Int. J. Dev. Biol. 39: 231-237 (1995)



# Root formation

H.F. THOMAS*

*Department of Pediatric Dentistry, University of Texas Health Science Center, San Antonio, Texas, USA*

**ABSTRACT This paper provides an overview of recent studies that have enhanced our understanding of the biological mechanisms that operate during root development. For the most part, these studies have been performed on rodents. As significant species differences have been shown to exist, this data cannot necessarily be extrapolated to the human model. The events associated with root odontoblast differentiation are reviewed in comparison to similar events in coronal odontoblast differentiation. Morphological as well as phenotypic differences are outlined and the inductive role of the epithelial root sheath is discussed. Both acellular and cellular cementum formation are reviewed highlighting morphological and phenotypic differences. The potential influence of the epithelial root sheath in the formation of both tissues is compared and contrasted. Finally, a discussion of the fate of the epithelial root sheath is presented with emphasis placed upon the possible roles of apoptosis and epithelium-mesenchymal transition.**

KEY WORDS: *root dentin, acellular cementum, cellular cementum, epithelial root sheath*

## Introduction

Following the completion of crown morphogenesis and the elaboration of coronal dentin and enamel extracellular matrix, the developing tooth germ begins to form its root, a process that will establish its connection to the surrounding alveolar bone. The mesenchyme that surrounds the enamel organ (dental sac) and that situated within the developing pulp is contiguous and derived from cranial neural crest ectomesenchyme. This mesenchyme and particularly that portion situated in the apical portion of the tooth germ, proliferates throughout the period of root development, generating not only cell populations that will contribute to the developing radicular pulp but also those that will form the developing periodontium. Into this milieu, the epithelial root sheath (ERS), derived from cells of the cervical loop of the enamel organ, proliferates apically, thereby establishing the demarcation between pulp and periodontium.

The morphological events associated with root formation have been thoroughly described in a variety of animal species. Our understanding of the biological mechanisms involved in root formation is not as advanced. However, several important observations have been made recently that have enhanced our understanding. This paper will provide an overview of these recent studies in the context of our current understanding of the morphology of root dentin and cementum formation. For the most part, these studies have investigated the rodent model and, since species differences have been noted, the reader is cautioned against overextrapolation to the human model.

## Root dentinogenesis

Many excellent descriptions of root dentinogenesis in a variety of animal species have been published (Selvig, 1963; Owens, 1978, 1979; Ten Cate, 1978; Andujar *et al.*, 1985; Rademakers *et al.*, 1985). These descriptions reveal many similarities with the events seen in coronal dentinogenesis (Ten Cate, 1978; Hurmerinta and Thesleff, 1981). For example, pre-odontoblasts can be seen to align themselves along the basal lamina separating them from their respective epithelia (Fig. 1), with a gradient of differentiation of odontoblasts from the most apical (least differentiated) to the most coronal portion. Polarization of odontoblasts precedes predentin secretion (Fig. 2) with foci of mineralization appearing within the predentin matrix. Mineralization of the most peripheral layer of the predentin matrix is delayed in both crown and root (Figs. 4, 5). This unmineralized layer serves as the matrix into which initial enamel formation occurs in the crown and cementum formation occurs in the root, ensuring an intimate connection between the different mineralized tissues.

However, many important differences between crown and root dentin formation have been described (Ten Cate, 1978; Beertsen and Niehof, 1986), particularly during predentin secretion. For example, in the root, predentin matrix contains sparsely distributed collagen fibrils (Fig. 2). In coronal predentin, collagen fibrils are thicker, more densely packed and often arranged parallel to odontoblast processes (Fig. 3). Also, in the root, odontoblast processes retreat with the cell bodies away from the basal lamina; in the crown, they remain at the site of the future dentinoenamel junction and some even penetrate the ameloblast cell layer. The consequence of this latter observation is that peripheral dentin in the crown contains highly branched dentinal tubules whereas that in the root is atubular. Only after a certain amount of root dentin has been deposited do tubules form, a process that appears to occur rapidly and somewhat chaotically, resulting in the formation of the granular layer of Tomes (Weber, 1983). Finally it has been appre-

***Address for reprints:** Department of Pediatric Dentistry, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284-7888, USA. FAX: 210.567-3526.

0214-6282/95/$03.00



Printed in Spain

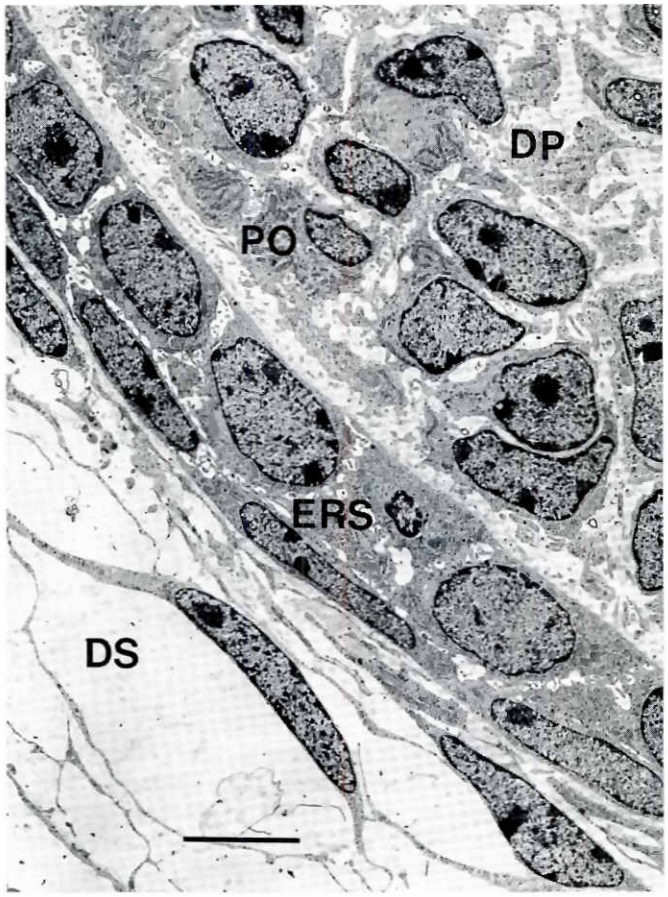


**Fig. 1. Transmission electron micrograph (TEM) of a developing root from an eight day old murine first molar.** *The epithelial root sheath (ERS) is visible as is the dental papilla (DP) and dental sac (DS). Note the gradient of differentiation within preodontoblasts (PO) as well as their alignment along the basal lamina. In contrast no gradient of differentiation is seen within cells of the dental sac. Scale bar, 5 μm.*

ciated for some time that morphological differences are apparent between fully differentiated odontoblasts in the crown and root. Coronal odontoblasts are columnar, root odontoblasts are cuboidal (Avery, 1986).

Similarly, differences have been described in the biochemical composition of root dentin when compared with crown (Steinfort *et al.*, 1989, 1990). For example, differences have been reported between crown and root odontoblasts in the quantity and quality of the phosphoproteins synthesized. Also, lower levels of both $\alpha_1$ and $\alpha_2$ chains of type I collagen mRNA have been described in root odontoblasts (Andujar *et al.*, 1991).

The above data describe morphological as well as phenotypic differences between crown and root odontoblasts. These differences may result from differences in the inductive mechanisms operating between crown and root. Although assumed to be the case for many years, the role of ERS in the induction of root odontoblasts was not confirmed until techniques were developed for isolating ERS from developing roots and then using it in tissue recombination experiments (Thomas and Kollar, 1989). Data from these studies demonstrated that ERS could indeed induce odontoblast differentiation from dental papilla, but only in papillae (from 18 day *in utero* murine tooth germs) in which a certain degree of commitment already existed. Further, no enamel organ formation was induced in ERS suggesting that it was refractory to any inductive signals from the papilla.

Taken together, these differences suggest that there may be qualitative as well as quantitative differences in the inductive mechanisms operating in the crown compared with the root. Our appreciation of these differences awaits a greater understanding of those mechanisms and subsequent comparison of their roles in crown and root dentin formation.

## Cementogenesis

The roots of teeth are covered throughout their length by cementum, the mineralized tissue that serves as the attachment for collagen fibers of the periodontal ligament. Two major types of cementum have been described, acellular (acellular extrinsic fiber cementum) and cellular (cellular mixed stratified cementum). For the most part, acellular cementum is found on the coronal and mid-portions of the root with cellular cementum on the apical and interradicular portions. The morphological events associated with the formation of both types of cementum have been comprehensively described in a variety of animal species (Selvig, 1964; Lester, 1969a,b; Lester and Boyde, 1970; Freeman and Ten Cate, 1971; Owens, 1978, 1979; Cho and Garant, 1988; Thomas and

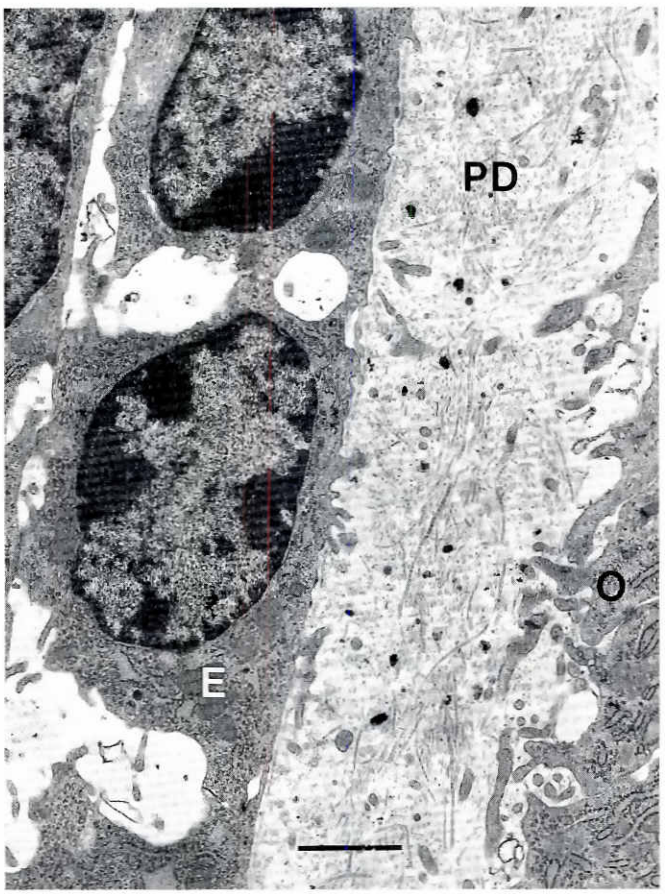


**Fig. 2. TEM of root predentin (PD) from eight day old murine first molar.** *The collagenous matrix of predentin is sparsely distributed. E, epithelial root sheath cell; O, odontoblast. Scale bar, 1.5 μm.*

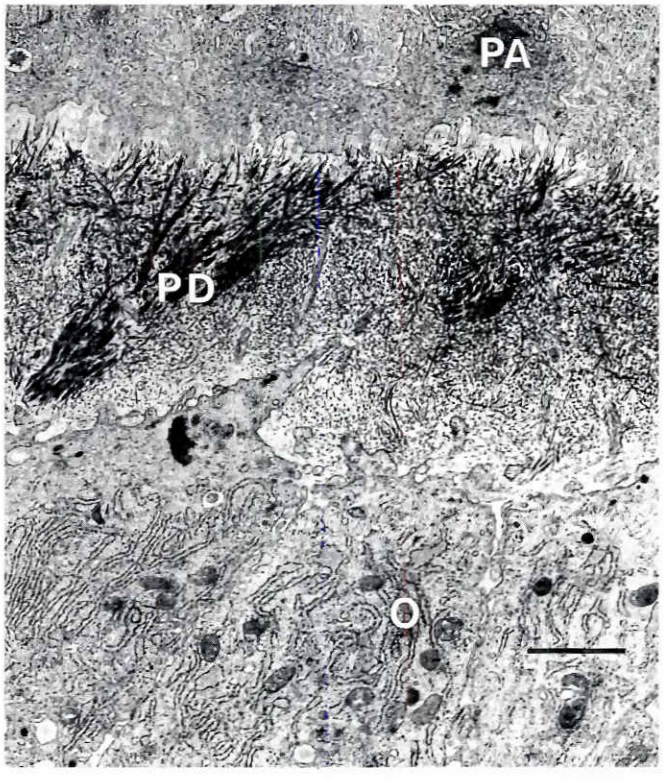


**Fig. 3. TEM of coronal predentin (PD) from one day old murine first molar.** *Note the denser predentin matrix than that seen in the root. O, odontoblast; PA, preameloblast. Scale bar, 1.5 μm.*

Kollar, 1988; Yamamoto and Wakita, 1991; Bosshardt and Schroeder, 1991, 1992).

Although it has been suggested that the ERS plays a role in the induction of cementoblasts (Orban, 1952), little experimental evidence exists to support this contention. Ultrastructurally, although a close association exists between ERS and dental sac cells (Fig. 1), little evidence for a progressive gradient of differentiation is seen within dental sac cells (Thomas and Kollar, 1988). Similarly, tissue recombination experiments between ERS and dental sac cells (Thomas *et al.*, 1995), although resulting in bone formation from dental sac cells, produce no different a result than when dental sac cells are explanted *per se* (the ability of dental sac cells to form mineralized tissue is presumably acquired during earlier inductive events).

Because of these findings, attention has been focused on the role of the peripheral root dentin surface as the inducer of cementogenesis. Certainly, ultrastructural data support this notion. Following fenestration of the ERS, cells of the dental sac insinuate themselves between the epithelial cells and contact the unmineralized surface of root dentin (Fig. 6). This important event precedes the establishment of the initial collagen fiber attachment and subsequent deposition of acellular cementum. Tissue separation and recombination experiments (MacNeil and Thomas, 1993a,b) support these findings. The unmineralized root dentin surface (attained by EDTA treatment of developing roots) is capable of supporting the attachment of cells, the establishment of a connective tissue attachment and the adherence of mineralized tissue (MacNeil and Thomas, 1993a). Although it has previously been suggested that enamel protein-like material may be deposited on the developing root surface by the ERS and act as an inducer of cementum (Slavkin, 1976), recent studies have discounted this possibility (Thomas *et al.*, 1986; Luo *et al.*, 1991). However, several studies (Paynter and Pudy, 1958; Grant and Bernick, 1971; MacNeil and Thomas, 1993a) have indicated that other epithelially-derived material (e.g. laminin) is present on the developing root surface. As epithelium and its products have long been known to be capable of inducing bone formation in susceptible mesenchyme (Hall, 1981; Hall and Van Exan, 1982), it seems reasonable to speculate that a similar mechanism may be functioning in root development. In support of this is the observation that trypsinization of developing roots prevents the development of adherence of mineralized tissue (MacNeil and Thomas, 1993a).

Although initial contact of dental sac cells with the developing root surface establishes the above described events, epithelial cells from the ERS have been shown, both by ultrastructural and immunohistochemical methods (Fig. 7), to remain on the developing root surface (Thomas and Kollar, 1988). What is the significance of this observation? Again, tissue separation and recombination experiments have begun to shed some light on this issue (MacNeil and Thomas, 1993b). Recombinations between developing root surfaces (treated with EDTA to remove cells) and dental sac result in an indiscriminate deposition of mineral on the root surface. Inclusion of ERS in these recombinations serves to limit the amount of mineral formed, such that a more normal periodontium is formed. Does ERS, by remaining in close proximity to the developing root surface, regulate the amount of acellular cementum deposited on the root surface? Further support for a role for

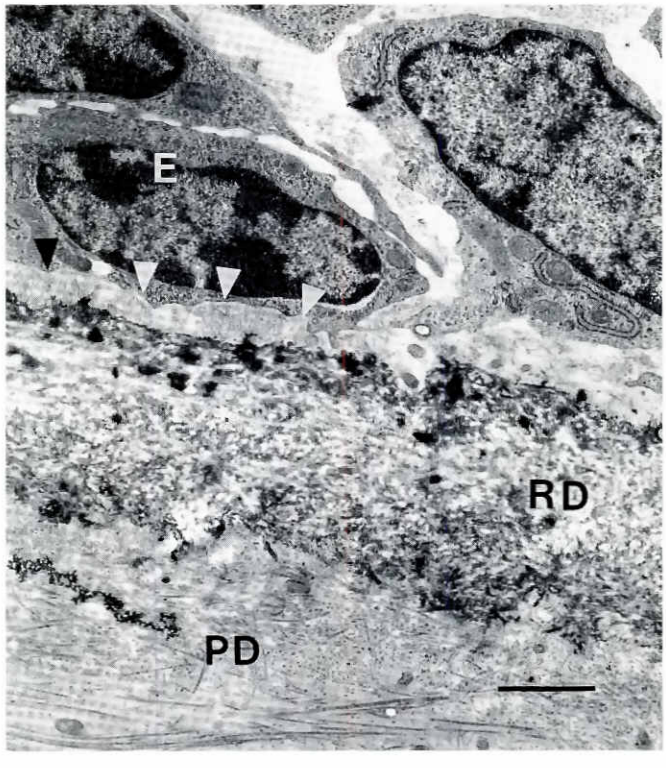


**Fig. 4. TEM of root dentin from eight day old murine first molar.** *The unmineralized surface zone of root dentin (RD) can be seen immediately subjacent to the cell membrane (arrowheads) of epithelial root sheath cells (E). PD, predentin. Scale bar, 1.5 μm.*

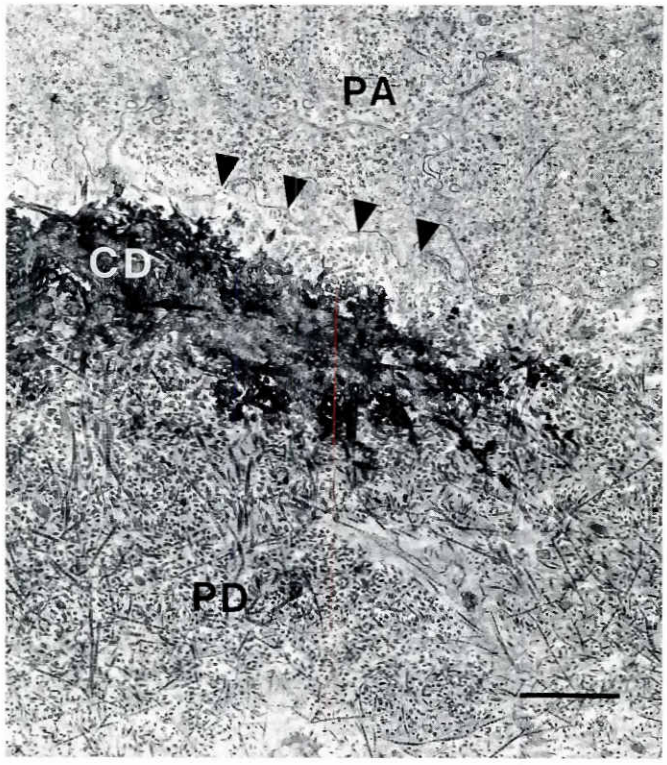


**Fig. 5. TEM of coronal dentin from one day old murine first molar.** *The unmineralized surface zone of coronal dentin (CD) can be seen immediately subjacent to the cell membrane (arrowheads) of preameloblasts (PA). PD, predentin. Scale bar, 1.5 μm.*

epithelium in cementogenesis and in the establishment of the periodontium comes from earlier recombination data (Andreasen and Kristerson, 1981) as well as from studies on tooth transplantation (Loe and Waerhaug, 1961; Lindskog *et al.*, 1988).

The manner in which acellular cementum is deposited deserves some comment. The tissue appears, from ultrastructural studies, to form as a slow accretion of mineral on the root dentin surface rather than the accumulation of mineral within a preformed collagenous matrix (in contrast to cellular cementum, see below). The mechanisms involved in this pattern of mineralization are unknown although it has been suggested that alkaline phosphatase plays a significant role in this process (Beertsen and Everts, 1990; Beertsen and Van Den Bos, 1991).

Recently, several studies have examined the distribution of a variety of adhesion proteins during the early events associated with cementogenesis (Bronckers *et al.*, 1994; MacNeil *et al.*, 1994). Included in these are the molecules osteopontin and bone sialoprotein. Both molecules have been associated with acellular cementum formation suggesting a potential role for each in the adhesion of cells to the peripheral root surface. Our understanding of the role of these molecules in root formation awaits further investigations.

The preceding discussion relates to the events occurring during the early stages of root formation in rodents, when acellular cementum is being formed. At later stages of development, significant changes in cellular behavior have been described resulting in the deposition of cellular cementum on the root surface (Lester, 1969a,b; Lester and Boyde, 1970). It appears from ultrastructural observations, that at some point during root formation (see below), foci of mineralization appear in the periodontium lateral to the ERS. As these foci coalesce, epithelial cells together with some cells of the dental sac, become entrapped within the newly deposited mineral, resulting in the formation of cellular inclusions within the cementum (i.e., cellular cementum). Indeed, at this stage the formation of cementum in an apical direction, has even been described as preceding that of root dentin. Ultrastructurally, not only does cellular cementum contain cells, but the cells (cellular cementoblasts) responsible for its formation also deposit a collagenous matrix (intrinsic fibers) in which mineralization occurs. These cementoblasts are frequently separated from the mineralized matrix by an unmineralized precementum layer (Furseth and Mjor, 1973). Additionally, (as is the case with acellular cementum) extrinsic collagen fiber bundles from the periodontal ligament are inserted into the matrix.

In support of the morphological differences between acellular and cellular cementum, a recent immunohistochemical investigation, that examined the distribution of a variety of adhesion molecules, has noted differences between the two tissues (Bronckers *et al.*, 1994). Whereas cells associated with acellular cementum stain only with antibodies against osteopontin, those associated with cellular cementum stain with antibodies against osteopontin and osteocalcin. While this observation reflects differences in

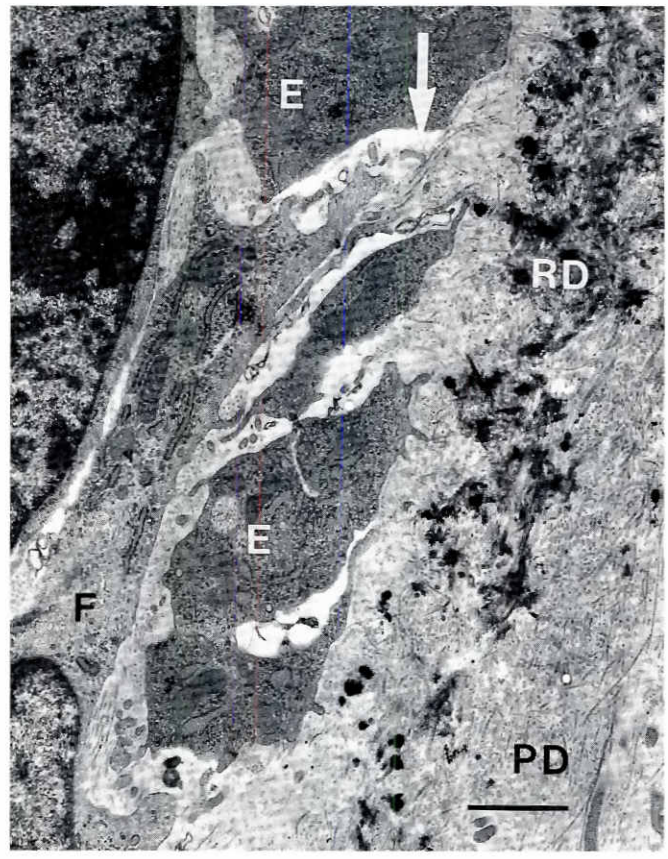


**Fig. 6. TEM of root dentin from eight day old murine first molar.** *Initial contact (arrow) can be seen between a dental sac cell (F) and the unmineralized surface zone of root dentin (RD). E, epithelial cell; PD, predentin. Scale bar, 1.5 μm.*

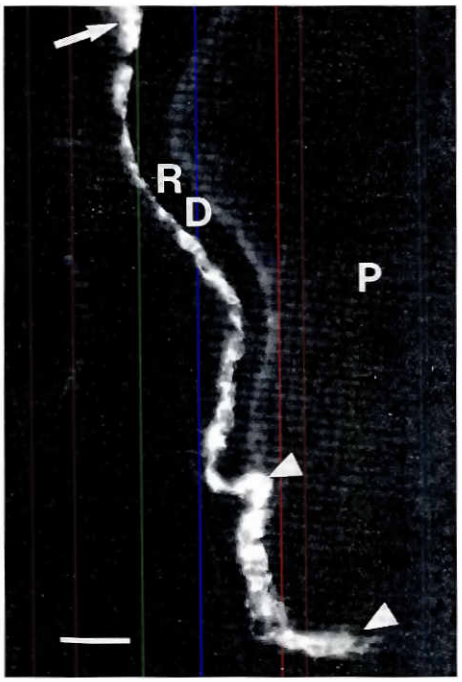


**Fig. 7. Immunofluorescence micrograph (IF) of the developing root from an eight day old first molar stained with antiserum against cytokeratins.** *The intact ERS is visible between arrowheads. All cells in contact with the root surface demonstrate positive staining. Arrow indicates cemento-enamel junction. RD, root dentin; P, pulp. Scale bar, 50 μm.*

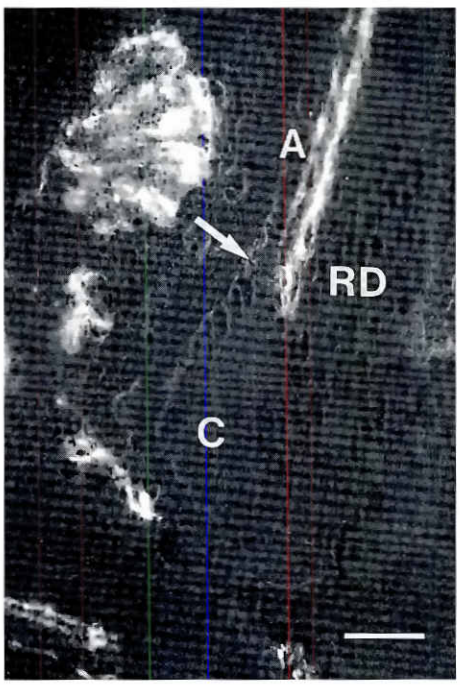


**Fig. 8. Fluorescence micrograph of a ground section through the root of a mandibular molar from an adult animal that received an intraperitoneal injection of tetracycline when the tooth reached occlusion.** *Extent of labeling within root dentin (RD) corresponds to the junction (arrow) between acellular (A) and cellular (C) cementum. Scale bar, 200 μm.*

cellular phenotype, it does not necessarily imply that different cells are involved in the elaboration of the two tissues. It may be due to the influence of epithelial cells on dental sac cells. In acellular cementum formation, epithelial cells remain in close proximity to dental sac cells and may influence their phenotype, whereas in cellular cementum formation, epithelial cells become entrapped within the cementum matrix, removing or lessening their potential influence on dental sac cell phenotype.

The stimulus for the change in the type of cementum formed on the root surface is unknown. It has been suggested that occlusal contact may provide this stimulus (Lester, 1969b), and a recent investigation (Thomas *et al.*, 1995) using tetracycline labeling of developing murine molars (Fig. 8) has confirmed that cellular cementun is only formed after the tooth has reached occlusion (the influence of enamel free areas on the cusps of rodent molars and the more rapid wear patterns of these teeth on this observation

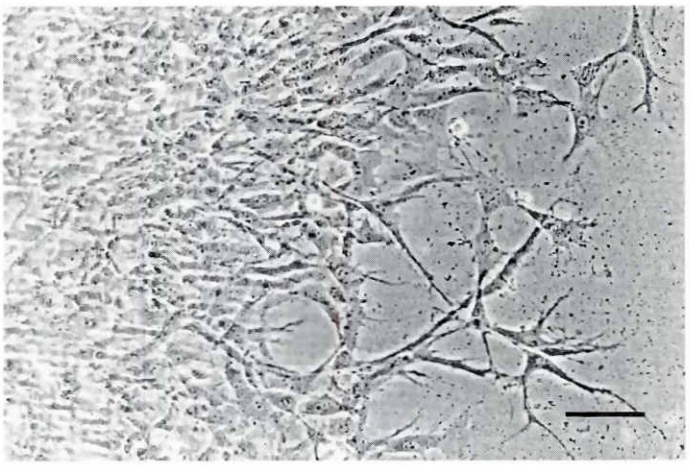

**Fig. 9. Phase contrast micrograph of culture of epithelial root sheath cells following media switch** *(see text). Cells at the periphery of the culture have assumed a bipolar, mesenchymal morphology. Scale bar, 65 μm.*

has yet to be determined). It is also interesting to note that radioautographic studies have shown that cellular proliferation within ERS essentially stops at the same time as the tooth reaches occlusion (Diab and Stallard, 1965). Are these latter two observations coincidental, or does occlusal contact signal an end to proliferation with resulting entrapment of epithelial cells and cellular cementum formation!

In summary, cementogenesis in rodent molars can be divided into two stages. The first occurs prior to and during the eruption of the tooth. At this time acellular cementum is formed on the root surface. Cells from the dental sac appear to establish the periodontal ligament attachment apparatus, while cells of the former ERS remain in close proximity to the root surface where they may play a role in limiting the formation of acellular cementum. The second stage begins as the tooth reaches occlusion. Proliferation of ERS is dramatically reduced, foci of mineralization appear lateral to it within the periodontium, and it becomes entrapped within the forming matrix of cellular cementum. The entrapment of epithelial cells during these later stages of root formation may result in a phenotypic change in dental sac cells and the formation of cellular cementum.

**Fig. 10. IF of culture similar to Fig. 9, double-stained with antibodies against vimentin (visualized with rhodamine) and keratin (visualized with fluorescein).** *Acquisition of staining for vimentin is apparent and accompanies the morphological change. Scale bar, 35 μm.*

## Epithelial root sheath (ERS)

It is apparent from the above discussion that the ERS plays a significant role not only in the induction of root dentinogenesis but also in cementogenesis and in the development of the periodontium. A long-held tenet of oral histology is that following these roles, ERS cells migrate into the periodontal ligament where they reassociate to form epithelial cell rests. Although this appears to be a reasonable assumption no direct evidence exists to support this notion.

Two additional fates for ERS cells should be considered. The first of these is apoptosis. Programmed cell death is becoming an increasingly more well understood phenomenon as a mechanism for an organism to eliminate cell populations during development (Hurle, 1988; Clarke, 1990). Certainly, as far as the rodent model is concerned, the number of cell rests is relatively sparse and has been reported to decrease with age (Wesselink and Beertsen, 1993), an observation not inconsistent with apoptosis. However, no consistent observation of cell death within ERS has been reported.

The second possibility to be considered is epithelium-mesenchymal transition (EMT, Boyer and Thiery, 1993). These transitions have been shown to occur during a variety of embryonic events as well as during wound healing and cellular metastasis in the adult. One of the most well characterized examples of EMT during orofacial development occurs during fusion of the palate (Griffith and Hay, 1992; Shuler *et al.*, 1992). Using fluorescent dyes to label medial edge epithelium and following the fate of labeled cells after palatal fusion, it has been conclusively shown that EMT is an integral component of palate formation. Following EMT former epithelial cells can be identified by morphological (bipolar, fibroblastic appearance) as well as immunohistochemical (expression of vimentin instead of keratin intermediate filament protein) methods as expressing a mesenchymal phenotype. In addition they appear (as judged by intracellular organelles) to be participating in the elaboration of the fibrous connective tissue matrix. Does the same phenomenon occur in cells of ERS? Do ERS cells undergo EMT and contribute to the mesenchymal cell population of the periodontal ligament? Certainly, ultrastructural evidence suggests that following fenestration of the ERS, the epithelial cells that remain on the root surface take on a more mesenchymal appearance, an observation supported by immunohistochemical data. We have recently been successful in effecting EMT in cultured ERS cells. This has been accomplished by culturing ERS cells on collagen gels in tissue culture media favoring epithelial cell growth. Following a few days of growth, media is switched to one favoring mesenchymal cell growth. This results in cells at the margin of the cultures assuming a more mesenchymal (bipolar) appearance (Fig. 9). Immunohistochemical observation (Fig. 10) shows that this morphological change is accompanied by a switch from keratin to vimentin expression. Further research is needed in this area to more thoroughly investigate this phenomenon and its role in root development.

In conclusion, although our understanding of the biological mechanisms involved in root formation has increased significantly during the last few years, our knowledge of these mechanisms is still in its infancy. However, with the technologies now available to address the important questions that remain, we should feel confident that this area of odontogenesis will yield important information in the near future.

## References


ANDREASEN, J.O. and KRISTERSON, L. (1981). Evaluation of different types of autotransplanted connective tissues as potential ligament substitutes. *Int. Oral Surg. 10:* 189-201.

ANDUJAR, M.B., COUBLE P., COUBLE M.L. and MAGLOIRE, H. (1991). Differential expression of type I and type III collagen genes during tooth development. *Development 3:* 691-698.

ANDUJAR, M.B., MAGLOIRE, H., HARTMANN, D.J., VILLE, G. and GRIMAUD, J.A. (1985). Early mouse molar root development: cellular changes and distribution of fibronectin, laminin and type IV collagen. *Differentiation 30:* 111-122.

AVERY, J.K. (1986). Pulp. In *Orban's Oral Histology and Embryology*, Tenth ed. (Ed. S.N. Bhasker). C.V. Mosby Co., St. Louis, pp. 135-174.

BEERTSEN, W. and EVERTS, V. (1990). Formation of acellular root cementum in relation to dental and non-dental hard tissues in the rat. *J. Dent. Res. 69:* 1669-1673.

BEERTSEN, W. and NIEHOF, A. (1986). Root-analog versus crown-analog dentin: a radioautographic and ultrastructural investigation of the mouse incisor. *Anat. Rec. 215:* 106-118.

BEERTSEN, W. and VAN DEN BOS, T. (1991). Alkaline phosphatase induces the deposition of calcified layers in relation to dentin: an *in vitro* study to mimic the formation of afibrillar acellular cementum. *J. Dent. Res. 70:* 176-181.

BOSSHARDT, D.D. and SCHROEDER, H.E. (1991). Initiation of acellular extrinsic fiber cementum on human teeth. *Cell Tissue Res. 263:* 311-324.

BOSSHARDT, D.D. and SCHROEDER, H.E. (1992). Initial formation of cellular intrinsic fiber cementum in developing human teeth. *Calcif. Tissue Res. 267:* 321-335.

BOYER, B. and THIERY, J.P. (1993). Epithelium-mesenchyme interconversion as example of epithelial plasticity. *Acta Pathol. Microbiol. Immunol. Scand. 101:* 257-268.

BRONCKERS, A.L.J.J., FARACH-CARSON, M.C., VAN WAVEREN, E. and BUTLER, W.T. (1994). Immunolocalization of osteopontin, osteocalcin, and dentin sialoprotein during dental root formation and early cementogenesis in the rat. *J. Bone Miner. Res. 9:* 833-841.

CHO, M.I. and GARANT, P.R. (1988). Ultrastructural evidence of directed cell migration during initial cementoblast differentiation in root formation. *J. Periodontol. Res. 23:* 268-276.

CLARKE, P.G.H. (1990). Developmental cell death: morphological diversity and multiple mechanisms. *Anat. Embryol. 181:* 195-213.

DIAB, M.A. and STALLARD, R.E. (1965). A study of the relationship between epithelial root sheath and root development. *Periodontics 3:* 10-14.

FREEMAN, E. and TEN CATE, A.R. (1971). Development of the periodontium: an electron microscopic study. *J. Periodontol. 42:* 387-395.

FURSETH, R. and MJOR, I.A. (1973). Cementum. In *Histology of the Human Tooth* (Eds. I.A. Mjor and J.J. Pindborg). Munksgaard, Copenhagen, pp. 97-119.

GRANT, D. and BERNICK, S. (1971). Morphodifferentiation and structure of Hertwig's root sheath in the cat. *J. Dent. Res. 50:* 1580-1588.

GRIFFITH, C.M. and HAY, E.D. (1992). Epithelial-mesenchymal transformation during palatal fusion: carboxyfluorescein traces cells at light and electron microscopic levels. *Development 116:* 1087-1099.

HALL, B.K. (1981). The induction of neural crest-derived cartilage and bone by embryonic epithelia: an analysis of the mode of action of an epithelial-mesenchymal interaction. *J. Embryol. Exp. Morphol. 64:* 305-320.

HALL, B.K. and VAN EXAN, R.J. (1982). Induction of bone by epithelial cell products. *J. Embryol. Exp. Morphol. 69:* 37-46.

HURLE, J.M. (1988). Cell death in developing systems. *Methods Achiev. Exp. Pathol. 13:* 55-86.

HURMERINTA, K. and THESLEFF, I. (1981). Ultrastructure of the epithelio-mesenchymal interface in the mouse tooth germ. *J. Craniofac. Genet. Dev. Biol. 1:* 191-202.

LESTER, K.S. (1969a). The incorporation of epithelial cells by cementum. *J. Ultrastruct. Res. 27:* 63-87.

LESTER, K.S. (1969b). The unusual nature of root formation in molar teeth of the laboratory rat. *J. Ultrastruct. Res. 28:* 481-506.

LESTER, K.S. and BOYDE, A. (1970). Scanning electron microscopy of developing roots of molar teeth of the laboratory rat. *J. Ultrastruct. Res. 33:* 80-94.

LINDSKOG, S., BLOMLOF, L. and HAMMARSTROM, L. (1988). Evidence for a role of odontogenic epithelium in maintaining the periodontal space. *J. Clin. Periodontol. 15:* 371-373.

LOE, H. and WAERHAUG, J. (1961). Experimental replantation of teeth in dogs and monkeys. *Arch. Oral Biol. 3:* 176-183.

LUO, W., SLAVKIN, H.C. and SNEAD, M.L. (1991). Cells from Hertwig's epithelial root sheath do not transcribe amelogenin. *J. Periodont. Res. 26:* 42-27.

MacNEIL, R.L. and THOMAS, H.F. (1993a). Development of the murine periodontium. I. Role of basement membrane in formation of a mineralized tissue on the developing root dentin surface. *J. Periodontol. 64:* 95-102.

MacNEIL, R.L. and THOMAS, H.F. (1993b). Development of the murine periodontium. II. Role of the epithelial root sheath in formation of the periodontal attachment. *J. Periodontol. 64:* 285-291.

MacNEIL, R.L., SHENG, N., STRAYHORN, C., FISHER, L.W. and SOMERMAN, M.J. (1994). Bone sialoprotein is localized to the root surface during cementogenesis. *J. Bone Miner. Res. 9:* 1597-1606.

ORBAN, B. (1952). The epithelial network in the periodontal membrane. *J. Am. Dent. Assoc. 44:* 632-635.

OWENS, P.D.A. (1978). Ultrastructure of Hertwig's epithelial root sheath during early root development in premolar teeth in dogs. *Arch. Oral Biol. 23:* 91-104.

OWENS, P.D.A. (1979). A light and electron microscopic study of the early stages of root surface formation in molar teeth in the rat. *Arch. Oral Biol. 24:* 901-907.

PAYNTER, K.J. and PUDY, G. (1958). A study of the structure, chemical nature, and development of cementum in the rat. *Anat. Rec. 131:* 233-251.

RADEMAKERS, L.H.P.M., SLOOTWEG, P.J. and VAN BLOKLAND, W.T.M. (1985). An ultrastructural study of cytodifferentiation in the developing human root tip. *Arch. Oral Biol. 30:* 655-660.

SELVIG, K.A. (1963). Electron microscopy of Hertwig's epithelial sheath and of early dentin formation in the mouse incisor. *Acta Odontol. Scand. 21:* 175-186.

SELVIG, K.A. (1964). An ultrastructural study of cementum formation. *Acta Odontol. Scand. 22:* 105-120.

SHULER, C.F., HALPERN, D.E., GUO, Y. and SANK, A.C. (1992). Medial edge epithelium fate traced by cell lineage analysis during epithelial-mesenchymal transformation *in vivo. Dev. Biol. 154:* 318-330.

SLAVKIN, H.C. (1976). Towards a cellular and molecular understanding of periodontics: cementogenesis revisited. *J. Periodontol. 47:* 249-255.

STEINFORT, J., DEBLAUWE, B.M. and BEERTSEN, W. (1990). The inorganic components of cementum- and enamel-related dentin in the rat incisor. *J. Dent. Res. 69:* 1287-1292.

STEINFORT, J., VAN DEN BOS, T. and BEERTSEN, W. (1989). Differences between enamel-related and cementum-related dentin in the rat incisor with special emphasis on the phosphoproteins. *J. Biol. Chem. 264:* 2840-2845.

TEN CATE, A.R. (1978). A fine structural study of coronal and root dentin-ogenesis in the mouse. Observations of the so-called "von Korff fibers" and their contribution to mantle dentine. *J. Anat. 125:* 183-197.

THOMAS, H.F. and KOLLAR, E.J. (1988). Tissue interactions in normal murine root development. In *The Biological Mechanisms of Tooth Eruption and Root Resorption* (Ed. Z. Davidovitch). EBSCO Media, Birmingham, AL, pp. 145-151.

THOMAS, H.F. and KOLLAR, E.J. (1989). Differentiation of odontoblasts in grafted recombinants of murine epithelial root sheath and dental mesenchyme. *Arch. Oral Biol. 34:* 27-35.

THOMAS, H.F., HEROLD, R.C. and KOLLAR, E.J. (1986). Enamel proteins do not participate in murine molar cementogenesis. *J. Dent. Res. 65:* 30.

THOMAS, H.F., MacNEIL, R.L. and HAYDENBLIT, R. (1995). The role of epithelium in the developing and adult periodontium. In *Proceedings of the International Conference on the Biological Mechanisms of Tooth Eruption, Resorption and Replacement by Implants* (Ed. Z. Davidovitch). EBSCO Media, Birmingham, AL. (In press).

WEBER, D.F. (1983). An improved technique for producing casts of the internal structure of hard tissues, including some observations on human dentine. *Arch. Oral Biol. 28:* 885-891.

WESSELINK, P.R. and BEERTSEN, W. (1993). The prevalence and distribution of rests of Malassez in the mouse molar and their possible role in repair and maintenance of the periodontal ligament. *Arch. Oral Biol. 38:* 399-403.

YAMAMOTO, T. and WAKITA, M., (1991). The development and structure of principal fibers and cellular cementum in rat molars. *J. Periodont. Res. 26:* 129-137.