze-fracture-replica images and ultrathin sections (Betti and Katchburian, 1982; Sasaki et al., 1982; Goldberg and Escaig, 1984; Sasaki et al., 1984; Kohling, 1987; Goldberg and Septier, 1989).

Some scanning electron microscopic studies (SEM) of intracellular organelles and the tubulo-vesicular system have been performed on ameloblasts (ABs). These studies have correlated the modified Golgi complex-endoplasmic reticulum-lysosome (GERL) system with the secretory and resorptive functions of ABs at different stages (Ohmi, 1987; Nanci et al., 1993). However, there have been only a few three-dimensional studies on the internal configuration of OBs (Sogarrd-Pederson and Boye, 1990; Iwai-Liao et al., 1992).

The aldehyde prefixation-osmium tetroxide (OsO₄) postfixation—dimethyl sulfoxide (DMSO) freeze-fracture—OsO₄ maceration (AODO) method is usually employed in three-dimensional studies on
the cellular morphoplasm and fibrous component after removal of the less-etching-resistant cytoplasm of soft tissues (Tanaka, 1981; Tanaka and Mitsushima, 1983). In the present study, we examined the dental pulp, which is surrounded by dense hard tissues, by SEM using the AODO method. The purpose of the present study was to perform a three-dimensional TEM findings using epon-embedded ultrathin sections.

Materials and Methods

Transmission electron microscopy (TEM)

Five rats (Wistar strain, 8 weeks old, males) were perfused through the heart with chilled aldehyde fixatives containing 2.5% glutaraldehyde (GA) and 2.0% paraformaldehyde (FA) (buffered with 0.1 M Na-cacodylate buffer solution, 0~4°C), and the lower incisors were dissected out with surrounding tissues. After cutting some shallow grooves in samples, which were repeatedly dipped in the chilled buffer solution, with a diamond disc mounted on a dental handpiece, the samples were separated into blocks with a snipper along the grooves and immediately immersed in the same aldehyde fixatives for an additional 2 hours (h). The samples were then rinsed with the buffer solution and postfixed in 1% OsO₄ solution (0~4°C; 2h), rinsed again, decalcified with 0.5% ethylene diamine tetra-acetic acid (EDTA; 1 week), dehydrated with ethyl alcohol, embedded in epon 812 and then ultrathin-sectioned according to conventional methods. The specimens were stained with uranyl acetic (20 min) and lead citrate (10 min), examined and photographed with a Hitachi H-7100 TEM at an accelerating voltage of 100 kV.

The developmental stages of the odontoblasts (OBs) and differentiation of the organelles are mainly based on the results of the previous conventional morphological TEM studies (Tsuboi, 1968; Takuma and Nagai, 1971; Weinstock and Leblond, 1974; Gartner et al., 1979). We defined the 30~45 μm thick layer of dentinogenic OBs in the segment between the levels showing 5-μm thick dentin formation, and 80-μm thick dentin and 60 μm enamel formation, where the predentin was 15~30 μm wide, as functioning cells. The less active OBs containing mostly flattened rER cisterns between osteodentin deeply invaded by a complicated capillary network and the region distal to the level showing formation of 140 μm dentin and 120 μm enamel, beneath the predentin less than 15-μm thick, were classified as resting OBs. The resting OBs were about 25 μm long.

Results

Transmission electron microscopy (TEM)

Small secretory granules and various types of cytoplasmic bodies such as lysosomes, multivesicular bodies (MVBs) and cytosomes of different forms and sizes were observed in both the functioning and resting odontoblasts (OBs). We noted that the occurrence of cytoplasmic bodies was more prominent in the clear area and distal portion of the OBs (Figs. 1-3). The well-developed granular (rough) endoplasmic reticulum (rER) was composed of distended, cisterns, in which a relatively electron-dense substance was evident not only in the functioning OBs but in active secretory cells distributed in the layer mostly containing resting OBs located beneath a thick layer of dentin and adjacent to the
and therefore have been elucidated to be the granules vesicles stained positive for acid phosphate activity, the Golgi complex. Furthermore, these abacus-like one type of cytoplasmic bodies closely related to containing fibrillar elements (the abacus-like vesicles) (Weinstock and Leblond, 1974). The granules containing membranous structures containing procollagen secretory granules of the odontoblasts (OBs), are observed in the distal end of the odontoblast cell bodies and in the odontoblast processes (Figs. 2, 7–9). The tubulo-vesicular elements containing small secretory granules and fine tubules—the network of agranular (smooth) endoplasmic reticulum (sER)—, and intracellular large membranous structures containing small vesicles (multi-vesicular bodies; MVBs) closely related to the tubulo-vesicular elements were found particularly in the odontoblast process and the distal end of the cells, respectively (Figs. 7–9). Membranous vesicles (measuring about 500 nm) were also noted in the predentin and in the vicinity of the cell membrane of the OBs (Fig. 9). Many thin lateral branchings (about 200 nm in diameter) of the OBs and the membranous vesicles (about 200 to 700 nm in diameter) running along the bundles of collagen fibres were observed in the mineralizing predentin of the circumpulpal dentin. The membranous vesicles found in the vicinity of the lateral branchings of OBs were morphologically similar to those found in the predentin by the TEM. They were identified as the vesicles micro-apocrine secreted vesicles by the OBs (Figs. 4 and 10).

Discussion

Some autoradiographic studies on the biosynthetic pathway for the precursor of collagen have shown that the distended Golgi saccules, presecretory and secretory granules of the odontoblasts (OBs), are membranous structures containing procollagen (Weinstock and Leblond, 1974). The granules containing fibrillar elements (the abacus-like vesicles) on the other hand, are morphologically similar to one type of cytoplasmic bodies closely related to the Golgi complex. Furthermore, these abacus-like vesicles stained positive for acid phosphate activity, and therefore have been elucidated to be the granules relating to the secretion and digestion of the procollagen in both the functioning and resting OBs by many previous studies (Tsubo1, 1968; Nagai, 1970a; Takuma and Nagai, 1971; Weinstock and Leblond, 1974; Leblond, 1989; Romagnoli et al., 1990; Linde and Goldberg, 1993). In the present SEM using the AODO method, we observed many empty vesicles (secretory-granule-like structures) near the nucleus and the cell membrane and some large membranous structures containing many small vesicles (multi-vesicular bodies; MVBs) in the functioning and resting OBs. Unfortunately, it was impossible to differentiate all types of cytoplasmic bodies, because some components in the fractured membranous structures were washed out during AODO treatment. The development of methods of preserving soluble substances in the intracellular organelles fractured for SEM investigation is the task we must confront.

The endocytotic function of the OBs has been demonstrated by observation of the formation of coated vesicles and resorption of the matrical components in the predentin into the cell process (Nagai, 1970a; Nagai, 1970b; Sasaki et al., 1982; Goldberg and Escaig, 1984; Sasaki et al., 1984; Köling, 1987; Leblond, 1989; Romagnoli et al., 1990; Linde and Goldberg, 1993). A previous study on OBs in tooth germ has also shown that the extensive lysosomal-vacuolar apparatus is linked to proteolytic activity and regulation of OBs in early dentinogenesis (Betti and Katchburian, 1982). The present SEM studies revealed that tubulo-vesicular elements containing a network of tubular smooth sER and strings of secretory granules, which have been elucidated to represent a modified Golgi-GERL system involved in both secretory and absorptive functions, are closely related to the multivesicular bodies (Sögaard-Pedersen and Boye, 1990; Nanci et al., 1993). The results of the present study seem to suggest that the digestive functions of the OBs also may be regulated by the tubulo-vesicular elements.

There have been many studies indicating that matrix vesicles are the sites of initial mineralization of certain connective tissues (Anderson, 1967; Ozawa and Yajima, 1972; Slavkin et al., 1974; Ozawa et al., 1975; Kim, 1976; Bonucci and Dearden, 1976; Rabinovitch and Anderson, 1976; Wuthier, 1976; Ozawa, 1977; Ozawa, 1985). The present SEM and TEM studies revealed some membranous vesicles in the predentin of the circumpulpal dentin, and vesicles were found in the vicinity of the basal portion and along the lateral branchings of the odontoblast process. The results of these examinations suggest that the microapocrine secretion of certain membranous vesicles into the predentin may be involved in the collagen-related mineralization of the dentin during
the matrix-deposition stage of the OBs.

References


Explanation of Figures

Plate I

Fig. 1. Electron-dense cytoplasmic bodies (arrows) of various sizes and shapes are observed in the elongated functioning odontoblasts. The odontoblastic layer is invaded by a capillary network (asterisks).

Fig. 2. The layer containing resting odontoblasts is deeply invaded by a capillary network (the asterisk indicates the lumen of a capillary). The cuboidal, resting odontoblasts near the capillary contain rough endoplasmic reticulum composed of distended cistern including electron-dense matrix (arrowheads). Small arrows indicate secretory granules in the odontoblast process and the distal clear area of the resting odontoblasts.

Fig. 3. A columnar lysosome (small arrow), cytosomes (small asterisks) and a large multivesicular body (arrowhead) are also seen in the distal clear area of the resting odontoblast.

Fig. 4. Transmission electron microscopy showing microapocrine secretion (budding; small arrow) from the odontoblast process (asterisk) in the mineralizing predentin (arrowhead) containing bundles of collagen fibers.
Plate II

Fig. 5. A scanning electron microscopy view showing some obliquely freeze-fractured functioning odontoblasts (OB). The supranuclear region contains parallel cisterns of the granular (rough) endoplasmic reticulum (rER), fractured granules (small arrows) and many long mitochondria (arrows). Asterisks indicate the nuclei of certain OBs and a cavity originally occupied by the nucleus of an OB.
Plate III

Fig. 6. Oblique- and cross-sections of the functioning odontoblasts (OB) indicate that the rough endoplasmic reticulum is composed of concentric coated laminations (small arrows) and cisterns (arrowheads). Mitochondria (small asterisks) are fractured and showed mitochondrial cristae in the inner chamber (intercristal space). They are interposed between the rough endoplasmic reticulum.

Fig. 7. Scanning electron microscopy (SEM) of the basal portion of a functioning odontoblasts; PD indicates the predentin containing many matrical fibers. The tubulo-vesicular elements containing small secretory granules and vesicles (small asterisks) adhering towards the cell membrane and fine tubules (arrows) are observed. A large cytoplasmic body (multivesicular body; arrowhead) closely related to the tubulo-vesicular elements is also seen.

Fig. 8. SEM showing an odontoblast (OP) containing tubulo-vesicular elements (arrowheads). Secretory granules (small arrows) adhering to the cell membrane of the OP are also noted.
Plate IV

Fig. 9. SEM view showing some multivesicular bodies (arrows) of different sizes and many small secretory granules (small asterisks) in the distal end of resting odontoblasts. A membranous vesicle (arrowhead) containing small granules is seen in the predentin near the basal portion of the odontoblast process.

Fig. 10. SEM view showing an oblique section through the predentin beneath the circumpulpal dentin. The matricial collagen fibers form a complicated network (asterisks indicate the spaces originally containing odontoblast processes). Membranous vesicles (arrow heads) and many thin lateral branchings (small arrows) of odontoblast processes are observed running along the collagen fibre bundles.