

Dental cementum: the dynamic tissue covering of the root

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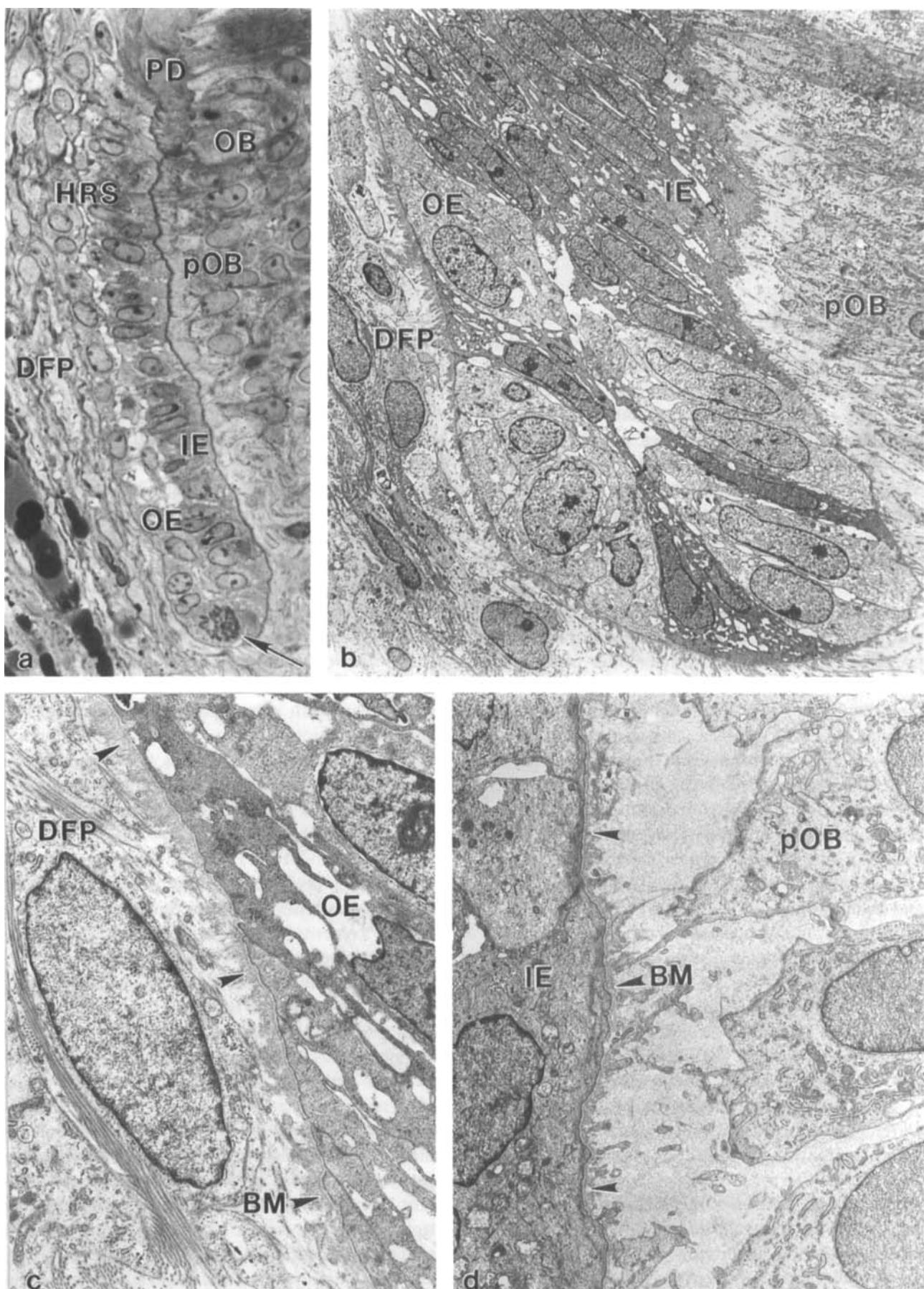
In humans and other mammals, the teeth are not attached rigidly to the alveolar bone. A soft connective tissue, referred to as the periodontal ligament, is interposed between the tooth root and the surrounding alveolar bone. In the mature periodontal ligament, principal fiber bundles span between the root and the bone surface. At the soft-hard tissue borders of the periodontal ligament, the principal periodontal ligament fibers are embedded in bone on one side and radicular cementum on the other side. The embedded terminations of these collagen fibers are referred to as Sharpey's fibers. Due to its intermediary position between the radicular dentin and the periodontal ligament, cementum is a component of the tooth itself, but belongs functionally to the dental attachment apparatus, that is, the periodontium. The latter tissue unit comprises the periodontal ligament, gingiva, alveolar bone and cementum. One of the main functions of cementum is to anchor the principal collagen fibers of the periodontal ligament to the root surface. Besides its indispensable role in tooth attachment to the surrounding alveolar bone, root cementum has important adaptive and reparative functions. The dynamic and highly responsive features of cementum are crucial for maintaining occlusal relationship and for the integrity of the root surface and its function in tooth support.

The morphogenesis of cementum has been comprehensively described in various mammalian species (38). In addition, the physical properties (170) and the basic chemical composition (125) of cementum are known. Cementum is a nonuniform, mineralized connective tissue. Several distinctly different cementum varieties are found on human teeth. They differ with respect to location, structure, function, rate of formation, chemical composition and degree of mineralization. In fully formed and functioning teeth, cementum is firmly attached to the radicular dentin and covers the entire surface of the root. In addition, small localized areas of enamel

close to the cementoenamel junction are frequently covered with a particular type of cementum. Cementum increases in thickness towards the apex and may extend partially into the apical foramen.

Although the morphogenesis and the established structure of the various cementum varieties have been described by many researchers, knowledge of cementum physiology still lags behind what is known about the other dental and periodontal tissues. The interest in cementum, however, has never been given up by researchers, and the ultimate goal of true periodontal regeneration after treatment for periodontitis has revived vigorously the interest in this unique mineralized tissue. Although recent studies have contributed to an understanding of the possible involvement of some of the molecular factors in cementum regeneration (119), cementogenesis, on a cell biological basis, continues to be poorly understood. Virtually nothing is known about the differentiation mechanisms of cementoprogenitor cells and the cell dynamics during normal development, repair and regeneration.

Radicular cementum is unique in that it is avascular, does not undergo continuous remodeling like bone, but continues to grow in thickness throughout life. New cementoblasts must, therefore, be continuously recruited from committed cementoprogenitor cells in order to replace cementoblasts that have reached the end of their life span. During initial periodontal wound healing, new cementoblasts have to be generated as well from ancestral cementoprogenitor cells, although much faster than for normal tissue maintenance. It is likely that new cementoblasts for both maintenance (homeostasis) and repair and regeneration take their origin in the same root-related portion of the intact periodontal ligament. In both circumstances, however, their precise origin and the molecular factors regulating new cell recruitment and differentiation are not known (38, 129). The clue to answer these questions lies in the still growing root and the non-pathologically altered



root surface. Basic knowledge of cementum development during normalcy is therefore of utmost importance.

The rodent has provided the most popular model for the study of tooth development in general. Although the tremendously fast growth rates of rats and mice may render these animals an exciting model for studying periodontal ligament development and tooth eruption, the rodent molar does not provide a good parallel for the human situation with regard to cementogenesis (38). Over recent years, an increasing quantity of data has accumulated that allows human cementogenesis to be described exclusively. The aim of this chapter is to give a comprehensive insight into the structure, function, physical properties and chemical composition of human cementum during undisturbed development and repair as well as under some pathological conditions.

Development

The formation of cementum can be subdivided into a prefunctional and functional developmental stage. The prefunctional portion of cementum is formed during root development. Since the formation of human tooth roots occurs over an extended period of time ranging between 3.75 and 7.75 years for permanent teeth, the prefunctional development of cementum is an extremely long-lasting process. During this period of time, the primary distribution of the main cementum varieties is determined for each root. The functional development of cementum, on the other hand, commences when the tooth is about to reach the occlusal level, is associated with the attachment of the root to the surrounding bone and continues throughout life. It is mainly during the functional development that adaptive and reparative processes are carried out by the biological responsiveness of cementum, which, in turn, influences the alterations in the distribution and appearance of the cementum varieties on the root surface with time.

Root formation

For an understanding of cementogenesis, the cellular events occurring during root formation are of utmost importance. Root formation commences when the enamel organ has reached its final size and the inner and outer cell layers of the enamel epithelium, which delineate the enamel organ, proliferate from the cervical loop to form Hertwig's epithelial root sheath (59). Continuous cell mitotic activity at the apical termination of Hertwig's root sheath leads to a coronoapical growth of this double cell layer. Its most apical portion, that is, the diaphragm, separates the dental papilla from the dental follicle (Fig. 1a,b). The inner and outer cell layer of Hertwig's root sheath is surrounded by a basement membrane (Fig. 1c,d). Similarly to the reciprocal epithelial-mesenchymal interactions occurring during crown formation (141, 158, 159, 209, 212), cells originating from the peripheral dental papilla differentiate along the internal basement membrane of the diaphragm into odontoblasts (Fig. 1d) (8, 215). Once the first matrix of radicular mantle dentin is formed by the maturing odontoblasts and before the mineralization of the dentin matrix reaches the inner epithelial cells, Hertwig's root sheath becomes discontinuous. Epithelial cell remnants of Hertwig's root sheath persist in the still developing and, later in time, in the aging periodontal ligament at an approximate distance of 30–60 µm remote from the root surface, where they are referred to as the epithelial rests of Malassez (123). Although seen in longitudinal sections as isolated cell clusters surrounded by a basement membrane, which separates them from the surrounding connective tissue, they apparently form a continuous network ensheathing the root at a certain distance (21, 41, 53, 143, 167, 182, 183, 184). Although the number of epithelial rests of Malassez decreases with age (86, 151, 154, 199, 220), cell mitotic activity has also been observed (90, 107, 220). Their existence in the periodontal ligament throughout life implies that they represent more than a

Fig. 1. Light (a) and transmission electron (b–d) micrographs showing the most apical portion of two still growing human premolar roots developed to 50% (a) and 75% (b–d) of their final length. a, b. Hertwig's root sheath (HRS) consists of an inner (IE) and outer epithelial cell layer (OE). Cell mitotic activity at the apical loop (arrow in a points at a dividing cell) results in an apical growth of Hertwig's root sheath, thereby separating the cells of the dental follicle proper (DFP) from the pre-odontoblasts

(pOB) of the dental papilla. Note the striking morphological diversity of the epithelial cells within Hertwig's root sheath in b. Source: Bosshardt & Schroeder (32) with permission from the publisher. c, d. A basement membrane (BM, arrowheads) separates the epithelial cells of Hertwig's root sheath from the surrounding mesenchyme. OB: odontoblasts; PD: predentin. Original magnification: a: ×800; b: ×2200; c, d: ×7700.

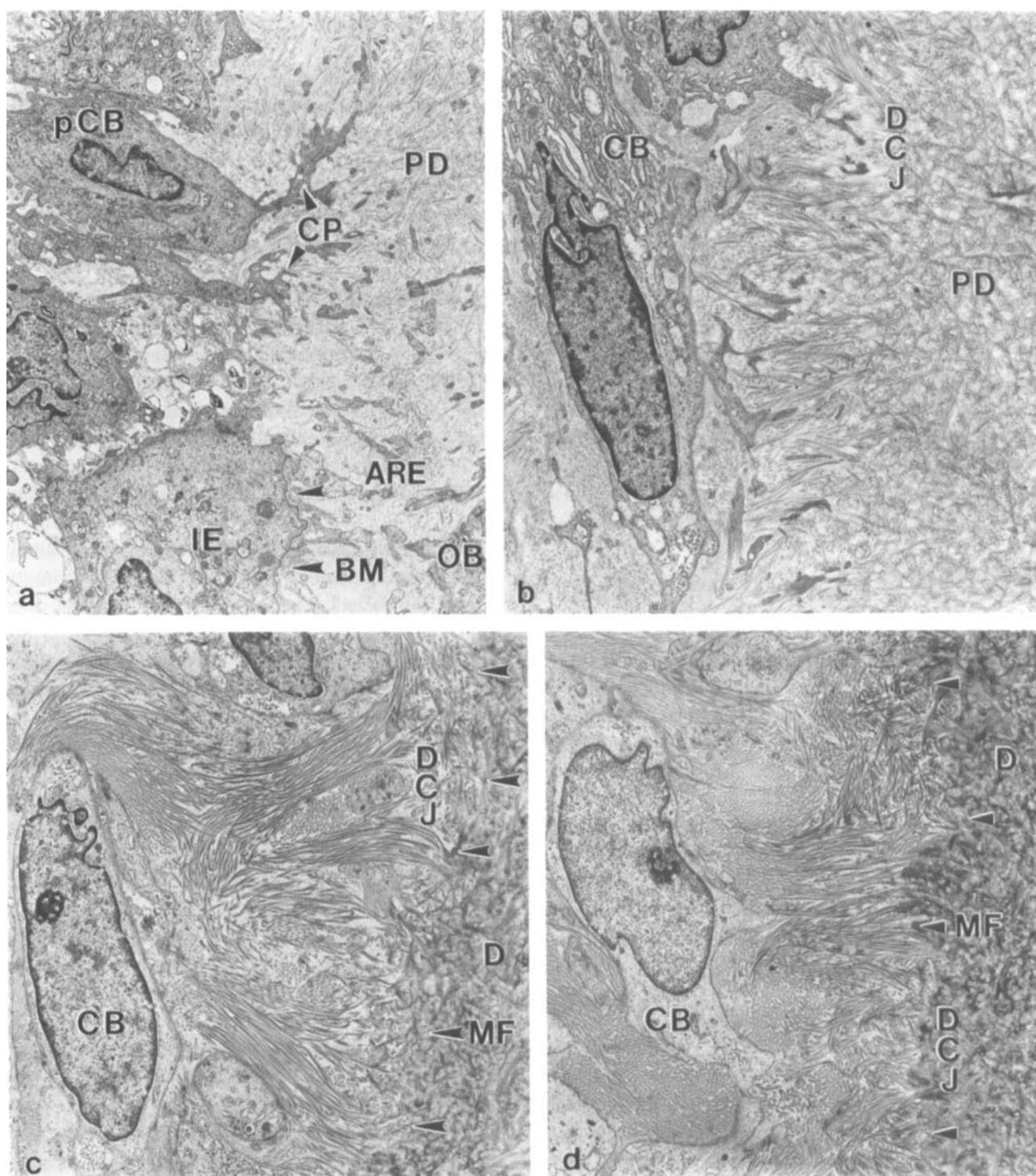


Fig. 2. Transmission electron micrographs illustrating the development of the dentinoocemental junction (DCJ) in the coronal half of a human premolar root developed to 50% of its final length. a. Following the disintegration of Hertwig's epithelial root sheath, cytoplasmic processes (CP) originating from pre-cementoblasts (pCB) penetrate the not yet mineralized matrix of the radicular mantle dentin, that is, the external predentin (PD), immediately coronal to the advancing root edge (ARE). The cell labeled with IE is separated from the newly deposited predentin by a basement membrane (BM) and represents the coronal termination of the intact inner epithelial cell layer of Hert-

wig's root sheath. b. When the pre-cementoblasts differentiate along the external surface of the predentin into cementoblasts (CB), they implant the initial collagen fibrils of the cementum matrix among those of the predentin. In this way, the intimate interdigititation of the collagen fibrils at the dentinoocemental junction is accomplished. Note that there is no intermediate layer interfacing the two different fibril populations. c. The external mineralization front (MF, arrowheads) in dentin (D) is about to reach the fibrillar dentinoocemental junction, when the dentinal matrix is almost completely covered by the cementum matrix. d. When the cementum matrix is established on

merely vestigial structure. Their function, however, continues to be unknown.

Cementoblast origin

The differentiation of cementoblasts from cementoprogenitor cells and the formation of the dentinoocemental junction are temporally and spatially closely related to dentin formation. The initiation of cementogenesis is, therefore, restricted to a narrow band encircling the forming root at its most apical portion. This circular band extends only 200–300 µm coronally from the advancing root edge and shifts in the apical direction while the root elongates (30, 32).

Based on transplantation and ³H-thymidine studies performed in mice (71, 96, 145, 201, 202, 204, 234) and supported by ultrastructural indications for directed cell migration towards the root surface in rat molars (46), it is widely accepted today that the cementoprogenitor cells arise from the dental follicle proper, which is of ectomesenchymal origin (that is, a derivative of the cranial neural crest). However, as pointed out by Thomas & Kollar (214), labeled cementoblasts could also be of epithelial origin, since cells of the enamel organ, which give rise to Hertwig's root sheath, also incorporate ³H-thymidine prior to transplantation. Recent ultrastructural and immunohistochemical studies support, indeed, the hypothesis that the cementoblasts originate from epithelial cells of Hertwig's root sheath when they undergo an epithelial-mesenchymal transformation (33, 38, 121, 214, 216). Such phenotypic transformations have been well documented during embryonic development (93) and include, for instance, neural crest (110), sclerotome (191) and cardiac cushion mesenchyme (126). Another example of phenotypic transformation has recently been shown during palatogenesis when the ectodermal cells of the palatal medial edge epithelium transform into mesenchymal cells (66, 87). This example is of particular interest in analogy to a possible epithelial-mesenchymal transformation of Hertwig's root sheath cells, since the epithelial cells of the palatal midline seam belong to the oral epithelium and the underlying mesenchymal cells are believed to be neural crest in origin.

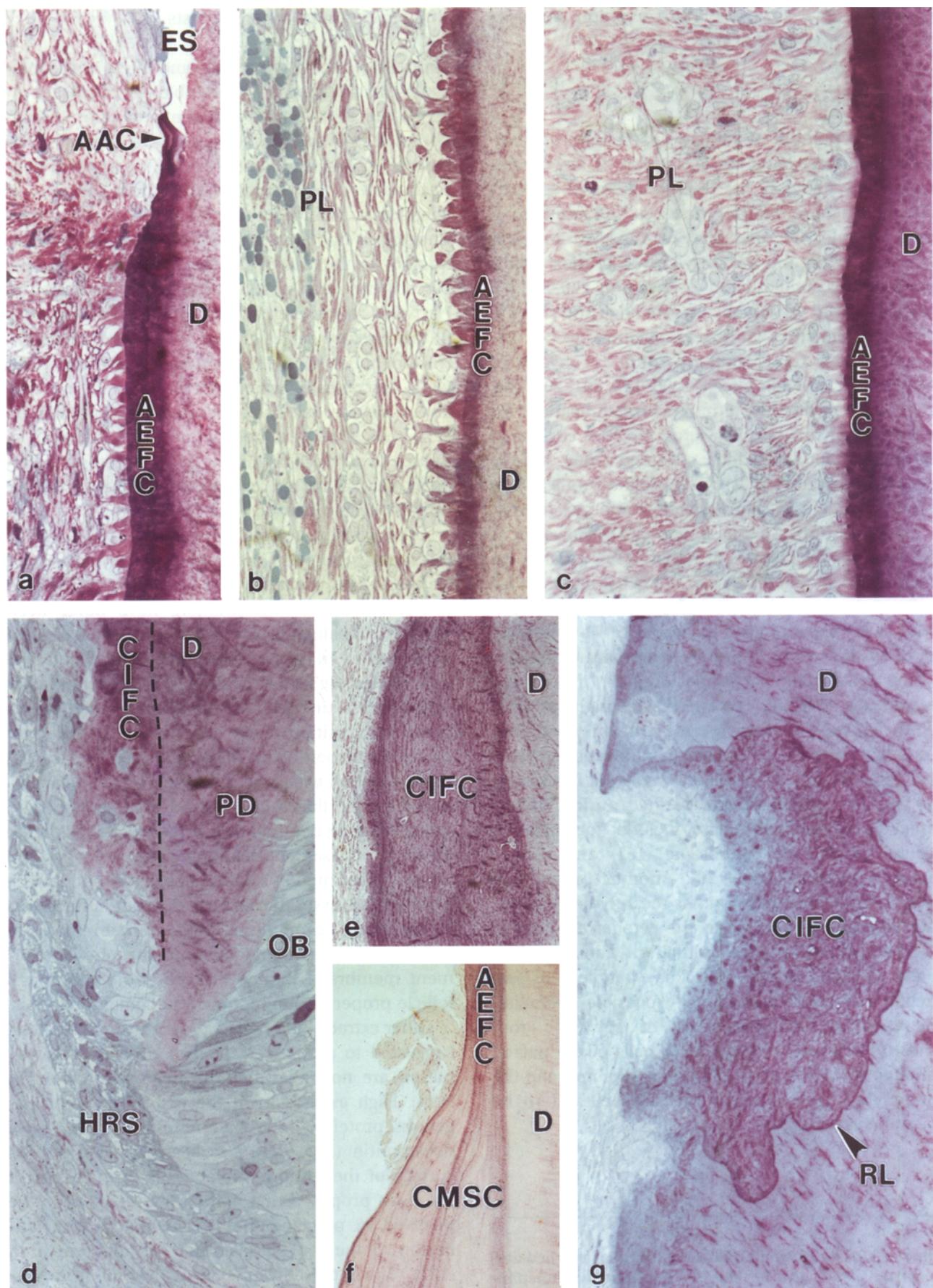
the root surface, the mineralization front has reached and partially passed the fibrillar dentinoocemental junction. OB: odontoblasts. Original magnification: a-d: ×6250.

Differentiation of cementoblasts

For the time being, the nature and origin of the molecules that trigger both a possible cell migration towards the root surface and cementoblast differentiation are not known. However, several possibilities have been suggested and, notably, all of them have been derived from experiments in rats and mice. A chemical substance produced early in rat molar dentinogenesis has been suggested to act as a chemoattractant for the cells of the dental follicle proper (46). Since it has been postulated that the disruption of Hertwig's root sheath appears to be a consequence of this directed cell migration (46), such a chemoattractant must have effect through the intact Hertwig's root sheath, which is composed of two cell layers surrounded by a basement membrane on each side. Although the dentin matrix is known to induce *in vitro* cell migration (168), it seems very unlikely that a matrix component can be effective through such a barrier.

Although repeatedly suggested (96, 113, 143, 165), interactions between the dental follicle proper and Hertwig's root sheath, which would eventually lead to cementoblast differentiation, have never been shown. In analogy to the reciprocal epithelial-mesenchymal interactions between the inner epithelial cells of Hertwig's root sheath and the differentiating odontoblasts, timed modulations in basement membrane composition could possibly act as inductive signals for cementoblast differentiation. Results from recombination experiments using murine molars indicate indeed that a mineralized tissue adhering to the developing dentinal root surface depends on the presence of basement membrane components (120). These experiments could, however, not clarify whether the tissue formed on the root surface was either bone or cementum. In addition, it remains to be determined whether components of the basement membrane induce the cells from the dental follicle proper to differentiate into cementoblasts.

Other extracellular matrix proteins that have been suggested to play a role in cementoblast differentiation are noncollagenous proteins also found in bone. High expression of the two major noncollagenous proteins, bone sialoprotein (122, 193) and osteopontin (192, 194), has been detected on the surface of the forming molar roots in mice, and it has been proposed that bone sialoprotein might be involved in precementoblast chemoattraction, adhesion to the root surface and cell differentiation (122). Since these results were derived from immunohistochemical studies of thick sections, they



do not allow a precise immunolocalization. The sequential appearance of bone sialoprotein and osteopontin during root development and their precise roles remain to be determined. The forming root comprises the initial mineralization of both dentin and cementum, a process that has to be precisely harmonized in time and space. The high expressions of bone sialoprotein and osteopontin are likely to be related to the mineralization process of mantle dentin and cementum, including their interface. The situation at this interfacial site is more complicated in the rodent molar, since an interfacial layer, which appears to be rich in glycoproteins, is frequently interposed between dentin and the cementum proper and may significantly contribute to the high immunoreaction on the root surface (see: The development of the dentinocecal junction). As a matter of fact, there exists no tissue intermediate between cementum and dentin in human teeth, and it remains to be determined whether bone sialoprotein and/or osteopontin expression precede and therefore induce cementoblast differentiation.

Another group of proteins, that are immunologically related to enamel proteins have also been proposed to be involved in early cementogenesis (164, 186, 187). They appear to be a normal feature on the root-analogue surface of rodent incisors and a frequent matrix constituent of the cervical root surface of rodent molars and have also been characterized biochemically from extracts of human cementum (188). The inner epithelial cells of Hertwig's root sheath, which are a derivative of the inner cells of the enamel organ, do, at least for a certain time, maintain the potential for the production and secretion of enamel or enamel-related proteins, as can be clearly seen in the extreme case of enamel drops and pearls occasionally covering the root surface. Although repeatedly suggested, it is still not clear

whether and how these proteins influence the initiation of cementogenesis.

The development of the dentinocecal junction

No matter what the factors are that trigger the differentiation of cementoprogenitor cells into fully activated cementum-producing cells, they differentiate along the newly deposited and not yet mineralized matrix of the radicular mantle dentin into cementoblasts. At the beginning of their maturation on the root surface, they extend numerous tiny cytoplasmic processes into the loosely arranged and not yet mineralized dentinal matrix (Fig. 2a). This enables the cementoblasts to position the initially secreted collagen fibrils of the cementum matrix among those of the dentinal matrix (Fig. 2b), and this crucial step leads eventually to an intimate interdigititation of the two different fibril populations (Fig. 2c) (30, 32). The mineralization of the outermost layer of the dentin matrix, that is, the mantle dentin, appears to be delayed and the mineralization front in dentin does reach the future dentinocecal junction, not before the implantation of the cementum matrix is established and the dentinal matrix is completely covered with the collagen fibrils of cementum (Fig. 2d). The term intermediate cementum appears repeatedly in the literature and in textbooks. Although originally described for the apical portion of human teeth (19), there exists no interfacial layer between dentin and cementum in human teeth (38). In rodent molars and incisors, on the other hand, an intermediate layer has frequently been observed, particularly between acellular extrinsic fiber cementum and dentin (112, 144, 146, 172, 225, 226). This layer appears to be rich in glycoproteins but contains sparsely distributed collagen fibrils (225, 226). The origin of this layer is still controversial. It is either

Fig. 3. Light micrographs showing the different cementum varieties found on human teeth. **a.** The acellular afibrillar cementum (AAC) shown in this micrograph overlaps the enamel on the crown (ES; enamel space), apposes to the dentin (D) and merges into acellular extrinsic fiber cementum (AEFC). **b, c.** The acellular extrinsic fiber cementum is usually found on the cervical half of the root. The extrinsic matrix fibers remain short during the pre-functional development (b) but become continuous with the principal fibers of the periodontal ligament (PL) when the tooth is in functional occlusion (c). **d, e.** Cellular intrinsic fiber cementum (CIFC) during its initial attachment to the root dentin at the apical portion of a human premolar root developed to 75% of its final length (d)

and at a more advanced stage of formation (e). **f.** Later in development, the cervically located acellular extrinsic fiber cementum splits into several layers which interface with the more apically located cellular intrinsic fiber cementum layers to form the cellular mixed stratified cementum (CMSC). Source: Schroeder (169). **g.** Cellular intrinsic fiber cementum is also found as a reparative tissue filling resorptive defects of the root. Note the reversal line (RL) demarcating the junction between old and new tissues. Source: Bosshardt (35) with permission from the publisher. HRS: Hertwig's root sheath; OB: odontoblasts; PD: predentin. Original magnification: **a-d:** $\times 440$; **e:** $\times 130$; **f:** $\times 80$; **g:** $\times 230$.

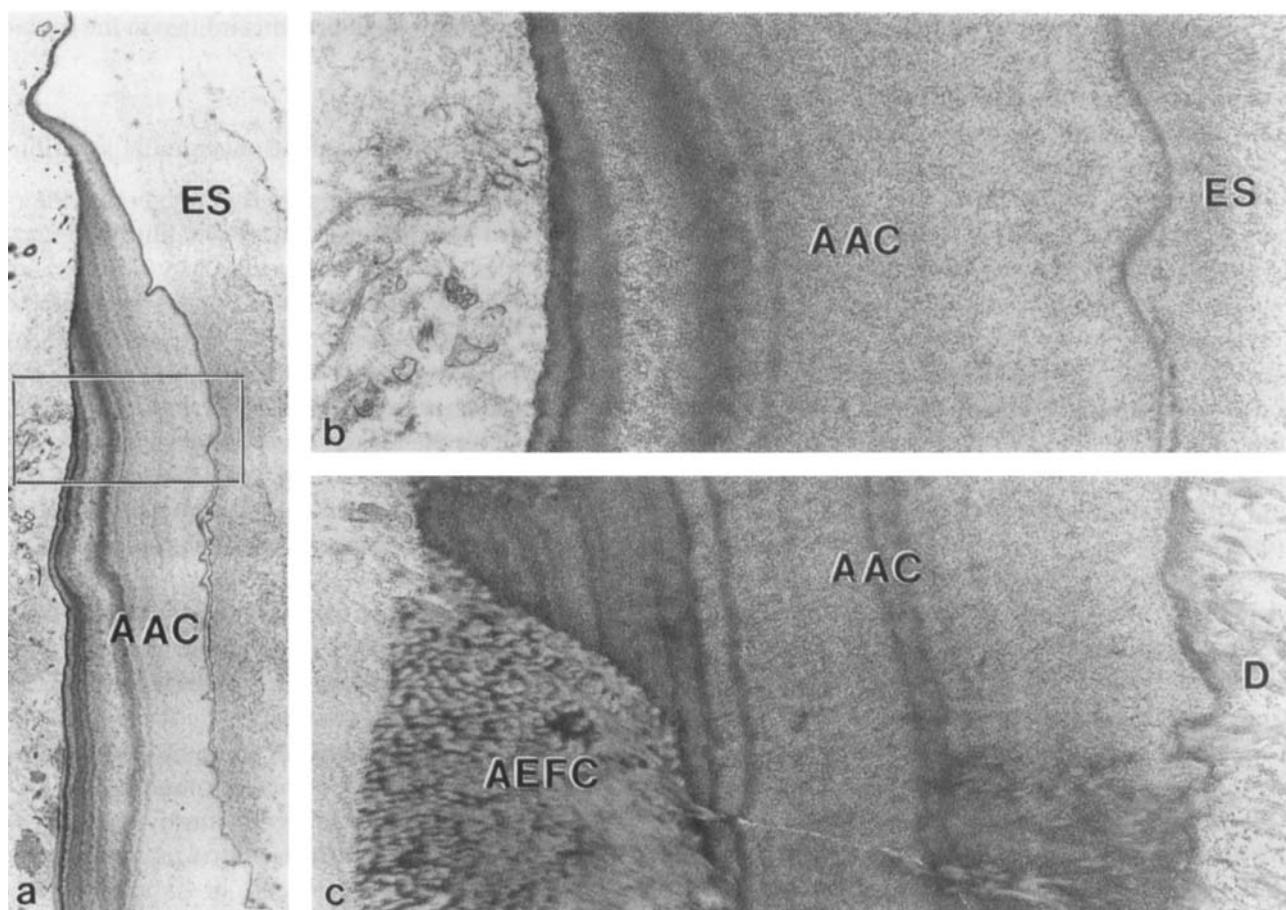


Fig. 4. Transmission electron micrographs of acellular afibrillar cementum (AAC) at the cementoenamel junction. The area outlined in a corresponds to b. a. The acellular afibrillar cementum apposes to the enamel space (ES), which contains residual enamel proteins, and tapers in the coronal direction. b. The acellular afibrillar ce-

mentum is characterized by numerous incremental layers with varying electron density and texture. c. In the apical direction, the acellular afibrillar cementum abuts against the dentin (D) and the acellular extrinsic fiber cementum (AEFC). Original magnification: a: $\times 3200$; b, c: $\times 13,000$.

formed by cementoblasts (47, 167, 225) or by the inner epithelial root sheath cells (144, 146, 161, 188, 194).

The cementum varieties: their location, formation, structure and function

Human teeth have three fundamentally different varieties of cementum. The location of these varieties shows tooth-type specific distribution patterns but may also vary along and around the surface of the same tooth. The following general rule applies to human teeth: acellular afibrillar cementum covers minor areas of the enamel, particularly at and along the cementoenamel junction (Fig. 3a). Acellular extrinsic fiber cementum is mainly found on the cervi-

cal and middle root portions (Fig. 3a-c). On front teeth, it may also cover part of the apical root portion, since the apical extension of acellular extrinsic fiber cementum on the root surface increases from posterior to anterior teeth. Cellular intrinsic fiber cementum is initially deposited on root surface areas where no acellular extrinsic fiber cementum has been laid down on the dentin (Fig. 3d). This may occur in the furcations and on the apical root portions (Fig. 3d,e). Cellular intrinsic fiber cementum may overgrow layers of acellular extrinsic fiber cementum, and acellular extrinsic fiber cementum, in turn, can overlay cellular intrinsic fiber cementum. The so formed mingled cementum is called cellular mixed stratified cementum and is confined to the apical root portions and to the furcations (Fig. 3f). In addition, mainly cellular intrinsic fiber cementum participates in the repair process of previously resorbed roots (Fig. 3g).

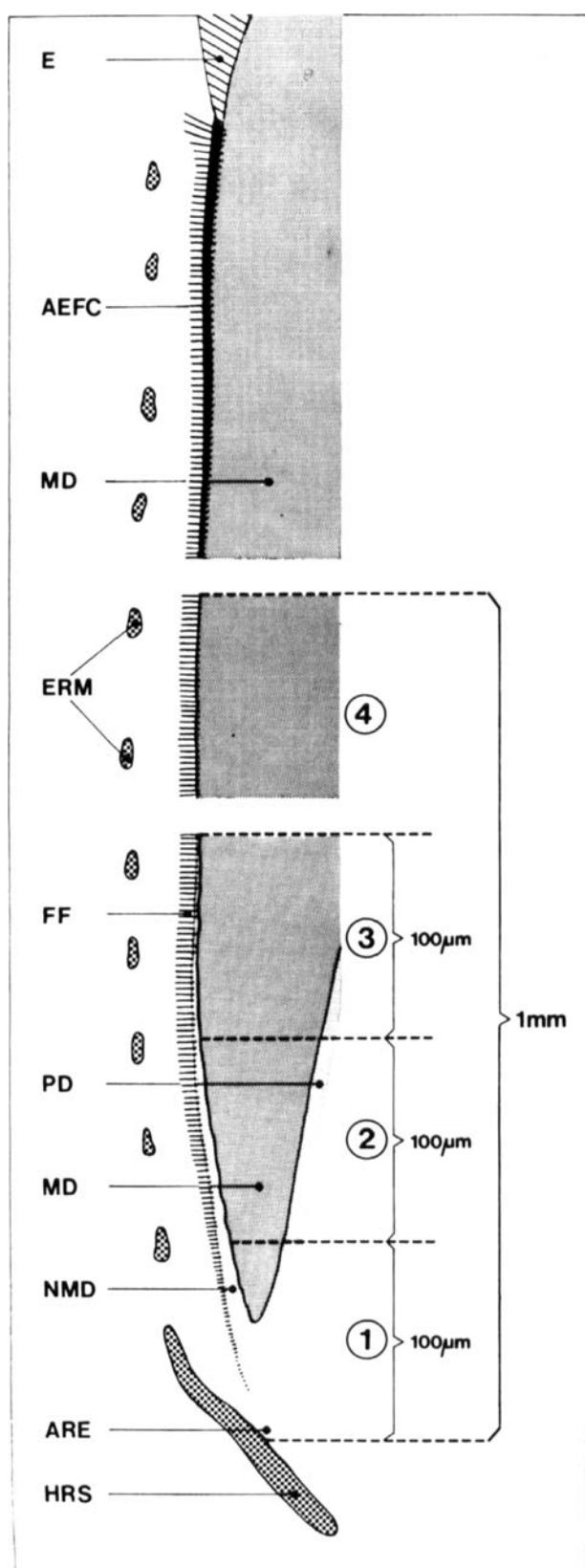


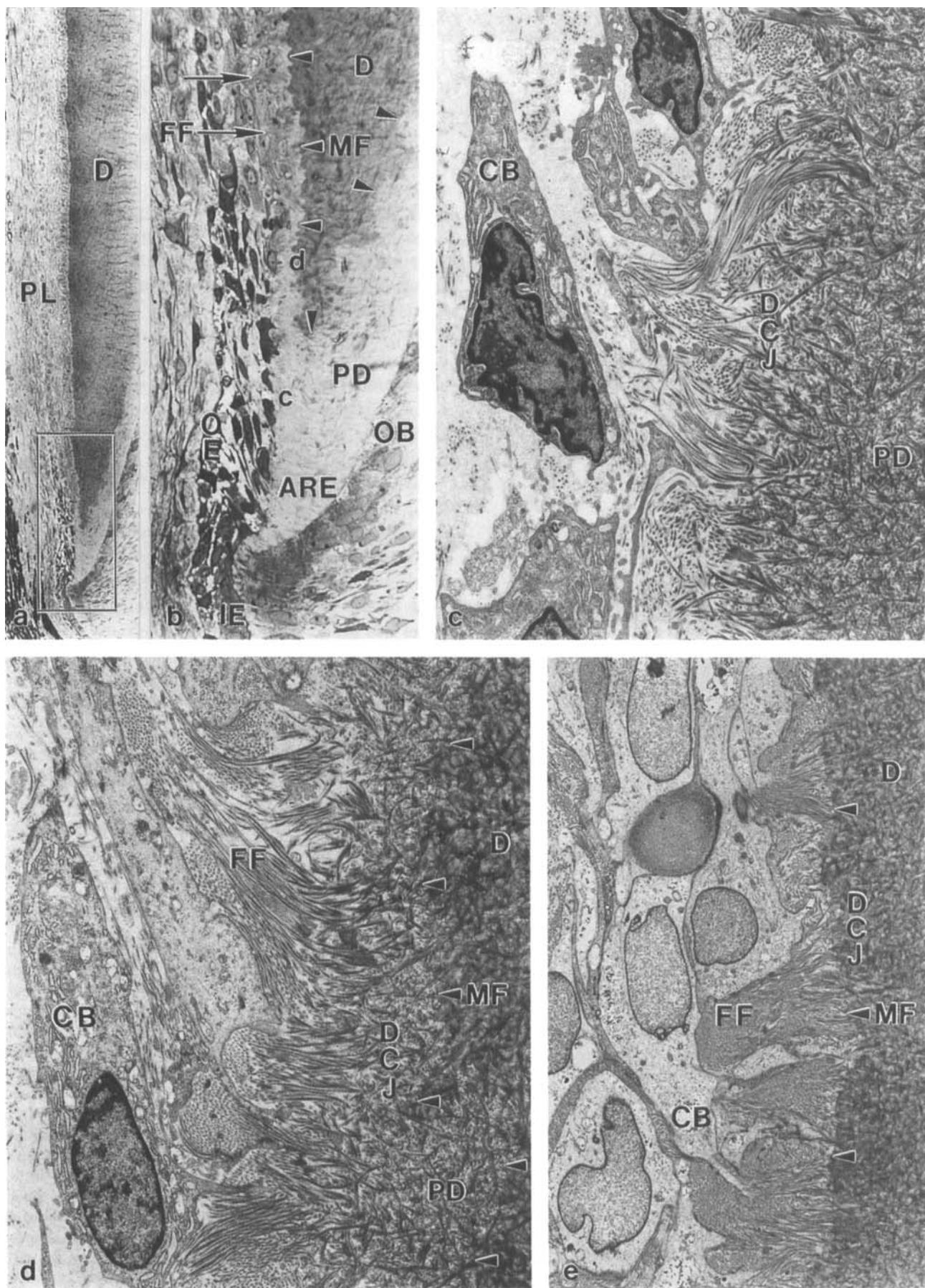
Fig. 5. Schematic diagram depicting the gradual development of acellular extrinsic fiber cementum during its pre-functional genesis along a human premolar root developed to about 50% of its final length. 1. Attachment of the acellular extrinsic fiber cementum matrix to the not

Acellular afibrillar cementum

The acellular afibrillar cementum consists of a mineralized matrix, which appears similar to the interfibrillar matrix of acellular extrinsic fiber cementum, but contains neither collagen fibrils nor embedded cells. The lack of collagen fibrils indicates that this cementum variety has no function in tooth attachment. The acellular afibrillar cementum can be identified by light and electron microscopy. Under the light microscope, the acellular afibrillar cementum stands out by its basophilia and its more or less uniform appearance (Fig. 3a). In the electron microscope, however, the structure of acellular afibrillar cementum is less homogeneous (Fig. 4a-c). A variable number of layers with varying electron density and different texture, which can be either granular or reticular, give the acellular afibrillar cementum a multifarious appearance.

Acellular afibrillar cementum is deposited as isolated patches over minor areas of enamel and dentin. Cementum islands represent isolated patches of acellular afibrillar cementum deposited on the enamel over small areas of the dental crown just coronal to the cementoenamel junction. Cementum spurs are found around the cementoenamel junction, where they cover minor areas of the enamel and the adjacent dentin of the root (Fig. 3a, 4a). Cementum spurs may be covered by acellular extrinsic fiber cementum and/or by junctional epithelium. The areas and location of acellular afibrillar cementum vary from tooth to tooth and along the cementoenamel junction of the same tooth. This unpredictable distribution pattern indicates that acel-

yet mineralized matrix of the radicular mantle dentin (NMD). The latter is continuous with the pulpal predentin layer (PD) and tapers in the coronal direction. Note that Hertwig's epithelial root sheath (HRS) is associated with the nonmineralized matrix of the radicular mantle dentin for an extremely short distance at the advancing root edge (ARE); 2. Establishment of the acellular extrinsic fiber cementum matrix on the root surface in form of a short collagenous fiber fringe (FF); 3. When the fiber fringe has attained its maximum numerical density, a cell-fiber fringe meshwork establishes on the root surface. 1-3. The external mineralization front in dentin (mineralized dentin, MD) is gradually approaching the base of the fiber fringe implantation on the root surface. 4. The external mineralization front in dentin has reached the future dentinocemental junction. E: enamel; ERM: epithelial cell rests of Malassez. Source: Bosshardt & Schroeder (30) with permission from the publisher.



lular afibrillar cementum formation is likely to be a developmental curiosity that deviates from the norm rather than an indispensable tissue.

The cells responsible for the formation of acellular afibrillar cementum have still not been determined with precision. Its formation commences at the end of enamel maturation and continues for an unknown period of time. It is believed that connective tissue cells are responsible for the acellular afibrillar cementum formation when they come in contact with the enamel surface (166, 167). To make this possible, cells of the reduced enamel epithelium must be lost or detached from the enamel. On the other hand, it cannot be completely ruled out that acellular afibrillar cementum is an epithelial product initially produced when the ameloblasts transform into the reduced enamel epithelium and when the cells of the inner enamel epithelium are about to generate the inner cells of Hertwig's root sheath. *In vitro* experiments showed that calcified layers, which morphologically resembled acellular afibrillar cementum, formed around demineralized dentin slices immersed in serum-containing culture medium supplemented with alkaline phosphatase and an organic source for phosphate such as the monophosphate ester β -glycerophosphate (17). The notion that such acellular afibrillar cementum-like layers formed also in the absence of cells suggests that this matrix represents a co-precipitate of medium- or serum-derived components and mineral. However, the enzyme alkaline phosphatase is required for mineralization to occur. In the *in vivo* situation, this enzyme is particularly associated with periodontal ligament cells in the vicinity to bone and cementum (88).

Acellular extrinsic fiber cementum

The acellular extrinsic fiber cementum is usually confined to the coronal half of the root. Its formation commences therefore shortly after crown formation is completed and always before cellular intrinsic fiber cementum starts to form on more apical root portions. The gradual development of acellular extrinsic fiber cementum can be followed along the forming root (Fig. 5, 6a,b) (30, 31, 33). The cementoblasts producing acellular extrinsic fiber cementum commence their cell differentiation in closest proximity to the advancing root edge. This may occur only about 20 to 30 μm coronal to the first deposited dentinal matrix. These cells resemble fibroblasts, reveal a well-developed rough endoplasmic reticulum, are interconnected by desmosome-like junctions and commence to produce and attach the collagenous cementum matrix as close as 50 μm coronal to the root edge (Fig. 6c). Further collagen deposition results in a complete covering of the not yet mineralized dentinal matrix along the next 100 μm of the root surface (Fig. 6d). About 200 to 300 μm coronal to the advancing root edge, the initial acellular extrinsic fiber cementum matrix is established on the dentinal matrix (Fig. 6e). The acellular extrinsic fiber cementum matrix consists of a dense fringe of short collagenous fibers that are implanted into the dentinal matrix and oriented about perpendicularly to the root surface (Fig. 6e, 7a). The outwardly progressing mineralization front in dentin (Fig. 6d) does not reach the future dentinocemental junction until the collagenous interdigitation of the two fibril populations is established (Fig. 6e). Since the mineralization of the dentinal matrix commences about

Fig. 6. Light (a, b) and transmission electron (c–e) micrographs illustrating the initial attachment and gradual development of the acellular extrinsic fiber cementum matrix (that is, fiber fringe; FF) along the apical portion of a human premolar root developed to about 50% of its final length. The area outlined in a corresponds to b. The positions of the micrographs c and d are indicated in b by the corresponding letters. a, b. Immediately coronal to the disintegration of Hertwig's epithelial root sheath (OE indicates the outer and IE the inner epithelial cell layer), intensely stained cells distend the space between the root surface and the outer epithelial cell layer. More coronally, the cells do no more reveal the same intense staining and a fiber fringe (arrows) appears on the root surface. The onset of dentin mineralization is seen 100 μm coronal to the advancing root edge (ARE) and somewhat away from the external root surface (lowest arrowhead). In the cor-

onal direction, the external mineralization front (MF, arrowheads pointing to the left) in dentin (D) is gradually approaching the root surface. c. The cementoblasts (CB) on the root surface commence to implant the fiber fringe among the collagenous matrix fibrils of predentin (PD) at the future dentinocemental junction (DCJ). Source: Bosshardt (35) with permission from the publisher. d. When the fiber fringe is covering almost the entire predentin, the external mineralization front in dentin is about to reach the fibrillar dentinocemental junction. Source: Bosshardt & Schroeder (30) with permission from the publisher. e. The established fiber fringe continues for a short distance into the space of the immature periodontal ligament (PL). The mineralization front has completely reached and partly passed the fibrillar dentinocemental junction. OB: odontoblasts. Original magnification: a: $\times 130$; b: $\times 440$; c, d: $\times 6700$; e: $\times 4400$.

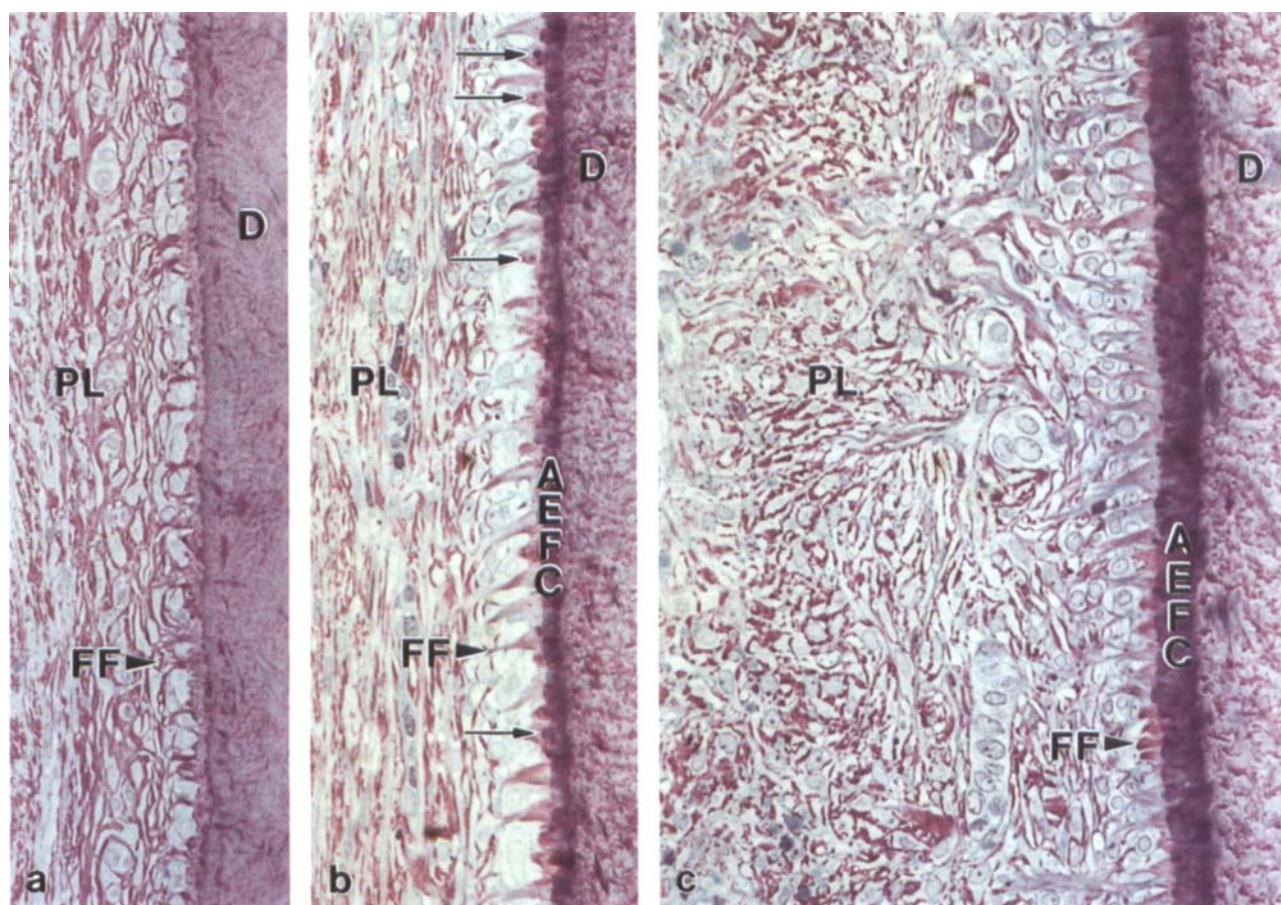


Fig. 7. Light micrographs demonstrating the growth of acellular extrinsic fiber cementum (AEFC) on human premolar roots. **a.** The acellular extrinsic fiber cementum matrix consists of short fringe fibers (FF) that emerge from the root surface and appose to a fibrocellular meshwork occupying the space of the immature periodontal ligament (PL). A cementum layer is not yet visible. **b.** Mineralization of the fiber fringe, which is indicated by round, basophilic dots within the fiber fringe base (arrows), proceeds very slowly. This acellular extrinsic fiber cementum

layer has developed over approximately 2 years. Note that the fiber fringe is still short and apposes to a fibrocellular meshwork running broadly parallel to the root surface. **c.** This 15-µm-thick acellular extrinsic fiber cementum layer has developed over approximately 5 years. Note the change in the orientation of the periodontal ligament fibers and the continuation of some of the fringe fibers with the developing principal periodontal ligament fibers. **D:** dentin. Original magnification: a-c: $\times 440$.

100 µm coronal to the advancing root edge and somewhat underneath the root surface (Fig. 6b), the mineralization of the mantle dentin seems apparently to be delayed. With the onset of cementum mineralization, the acellular extrinsic fiber cementum commences to grow in thickness (Fig. 7b,c). This growth is extremely slow but quite constant (Fig. 10a) (33, 179). The extrinsic fibers remain short until the tooth is about to reach the occlusal level (Fig. 7c). On cervical root surfaces of human premolars, the prefunctional acellular extrinsic fiber cementum development may last 5 years or more, that is, until acellular extrinsic fiber cementum has reached a thickness of about 15 µm (Fig. 7c) (31, 33, 38). How the short fiber fringe becomes elongated and eventually continuous with the principal peri-

odontal ligament fibers is still an open question. The acellular extrinsic fiber cementum continues to grow as long as the adjacent periodontal ligament remains undisturbed. The extraordinarily high numerical density of fibers inserting into acellular extrinsic fiber cementum (approximately 30,000/mm²; (167)) is a reflection of the significant function of this cementum variety for tooth anchorage to the surrounding bone. Due to posteruptive tooth movements, changes can occur in the direction of the Sharpey's fibers. These changes are accentuated by individual acellular extrinsic fiber cementum layers interfaced by growth lines, also known as resting or incremental lines. Although acellular extrinsic fiber cementum is a quite constantly growing tissue, these lines appear to represent the periodic deposition of

cementum layers in frequent association with an abrupt change in the direction of Sharpey's fibers. Moreover, as can be deduced from faster growth rates on distal (4.3 $\mu\text{m}/\text{year}$) than on mesial (1.4 $\mu\text{m}/\text{year}$) root surfaces (56), acellular extrinsic fiber cementum has the potential to adapt to functionally dictated alterations such as mesial tooth drift.

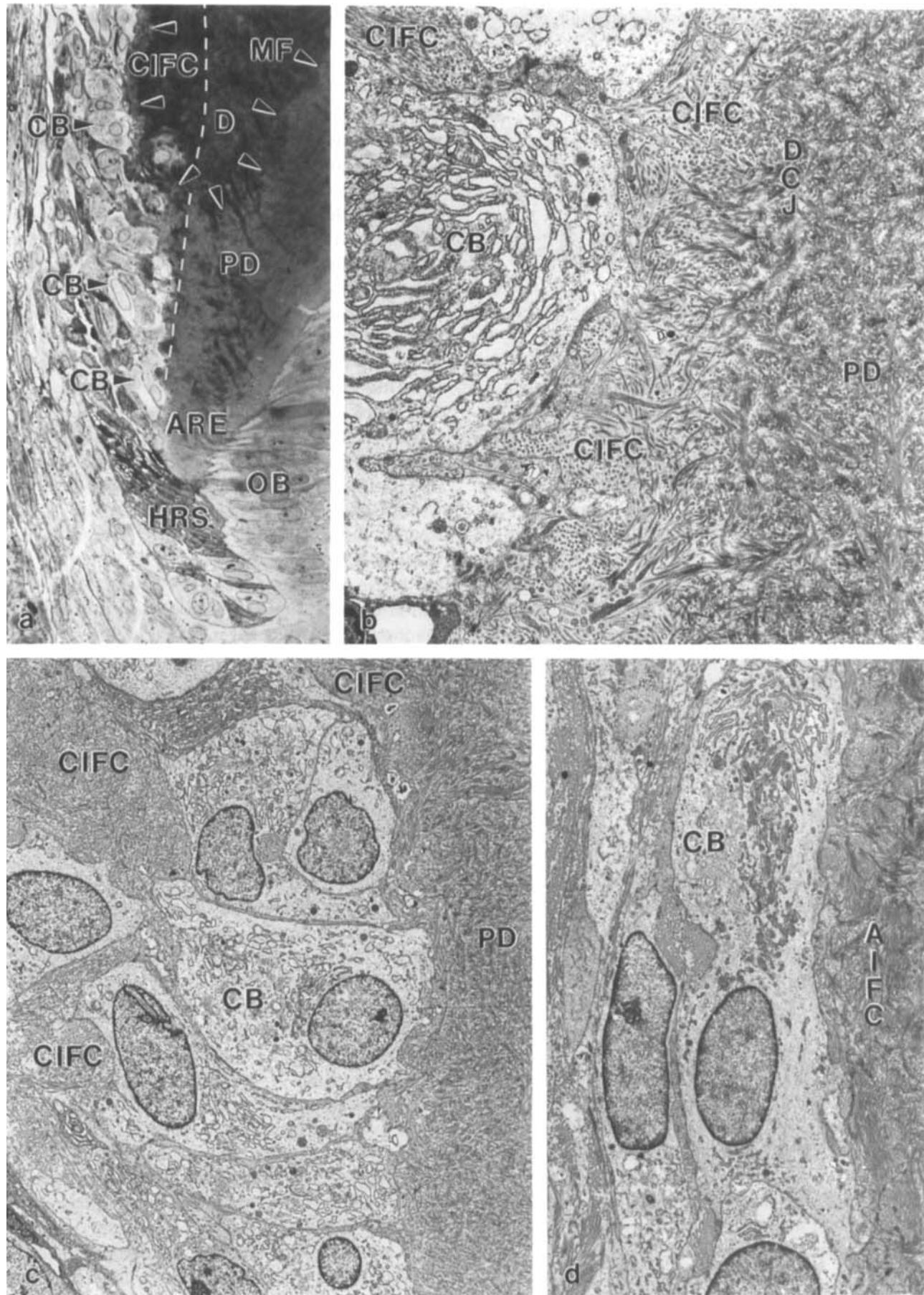
Cellular and acellular intrinsic fiber cementum

Although the intrinsic cementum alone has no immediate function in tooth attachment, its important role as an adaptive tissue (167) that brings and maintains the tooth in its proper position should not be underestimated. In addition, only cellular intrinsic fiber cementum can repair a resorptive defect of the root in a reasonable time due to its capacity to grow much faster than any other known cementum type. Functional stimuli, that is, the force generated by tooth contact and mastication, are widely held responsible for the onset and appositional growth of cellular intrinsic fiber cementum. This assumption probably originates from observations showing that the genesis of this cementum variety coincides with the first occlusal tooth contact (51, 58, 95, 140) and that functioning teeth appear to have thicker cementum layers than teeth which are not in function (84, 99). Several observations, however, are not in keeping with this concept. As already suggested by Kronfeld (105, 106) and Kellner (104), mastication is apparently not a prerequisite for cellular intrinsic fiber cementum genesis, since i) the furcations of human teeth are covered with thick cementum layers before they emerge into the oral cavity (105), ii) impacted and erupted teeth without antagonists appear to have thicker cementum layers than fully erupted and functioning teeth (9, 103, 104, 106, 181) and iii) over-compression of the periodontal ligament causes root resorption. Thus, it seems that the initiation of cellular intrinsic fiber cementum genesis does not depend on stimuli transmitted by masticatory forces and that influence by pressure may reduce the rate of matrix deposition.

As for acellular extrinsic fiber cementum, the initiation of cellular intrinsic fiber cementum genesis on the forming root commences in closest proximity to the advancing root edge (32, 34). Pre-cementoblasts differentiate along the not yet mineralized dentinal matrix into large, basophilic cells (Fig. 8a). They first project numerous cytoplasmic processes into the loose dentinal matrix and immediately commence to implant the initial collagen fibrils

among those of the dentinal matrix (Fig. 8b). Additional cementoblasts, which are remote from the dentinal surface, deposit their cementum matrix at various locations around themselves (Fig. 8c). This multipolar and fast matrix deposition, which occurs in the space between deviating epithelial cells of Hertwig's root sheath and the dentinal surface (Fig. 8a), appears to be the reason for the incorporation of some of the cementoblasts (32, 67, 146). The cells entrapped in the mineralized cementum are referred to as cementocytes and occupy lacunae, which are interconnected through canaliculi (see: Physiological activity of cementocytes) (Fig. 16, 17). The cementoblasts attain their full synthetic activity approximately 100 μm coronal to the advancing root edge (Fig. 9a). They are large, basophilic cells with an euchromatin-rich nucleus and an abundant endoplasmic reticulum. The rapid matrix deposition slows down soon, and further collagen matrix is deposited in a more unipolar mode of secretion (Fig. 9b-e). In rare cases, the intrinsic cementum is formed in an extremely unipolar mode of matrix deposition and completely lacks cementocytes (Fig. 8d, 9d,e). This particular tissue consists of densely bundled collagen fibrils and is named acellular intrinsic fiber cementum (29). The collagen fibrils produced during the fast, multipolar cellular intrinsic fiber cementum initiation show a more random orientation than those of the subsequently deposited matrix. Therefore, the bulk of the intrinsic collagen fibrils form discrete bundles oriented mainly parallel to the root surface (Fig. 11a). Polarized light microscopic investigations in deciduous (102), permanent (163) and impacted human teeth (99) suggest that these intrinsic fibers are arranged in an orderly fashion around the root (Fig. 11b).

Unlike in rat molars (227, 228), initial cellular intrinsic fiber cementum deposition on human tooth roots is not necessarily associated with the simultaneous formation of extrinsic fibers, that is, the future Sharpey's fibers. In humans, the extrinsic fibers are oriented about perpendicularly to the root surface and traverse the intrinsic cementum variety either sporadically or densely arrayed in parallel. Although the numerical density of these highly aggregated extrinsic fibers may be distinctly less than in pure acellular extrinsic fiber cementum (167), they are considered as the matrix of acellular extrinsic fiber cementum that intermingles or alternates with the intrinsic fibers. This mixed cementum is referred to as cellular mixed stratified cementum. When the extrinsic fibers are continuous with the functionally oriented principal fibers of the periodontal ligament,



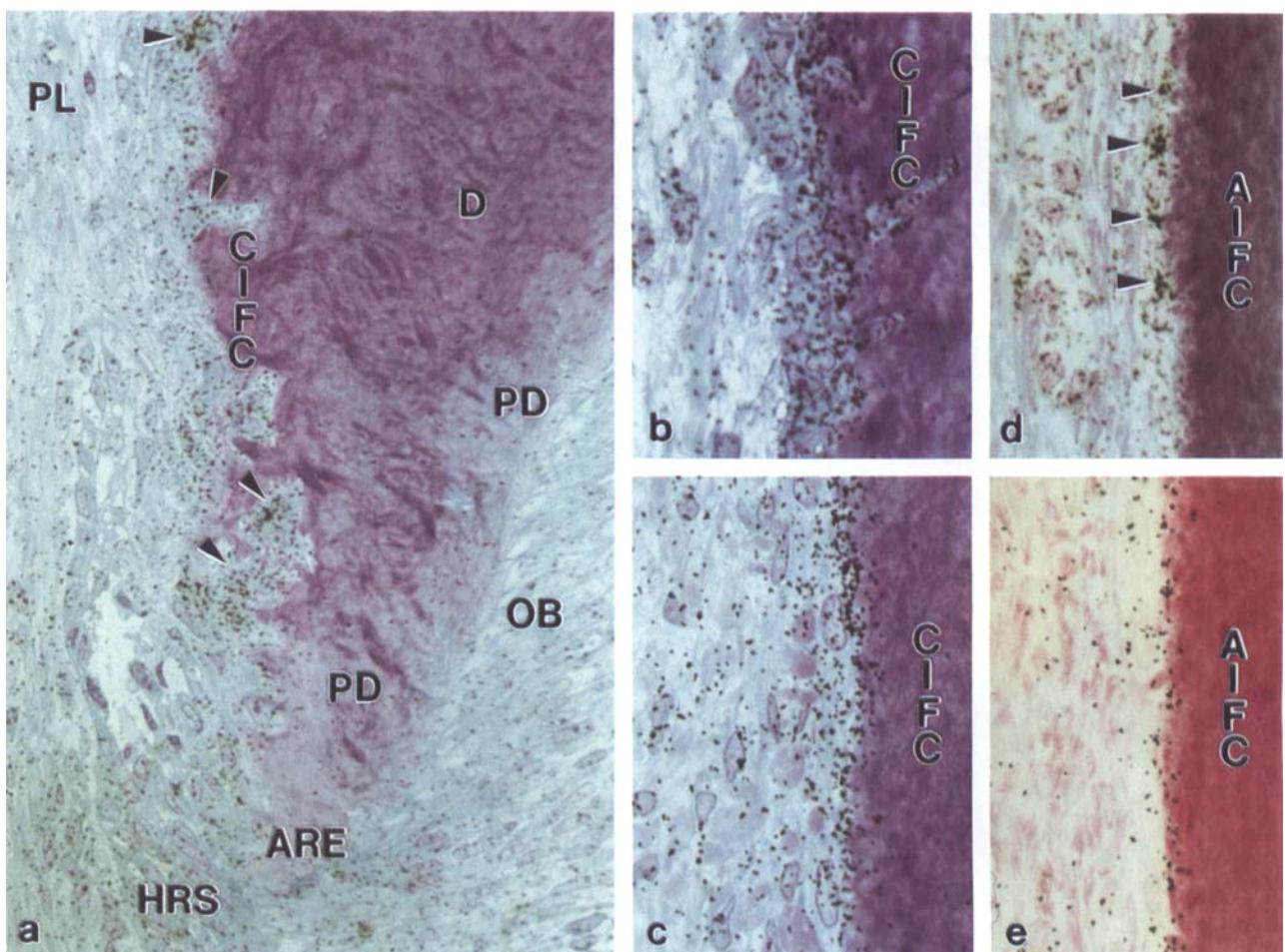


Fig. 9. Light microscopic radioautographs demonstrating the prefunctional deposition of cellular intrinsic fiber cementum (CIFC) and acellular intrinsic fiber cementum (AIFC) close to (a) and more remote from the advancing root edge (ARE) (b–e). These human premolars with roots developed to 75–95% of their final length were *in vitro* pulse-labeled with ^3H -proline for 15 min followed by a chase incubation for 45 min (a, d), 1 h 45 min (b), 4 h 45 min (e), and 23 h 45 min (c). Silver grains are, first, local-

ized in clusters over the paranuclear cytoplasm of cementoblasts (arrowheads in a, d), appear, later, over the peripheral cytoplasm of cementoblasts and the subjacent cementum matrix (b), and eventually cover the peripheral cementum matrix (c, e). Source: Bosshardt & Schroeder (34) with permission from the publisher. D: dentin; HRS: Hertwig's epithelial root sheath; OB: odontoblasts; PD: predentin; PL: periodontal ligament. Original magnification: a: $\times 440$; b–e: $\times 700$.

Fig. 8. Light (a) and transmission electron (b–d) micrographs illustrating the genesis of cellular intrinsic fiber cementum (CIFC) and acellular intrinsic fiber cementum (AIFC) at the advancing root edge (ARE) (a–c) and on the established cementum layer (d), respectively. Human premolar roots developed to 75% of their final length. a. At the advancing root edge, the inner cells of Hertwig's epithelial root sheath (HRS) cover not more than 20 μm of the newly deposited predentin (PD), whereas the outer cell layer of Hertwig's epithelial root sheath continues for about 150 μm in the coronal direction. Cementoblasts (CB) distend the space between the root surface and the deviating outer epithelial cell layer. The cellular intrinsic fiber cementum thickness increases rapidly in the coronal direction. Arrowheads point at the mineralization front (MF) of both dentin (D) and cellular intrinsic fiber ce-

mentum. The interrupted line corresponds to the dentinocecal junction. b. Large cementoblasts commence to produce and attach the initial cellular intrinsic fiber cementum matrix to the external predentin matrix about 50 μm coronal to the advancing root edge. Note that there is no intermediate layer between the two matrices at the dentinocecal junction (DCJ). c. The cellular intrinsic fiber cementum matrix is also produced away from the predentin among the cementoblasts. d. Further matrix is deposited by cementoblasts, which form a continuous cell layer when they are associated with acellular intrinsic fiber cementum. Sources: a–c: Bosshardt & Schroeder (32); d: Bosshardt & Schroeder (34) with permission from the publisher. OB: odontoblasts. Original magnification: a: $\times 440$; b: $\times 7700$; c: $\times 3200$; d: $\times 4400$.

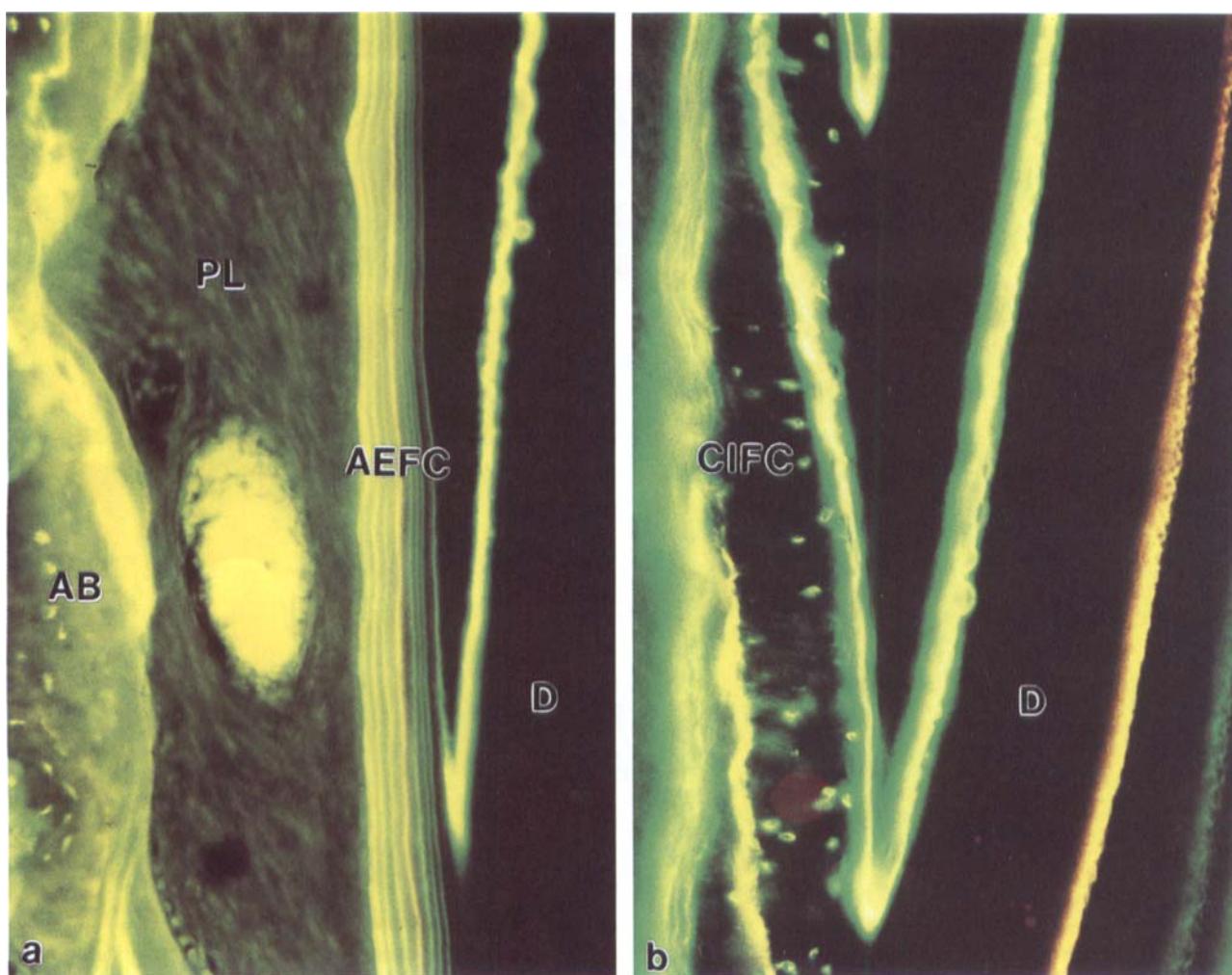


Fig. 10. Fluorescence lines in cementum, dentin (D) and alveolar bone (AB) of a mandibular second deciduous molar of a *Macaca fascicularis* monkey. The animal received sequential injections of calcein (green lines) and xylol orange (orange lines) on average every 33 days. The two fluorochromes bind to sites of ongoing mineralization and produce clear fluorescence lines. Note the difference in the labeling pattern between acellular extrinsic fiber ce-

mentum (AEFC) and cellular intrinsic fiber cementum (CIFC). The labeling pattern and the difference in the growth rates between acellular extrinsic fiber cementum and cellular intrinsic fiber cementum are basically the same in human teeth, albeit acellular extrinsic fiber cementum and cellular intrinsic fiber cementum grow more slowly. Source: Bosshardt et al. (28). PL: periodontal ligament. Original magnification: a, b: $\times 250$.

they can be regarded as Sharpey's fibers. As layers of acellular extrinsic fiber cementum and cellular and acellular intrinsic fiber cementum develop unpredictably in time, space and thickness (167, 169), particular root surface areas covered with cellular mixed stratified cementum may temporarily remain unsupported by periodontal fibers.

Like pure acellular extrinsic fiber cementum on the coronal half of the root, cellular mixed stratified cementum increases in thickness throughout life (236). However, the unpredictable dynamics of the tissue alternations renders growth rate determination much more difficult for cellular mixed stratified cementum. Nevertheless, the initial cel-

lar intrinsic fiber cementum growth has been measured in deciduous teeth of a non-human primate (28). It may be up to 30-fold faster than the more regular acellular extrinsic fiber cementum deposition (Fig. 10b). The dynamic tissue alternations and the variations in growth rates are reflected by a tissue layering of cellular mixed stratified cementum with layers of acellular extrinsic fiber cementum and incremental lines (that is, growth lines or resting lines) interfacing layers of cellular and acellular intrinsic fiber cementum. The patch-wise deposition of cellular mixed stratified cementum results in great circumferential variations in cementum thickness and reflects periods



Fig. 11. Polarized light photomicrographs of cementum. a. In a section made parallel to the root surface, intrinsic collagen fibers in cellular intrinsic fiber cementum appear as wide sheets of alternating directions. b. Cementum on

an impacted third molar. Collagen fibers are almost exclusively of the intrinsic type arranged in an orderly fashion parallel to the root surface (arrow). D: dentin. Original magnification $\times 130$.

of accelerated deposition of cellular intrinsic fiber cementum, which are probably due to functional demands in order to reposition the tooth when it is shifting in its bony socket during its post-eruptive tooth movements.

Mineralization

Mineralization begins in the depth of pre cementum. Fine hydroxyapatite crystals are deposited, first between and, secondly, within the collagen fibrils (Fig. 12) by a process which, apparently, is identical to the mineralization of bone tissue. Zander & Hürzeler (236) examined the thickness of cementum on human teeth extracted from individuals of varying ages. From their data it can be calculated that the mean, linear rate of cementum deposition on single-rooted teeth is about 3 μm per year, but varying greatly with tooth type, root surface area, and type of cementum being formed. A similar rate has been found for acellular extrinsic fiber cementum in young human premolars (33) and in nonfunctioning, impacted teeth (9). Cementum forms at a much higher rate in deciduous teeth of the *Macaca fascicularis* monkey (0.1 $\mu\text{m}/\text{day}$ for acellular extrinsic fiber cementum and

up to 3.1 $\mu\text{m}/\text{day}$ for cellular intrinsic fiber cementum (28)), and, presumably, in most other mammals.

The width of the pre cementum layer in the human is about 3–5 μm (74, 173). The mineral crystals reach mature size similar to mineral crystals in bone and dentin within 1 to 4 μm from the calcification front (173). Thus, it appears that the processes of establishing the appropriate condition for crystallization as well as for the appositional growth of the individual crystals in cementum normally are extremely slow and extend over a period of several months.

The distribution of mineral within the mature tissue shows a great deal of variability. Studies by microradiography, using a technique that basically reflects the distribution of calcium in the tissue, have shown that cellular mixed stratified cementum generally has a lower mineral content than acellular extrinsic fiber cementum. This difference can in part be accounted for by the nonmineralized structures present in cellular intrinsic fiber cementum. These may include cementocyte lacunae as well as larger inclusions of cellular elements. In addition, the Sharpey's fibers of cellular mixed stratified cementum generally retain an unmineralized core (Fig. 13) (61,

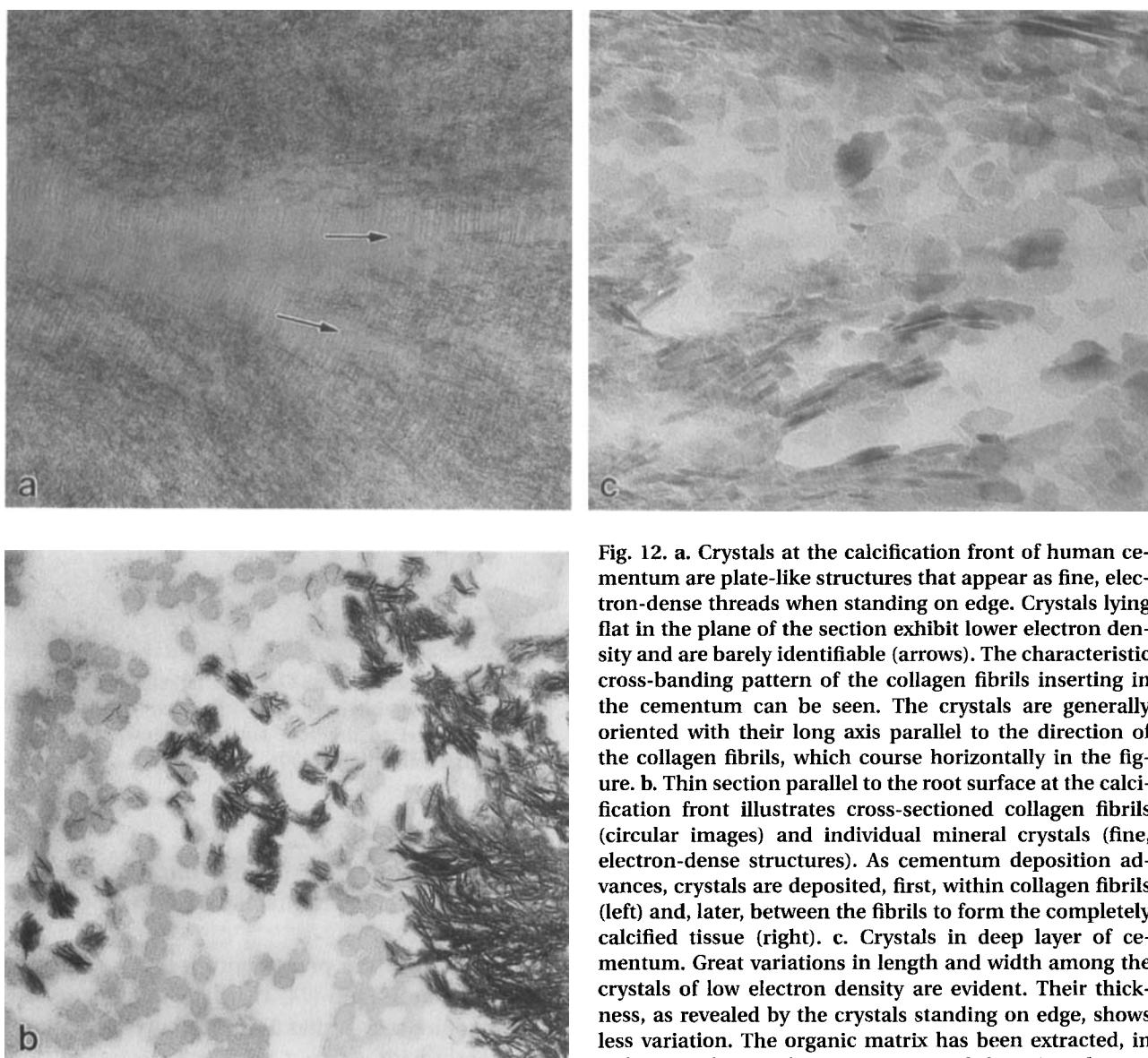


Fig. 12. a. Crystals at the calcification front of human cementum are plate-like structures that appear as fine, electron-dense threads when standing on edge. Crystals lying flat in the plane of the section exhibit lower electron density and are barely identifiable (arrows). The characteristic cross-banding pattern of the collagen fibrils inserting in the cementum can be seen. The crystals are generally oriented with their long axis parallel to the direction of the collagen fibrils, which course horizontally in the figure. b. Thin section parallel to the root surface at the calcification front illustrates cross-sectioned collagen fibrils (circular images) and individual mineral crystals (fine, electron-dense structures). As cementum deposition advances, crystals are deposited, first, within collagen fibrils (left) and, later, between the fibrils to form the completely calcified tissue (right). c. Crystals in deep layer of cementum. Great variations in length and width among the crystals of low electron density are evident. Their thickness, as revealed by the crystals standing on edge, shows less variation. The organic matrix has been extracted, in order to enhance electron contrast of the mineral component. Transmission electron micrographs. Original magnification: a, c: $\times 120,000$; b: $\times 60,000$.

173). The latter feature is in contrast to the intrinsic fibers and to the Sharpey's fibers in acellular extrinsic fiber cementum which exhibit a more complete degree of mineralization. A similar difference in mineralization pattern between Sharpey's fibers and intrinsic fibers is present in the alveolar bone as well. Selvig (173) has pointed out that Sharpey's fibers are derived from periodontal fibers, which are not calcifiable in their original location and that, therefore, these fibers will calcify after they have become embedded in bone and cementum only if they have acquired the concentration of inorganic ions and other components required for calcification, and if possible inhibiting substances have been removed. This

exchange would be less complete in regions where hard tissue formation progresses at a more rapid rate, such as during formation of alveolar bone or cellular intrinsic fiber cementum. More recent data seem to indicate that these fibers have a coating of type III collagen, which may prevent mineralization of the type I collagen in the core (218).

Although additional cementum is laid down throughout life, the mineral content of this tissue, once formed, does not seem to change significantly with age (136, 170, 197). This is in contrast to root dentin, which increases in mineral content and root transparency with age by obliteration of the dentinal tubules.

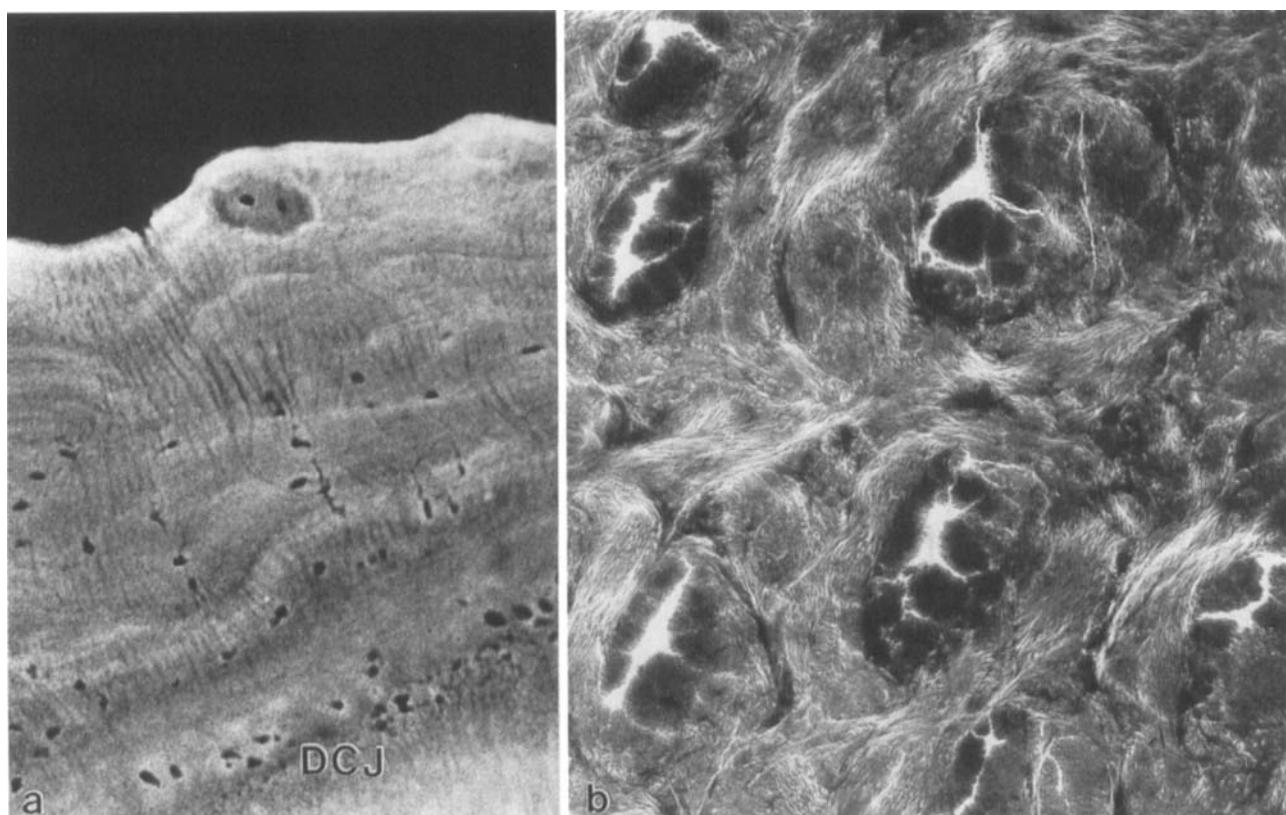


Fig. 13. a. Microradiograph illustrating uncalcified cores of Sharpey's fibers (dark, radially oriented structures) and lacunae in cellular cementum. b. Thin section prepared parallel to the root surface of cellular cementum, illustrating Sharpey's fibers in cross section. Each Sharpey's fiber

exhibits an irregularly shaped, uncalcified core, surrounded by an electron-dense peripheral part. The fibers in this section are 10 μm or less in diameter. Transmission electron micrograph. DCJ: dentinocemental junction. Original magnification: a: $\times 200$; b: $\times 4000$.

Biochemistry

Since cementum is not a uniform, mineralized connective tissue, differences in the proportional composition of the chemical constituents exist between the various cementum varieties. Thus, the percentages of its chemical components may vary from sample to sample, particularly so when they originate from different species. Biochemical studies have shown that "cementum" has a chemical composition similar to bone. To about equal parts per volume, cementum is composed of water, organic matrix and mineral. About 50% of the dry mass is inorganic and consists of hydroxyapatite crystals. The remaining organic matrix contains largely collagens and to a lesser degree mainly glycoproteins and proteoglycans.

Organic matrix

Collagens. The organic matrix of cementum consists primarily of collagens. Like in bone and periodontal

ligament, the two typical fibril-forming collagens type I and III are also found in cementum. Biochemical analyses of bovine (26, 27) and human (48) cementum have revealed that approximately 90% of the organic matrix is type I collagen and a minor proportion of approximately 5% accounts for type III collagen. It has been suggested that the collagen type I fibrils are coated by type III collagen (218). On the other hand, immunocytochemical and biochemical (14, 83, 94, 97, 101, 137) as well as *in vitro* studies (108), using different tissues, have shown that collagen type I is apparently co-localized with collagen/procollagen Type III in the same fibril rather than surrounded by it. The collagens are composed of three polypeptide alpha chains coiled around each other to form the classic triple helix configuration. The procollagen molecules are secreted and aggregate extracellularly to form cross-striated collagen fibrils with the typical 67 nm banding pattern. This striking banding pattern stands out in electron micrographs and is partly obscured when the collagenous matrix is mineralized.

In most tissues, the collagens play important structural and morphogenic roles (93). In mineralized tissues, they provide also a scaffold for the mineral crystals (49). Banded collagen fibrils are frequently observed in membrane-bounded compartments within the cementoblast cytoplasm (32). Their role is still a controversial subject (32, 109, 124). These compartments appear to be continuous with the extracellular space and serve to position newly produced fibril segments to already existing fibril bundles (22–25). However, in most studies, primarily concerning fibroblasts from the periodontal ligament and the gingiva, these membrane-bounded collagen fibrils have been associated with enzymatic collagen degradation (57, 69, 77, 78, 162, 180, 203, 205, 206, 229), whereas only a few authors associated them with polymerization and secretion of collagen (43–45, 79).

Noncollagenous proteins. Cementum is rich in glycoconjugates, which represent either glycolipids, glycoproteins or proteoglycans, and harbors a variety of other proteins.

Like in bone, the predominant noncollagenous proteins are bone sialoprotein and osteopontin (see also: Differentiation of cementoblasts). Both are phosphorylated (70, 150) and sulfated (62, 133) glycoproteins. They bind tightly to collagenous matrices and hydroxyapatite, participate in the mineralization process and reveal cell attachment properties through the tripeptide sequence Arg-Gly-Asp (RGD) that binds to integrins (42, 65, 189). Since their definite roles in the mineralization process are not known, their concerted functions in mineralization are currently being investigated by several research groups. As revealed by immunocytochemistry, acellular afibrillar cementum and acellular extrinsic fiber cementum appear to contain much more of these two glycoproteins than cellular intrinsic fiber cementum (37). The structural organization and the much slower rate of formation may possibly account for the higher immunoreaction and the higher degree of mineralization found in acellular extrinsic fiber cementum.

Osteonectin is another glycosylated protein found in the extracellular matrix of mineralized tissues. As shown in bone, a close relationship between osteonectin and collagen seems to exist in the mineralization process (60, 64, 157, 208). An immunohistochemical study of human cementum showed that osteonectin is expressed by acellular extrinsic fiber cementum- and cellular intrinsic fiber cementum-producing cementoblasts and cementocytes,

whereas the reaction in both cementum varieties was negative (153).

The two glycoproteins fibronectin (98) and tenascin are more widely distributed, high-molecular-weight and multifunctional proteins of the extracellular matrix. One function of fibronectin is to bind cells to components of the extracellular matrix. During tooth development, fibronectin and tenascin are present in the basement membrane of Hertwig's root sheath at the time of odontoblast differentiation (111, 131, 210, 211, 213). Later in development, they are also found at the attachment site of the periodontal ligament to the cementum surface but not in the cementum layer itself (117).

Enamel-related proteins have been detected in cementum by immunohistochemistry and biochemical analysis (see: Differentiation of cementoblasts). Since the presence of these proteins in cementum is controversial, their functions in cementogenesis await further clarification by high-resolution immunodetection.

The proteoglycans, which are widely distributed in mammalian tissues, consist of a core protein to which sulfated polysaccharides (that is, glycosaminoglycans) are covalently linked. Their functions in the extracellular matrix are manifold. The proteoglycans of cementum are small proteins (125). Biochemical analyses of extracts from human cementum have identified chondroitin sulfate, dermatan sulfate and hyaluronic acid as the glycosaminoglycan constituents of these proteoglycans (11).

Osteocalcin, a very small protein found in abundance in the extracellular matrices of bone, dentin and cementum, appears to be involved in the mineralization process (92). An immunohistochemical study of rat molars has shown that cellular intrinsic fiber cementum and associated cementoblasts and cementocytes stained for osteocalcin, whereas acellular extrinsic fiber cementum and its associated cementoblasts did not (39). Another immunohistochemical study of the rat molar (207), however, found that acellular extrinsic fiber cementum but not the associated cementoblasts stained positively for osteocalcin, whereas cellular intrinsic fiber cementum and its associated cementoblasts showed moderate and weak staining, respectively. Although these results are controversial, in both studies a phenotypic difference between cementoblasts has been suggested, with the cellular intrinsic fiber cementum-producing cementoblasts and cementocytes expressing a more osteoblast-like phenotype.

The enzyme alkaline phosphatase is believed to participate in cementum mineralization (16). Super-

saturation of phosphate ions, released from organic phosphate esters, would result in the precipitation of calcium phosphate salts (15, 17, 18). Although alkaline phosphatase exists in a plasma membrane-bound form, part of the enzyme may also be bound to the extracellular matrix (89). In rat molars, the enzyme is heterogeneously distributed in the periodontal ligament, with the highest activity being found adjacent to alveolar bone and cementum (88). The enzyme activity adjacent to cellular intrinsic fiber cementum is higher than that to acellular extrinsic fiber cementum, and the thickness of the latter correlates positively with the enzyme activity (88).

Mineral component

The composition of dental cementum has not been studied to the same extent as that of other mineralized tissues. Cementum is generally less mineralized than root dentin from the same teeth (50, 139, 170), although this may not be without exception (72). Acellular extrinsic fiber cementum appears more highly mineralized than cellular intrinsic fiber cementum and cellular mixed stratified cementum (195). The difference can in part be explained by the presence of uncalcified spaces, such as lacunae and by the uncalcified core of Sharpey's fibers. In addition, the matrix of acellular extrinsic fiber cementum may be more completely mineralized because its formation is a slow process that allows longer direct contact of tissue fluids (195). Röckert (156) examined cementum of monkey teeth by quantitative X-ray microscopy and found that the concentration of Ca varied within a wide range, from 0.10 to 0.83 mg Ca/mm³. This variation confirms that cementum is not a completely mineralized or a homogeneously mineralized tissue.

The region of the dentinocemental junction may demonstrate a variable appearance but commonly shows a zone of high mineral content and low organic content delineated by zones of low mineral content on the dentin side and sometimes on the cementum side as well (2, 61, 73, 155, 223).

Chemical analysis and physicochemical studies indicate that the mineral component is the same as in other calcified tissues; that is, hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), with small amounts of amorphous calcium phosphates present. Transmission electron microscopy and electron diffraction analyses have confirmed that the mineral crystals are arranged with their crystallographic *c*-axis parallel to the long axis of the collagen fibril with which they are associ-

ated (173) (Fig. 12). Such studies also indicate that the crystallinity of the mineral component is lower in cementum than in the other hard tissues. The minute size of the mineral crystals compared with enamel results in a much larger specific surface area of the mineral component. As a consequence, cementum has a greater capacity for adsorption of fluoride and other elements over time but also more readily decalcifies in the presence of acidic conditions.

As in other hard tissues, the hydroxyapatite of cementum is not chemically pure but contains other elements that have been taken up from the tissue fluid during initial crystallization. The amount of these ions initially incorporated into the mineral phase reflects their concentration in the fluid environment during mineralization. Over time, the concentration may change by additional uptake or substitution by other ions.

Thus, cementum contains 0.5–0.9% magnesium (91, 136, 139). Both physicochemical considerations and analyses of dental hard tissues indicate that the Mg ion occupies the place of an equal number of Ca ions in the hydroxyapatite crystal lattice. The Mg content in cementum is about half that in dentin. The significance of the low Mg content remains obscure, but the finding is in harmony with the notion that the composition of cementum is more similar to bone tissue than to dentin. The concentration of Mg appears to be lower at the surface than in deeper layers of cementum (Fig. 14a).

The distribution of fluoride shows the opposite trend (91, 132, 134, 219, 232) (Fig. 14b). Cementum appears to have a high fluoride content compared with other hard tissues. Concentrations up to 0.9% ash weight have been reported, but some authors have found considerably lower values (40, 82, 134, 135, 185, 198, 230, 232). The ash content of cementum is 45–65% of the dry weight. Previously reported values must therefore be reduced correspondingly in order to arrive at the dry weight (136). Fluoride concentration in cementum shows a general increase with age and varies with the nutritional fluoride supply to the individual (134, 136, 219, 233). An increase in fluoride concentration with increased fluoride exposure is found in bone, dentin and enamel as well (40, 81, 100, 219).

Cementum contains 0.1–0.3% sulfur as a constituent of the organic matrix. Sulfur shows a more even distribution than the inorganic components and does not exhibit consistent variation trends within the normal tissue (91) (Fig. 14b). This is consistent with the observation by microradiography of calcified and de-

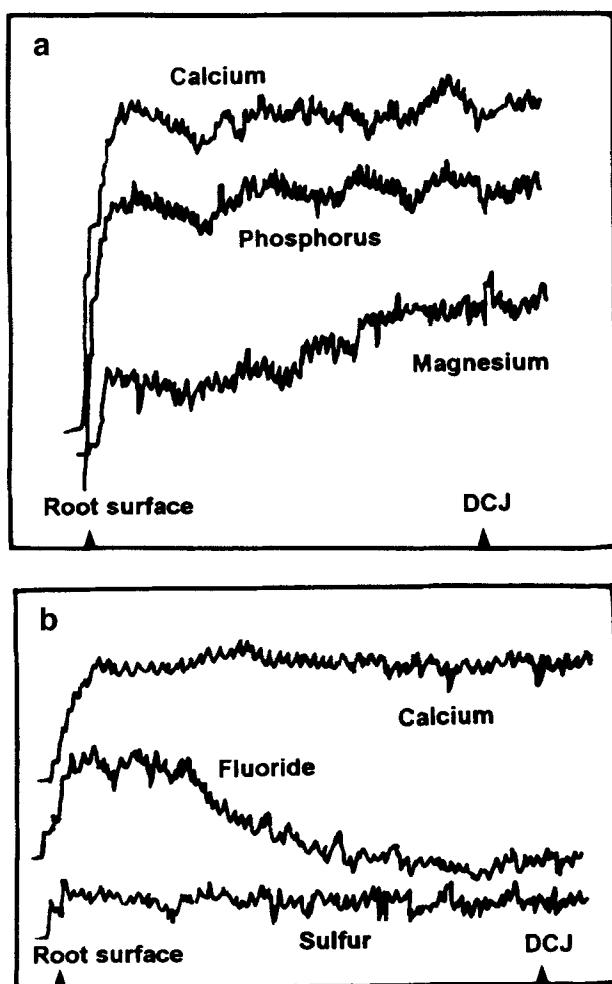


Fig. 14. Typical electron microprobe scan through normal cementum from the surface to the dentinocemental junction. a. The concentration of phosphorus closely parallels that of calcium. Magnesium exhibits lower concentration near the root surface than in the deep layer of the cementum and the underlying root dentin. b. Fluoride exhibits a distinct concentration gradient. Sulfur, which illustrates the distribution of the organic matrix, shows less variation in concentration than the inorganic components.

calcified sections that incremental lines do not correspond to variations in organic content (2).

A number of trace elements may also be present in normal cementum in concentrations detectable by electron microprobe analysis, in particular Cu, Zn and Na (12, 40); however, their distribution and significance do not seem to have been studied in any detail.

Metabolism (turnover) at the tissue and molecular levels

Although dental cementum is usually referred to as a bone-like tissue, there are obvious differences in

regard to vascularity, cellular components, and rate of turnover. Bone tissue, including alveolar bone, participates actively in the metabolism of the body and constitutes a reservoir of calcium and other elements that the body can draw upon as needed. The cementum is largely excluded from such processes.

A variety of noncollagenous proteins are stored in the mineralized matrix of cementum (see: Organic matrix). Whereas most of these extracellular proteins are typical matrix constituents of collagen-based mineralized tissues, others appear specific for cementum. Among these is a cementum-derived attachment protein that mediates the attachment of connective tissue cells (128, 142, 148). Some data indicate that this protein is different from both bone sialoprotein and osteopontin. On the other hand, the possibility cannot be ruled out that this protein may represent a degradation product or a posttranslational variant of known proteins. Another protein believed to be specific for cementum is a cementum-derived growth factor (231). During root resorption and surgical instrumentation, proteins exposed to the root surface and/or released from cementum and dentin could possibly influence the initiation of the repair process by cell migration, division, attachment and differentiation.

Fluoride accumulates in the surface layer of cementum, which is exposed to the circulating tissue fluids in the periodontal ligament (134, 200). Since the F ion reacts aggressively with hydroxyapatite, fluoride concentrates near the surface and shows limited diffusion into deeper layers of the tissue. Thus, the mean fluoride content of the fine layer of cervical cementum is higher than that of the thicker apical cementum (40, 198). As a consequence of its longevity, cementum appears to be the most fluoride-rich tissue of the body (82, 185, 232). By contrast, the bone tissue facing the periodontal ligament is constantly being remodeled and, therefore, has no chance to accumulate the same amount of fluoride. For the same reason, the fluoride concentration of alveolar bone is lower than that of the cortical lamellae of the maxilla and mandible (232) and of most other bones in the body. The relatively high fluoride content of the surface layer compared with deeper layers of cementum and root dentin may help explain why any root resorption tends to be of an undermining character (Fig. 15). Root caries as well seems to progress in a similar pattern.

The two opposing hard tissues, dental cementum and alveolar bone, are strikingly different in histological structure, physiological activity, biochemical composition, as well as in age and reactivity of their



Fig. 15. Root resorption following application of excessive orthodontic force. After a 4-month remission period, the lesion has been partially repaired by cellular intrinsic fiber cementum. Photomicrograph. Original magnification $\times 150$.

organic and inorganic components. These differences may explain why cementum, to a large extent, is excluded from the normal metabolism of the body and, consequently, remains unaffected by many pathological conditions which involve bone tissue.

Age changes

Continuous deposition

Cementum formation on the roots of human teeth continues throughout life unless disturbed by peripapal or periodontal pathology. When mean values of cementum thickness are computed for a large sample, it appears that cementum is deposited at a linear rate (9, 236). More cementum is formed apically than cervically. In addition, cementum thickness shows characteristic variations among tooth groups and tooth surfaces (33, 190). There is a tendency for cementum to reduce root surface concavities. Thus, thicker layers of cementum may form in root surface grooves and in the furcations of multirooted teeth. Also, great variations in width of incremental layers indicate that the rate of cementum formation may vary from time to time. The reasons for these variations are not completely clear. However, changes in tooth position may exert temporal and spatial variations in pressure and tension on root and bone surfaces. The biological responsiveness of cementoblasts to these stimuli may influence the rate as well as pattern of cementum deposition. This regulatory

mechanism would, in turn, maintain the tooth in its proper position in relation to its antagonistic and neighboring teeth.

Nonfunctioning, impacted teeth generally appear to have thicker cementum than functioning teeth (9), and the structural architecture is different. In the cementum of impacted teeth, Sharpey's fibers may be nearly completely absent, and the cementum is built up mainly by intrinsic fibers arranged parallel to the root surface (99) (Fig. 11). In the periodontal ligament of such teeth as well, the fiber arrangement may be predominantly parallel to the root surface.

Physiological activity of cementocytes

Deposition of cellular intrinsic fiber cementum is characterized by the entrapment of cementoblasts as they become surrounded by the matrix which they have formed. It appears that the number of cells that become incorporated is proportional to the rate of cementum deposition (29, 67, 146). The density of cells in cellular intrinsic fiber cementum on human teeth is, however, much lower than in bone tissue. Also, the system of interconnecting canaliculi that might serve to maintain nutritional supply and cell contacts is more sparse. Cementocytes close to the cementum surface may resemble cementoblasts; however, the amount of cytoplasm is reduced and they contain less endoplasmic reticulum and fewer mitochondria (72, 74) (Fig. 16). Characteristically, the most well-developed cell processes of the cementocytes point toward the root surface (167, 176) (Fig. 17). These observations indicate that the exchange of metabolites through cellular intrinsic fiber cementum is limited. In deeper layers of cellular intrinsic fiber cementum, more advanced nuclear and cytoplasmic changes may occur (72, 74), or the lacunae may appear empty. Whether the eventual cell death is due to starvation or is a consequence of age is, however, not known.

Although early studies of root permeability have indicated that the dentinocemental junction represents a barrier against permeation of substances experimentally applied to the root surface, Erasquin & Muruzábal (63) have observed necrosis of cells in the deep layers of cementum after root canal treatment in the molar teeth of rats.

Cementum reactions to physiological tooth movement and occlusal forces

The distribution of cementum on impacted teeth tends to indicate that occlusal forces are not

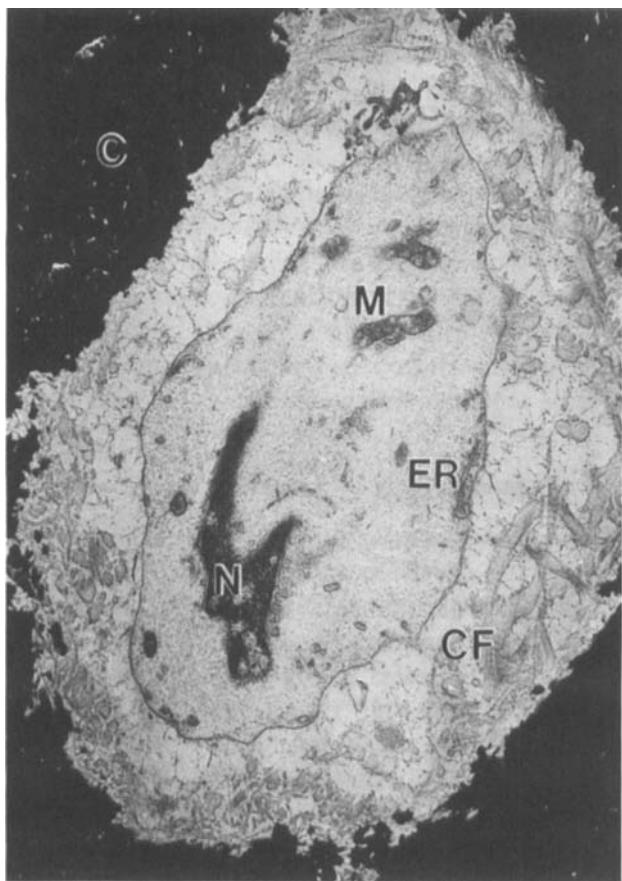


Fig. 16. Electron micrograph of cementocyte located approximately 30 µm from the cementum surface. Note the paucity of organelles. A pyknotic nucleus (N), a few mitochondria (M) and traces of endoplasmic reticulum (ER) can be seen. The cytoplasm is filled with a structureless material. The lacunar wall is lined by scattered nonmineralized collagen fibrils (CF). Source: Furseth (74) with permission from the publisher. Original magnification $\times 14,000$.

necessary to stimulate cementum deposition. In posterior teeth in the human, cementum is markedly thicker on the distal than on the mesial root surface, indicating a relationship to mesial drift (56). It has been suggested that cementum is thicker in areas exposed to tensional forces on labial and lingual surfaces of incisors (84, 167). The deposition of considerably more new cementum has been noted on the tension side compared with the pressure side of the root surface of teeth undergoing orthodontic tooth movement in rhesus monkeys (149). This finding correlates with the observation of appositional layers of bone lining the distal wall of alveolar sockets, and indicates that cementum, like bone tissue, has the potential to be dynamically responsive and that its growth may be stimulated by tensional forces (56).

Resorption and repair

Types of resorption

Although physiological root resorption is a normal phenomenon of deciduous teeth during tooth shedding, permanent teeth do not undergo physiological resorption. A variety of other factors, however, can induce root resorption on teeth of either dentition. These factors can be either pathological or not. In the former case, infectious and systemic diseases as well as tumors may cause root resorption. Under nonpathological circumstances, trauma (either mechanical, chemical or thermal) or sustained over-compression of the periodontal ligament can result in the resorption of cementum and dentin. In the vast majority of cases, however, idiopathic resorption does occur (127). Root resorption can be further classified by location into internal and external and by the degree of persistence into transient or progressive.

Although it is widely accepted that the root surface is more resistant to resorption than alveolar bone, it is also known that the number of teeth resorbed and the severity of resorption are markedly increased by orthodontic treatment (127). However, the frequency of resorative defects is likely to be higher than generally believed, since very superficial resorptions are too small to be detected radiographically. In most cases, however, these resorptions are reversible and therefore of minor clinical significance. When the resorative activity of odontoclasts has ceased and the stimulus for new odontoclast recruitment disappears, they become filled by repair cementum.

Repair

The non-pathologically resorbed root (Fig. 18) is a particularly good model for studying the repair process and the adaptation of the adjacent periodontal ligament, because complications with an infectious disease process do not exist. It can be assumed that the origin and differentiation mechanism of the cells involved in the repair process do not differ in these two situations.

Morphological studies have shown that two different repair matrices become attached to the resorbed root surface (35, 36). Following the detachment of odontoclasts from the root surface, cementogenic cells repopulate the Howship's lacunae (Fig. 18a) and attach the initial repair matrix to a thin decalcified layer of residual and exposed col-

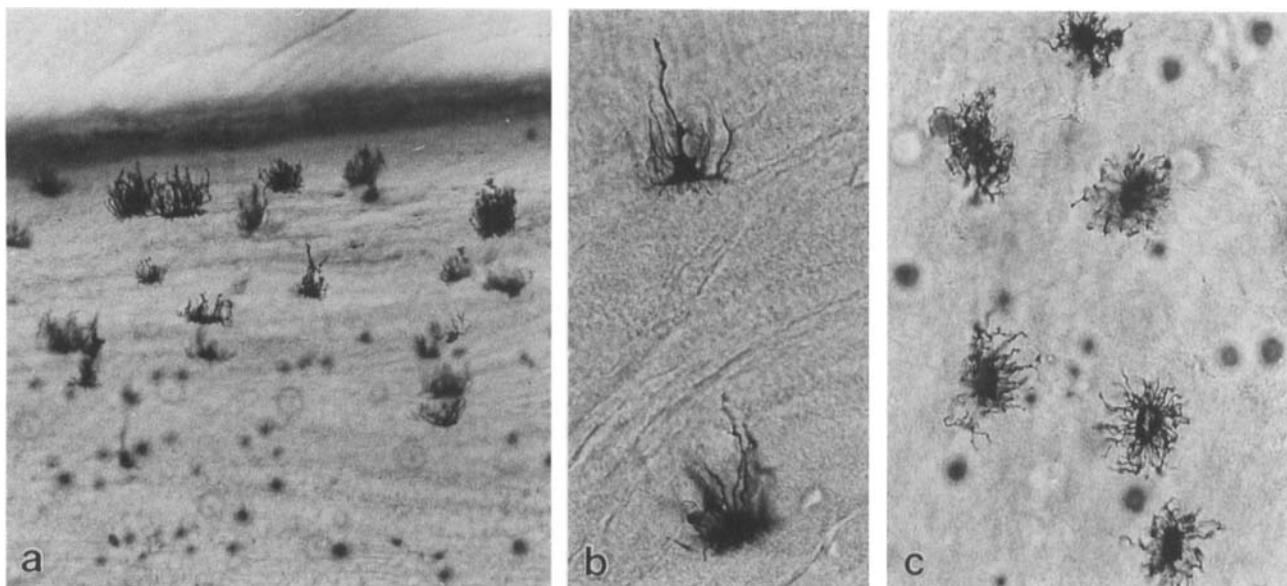


Fig. 17. Cementocyte lacunae and canaliculi as seen in ground section. a. The cells incorporated in cellular cementum are more widely spaced than in bone tissue and exhibit few intercellular connections. Most cell processes point toward the cementum surface (top). Out-of-focus lacunae appear as dark dots. b. Detail illustrating canaliculi

extending from two cementocyte lacunae toward the cementum surface which is just outside the top of the photomicrograph. c. Ground section prepared parallel to the cementum surface. In this projection the lacunae have a symmetrical appearance. Original magnification: a: $\times 150$; b, c: $\times 400$.

lagen fibrils. These cells and their respective repair tissues reveal remarkable homologies to the initial genesis of the two major cementum varieties (that is, acellular extrinsic fiber cementum and cellular intrinsic fiber cementum) on growing human roots (Fig. 18b,e,f and c,g, respectively). In analogy to the formation of the genuine dentinocemental junction, the interdigitation of the newly formed collagen fibrils with the residual dentinal matrix fibrils occurs before (Fig. 18e,g) the new attachment site becomes obscured by electron-dense material (Fig. 18h) the globular accumulation of which is indicative of mineralization. Eventually, a basophilic and electron-dense reversal line forms at the fibrillar junction (Fig. 18d). Subsequently deposited repair matrix usually resembles cellular intrinsic fiber cementum formed on nonresorbed roots (Fig. 18d). Since the cement line found between old and new bone appears to be laid down before the collagenous matrix of new bone is deposited (10, 130, 237), the sequential steps of initial bone and root repair may be different.

The strong resemblance of the initial formation of the two repair matrices with the initiation of acellular extrinsic fiber cementum and cellular intrinsic fiber cementum on the forming root indicates that repair cementogenesis recapitulates the events occurring during root development, a notion that is in

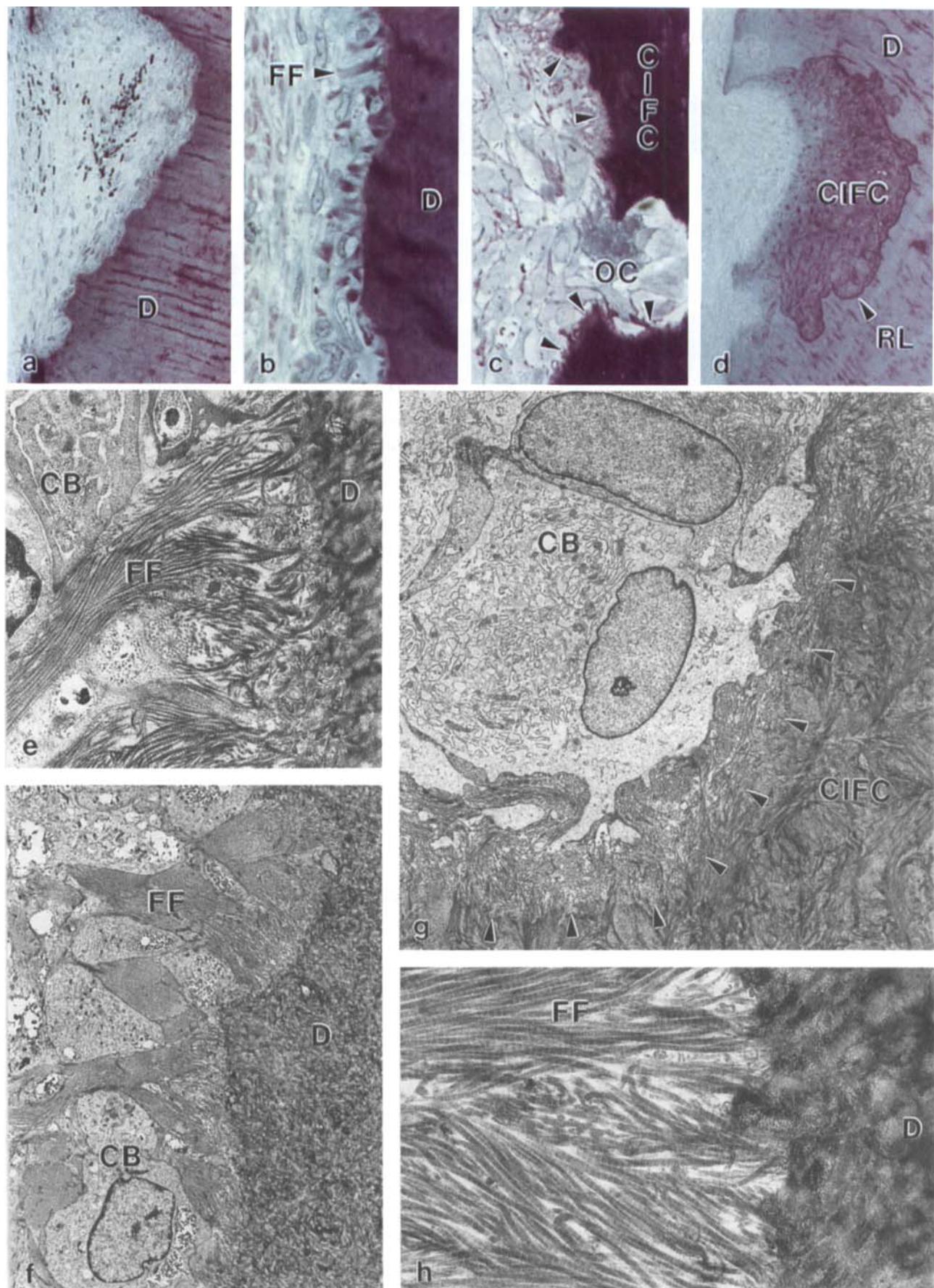
line with the views of Aukhil (7) and MacNeil & Somerman (119) but in contrast with the concept hold by Pitaru et al. (147). However, since the precise origin of the cementoprogenitor cells and the molecular factors that trigger their differentiation are not known, future studies are still imperative to shed light into the cell dynamics occurring during root repair.

Alterations resulting from periodontal pathology

Effect of gingival inflammation

Subsurface alterations. Cementum may undergo alterations in structure as well as in the composition of its organic and inorganic components consequential to pathological changes in the immediate environment. Several in-depth reviews of this subject have been presented (5, 54, 80). The cementum may also become affected by pulpal pathology and root surface caries; however, these processes are not discussed here.

The longstanding presence of an inflammatory process in the gingival connective tissue results in a net loss of collagen and in breakdown of dentogingival fibers. Although the enzymatic breakdown



of collagen fibers is obvious in the gingival soft tissue, the extension of this process into the hard tissue of the root, with loss of collagen cross-banding and dissolution of mineral crystals, has also been described (174, 175). This process, however, is rather surface-limited with a diffuse transition to subjacent unaffected tissue, which explains why it has been detected and described only by electron microscopy.

Cervical root resorption. The development of large root resorption defects in the cervical region is, most likely, also triggered by inflammatory processes in the adjacent connective tissue. Most frequently, cervical resorption is seen in cases of hyperplastic gingivitis (152). Such resorption generally has an undermining character.

It has not been explained why alveolar bone often is resorbed before the teeth, as is the case in apical and marginal periodontitis, as well as in orthodontic tooth movement. For the lack of more plausible alternatives, the epithelial rests of Malassez have been ascribed a protective function (116, 143, 196, 217). More commonly, immunity to resorption has been linked to the presence of an uncalcified, "vital" layer of precementum on the root surface (85, 181). This appears to be an attractive, although somewhat simplistic, explanation based upon the observation that, on the periodontal surface of the alveolar bone, resorption occurs at sites where the bone surface is not covered by osteoid, that is, where bone apposition has ended or where inflammatory breakdown of adjacent collagen fibers has occurred. Studies by Lindskog et al. (115) indicate that the cells lining the root surface

do not respond to parathyroid hormone as do cells lining bone, thus reinforcing previous studies suggesting a protective function for these cells against root resorption (114). One plausible explanation for a delay in root resorption is the fact that cementum is not vascularized. As the odontoclasts most likely take their origin from the bone marrow, these cells cannot attack the root surface as fast as the osteoclasts reach the bone surface.

Some authors have pointed out that resorption of alveolar bone often occurs adjacent to the orifice of vascular canals where branches of the interalveolar vessels enter the periodontal ligament, indicating an association with "circulatory disturbances" (152). The latter hypothesis is supported by the observation that root resorption following clearing-up of a hyalinized zone consequent to the application of excessive orthodontic force is often associated with the ingrowth of a large blood vessel close to the root surface (152, and our own unpublished material).

Exposure to the oral environment

Bacterial contamination. More obvious alterations may occur following exposure of the cementum surface to the environment of the periodontal pocket or the oral cavity. This includes the development of overt cementum caries, which is not described here. Even the noncarious cementum is, however, sufficiently porous to allow permeation of organic substances derived from saliva or bacterial plaque, as well as of inorganic ions. Permeation of particulate matter, including bacteria, is facilitated by the occurrence of minifractures and cracks,

Fig. 18. Light (a-d) and electron (e-h) micrographs illustrating the sequences of repair following the resorption of human premolar roots. Two different repair matrices become attached to the resorbed root surface, one resembling acellular extrinsic fiber cementum (b, e, f) and the other one resembling cellular intrinsic fiber cementum (c, g). a. The resorption has penetrated deeply into the dentin (D). The profile of Howship's lacunae is seen. b, c. The initial repair matrix consists of either a short fiber fringe (FF in b) or a thin layer of cementoid (arrowheads in c). Note that the attachment of the repair matrix can occur side-by-side with odontoclasts (OC in c). d. The bulk of the resorative defect is usually filled with cellular intrinsic fiber cementum (CIFC). Note that a reversal line (RL) is seen at the junction between dentin and cellular intrinsic fiber cementum. e. Cementoblasts (CB) attach the colla-

gen fibrils of the fiber fringe matrix to a thin residual seam of exposed dentinal matrix (star). f. Further development of this type of initial repair matrix results in a dense collagenous fiber fringe resembling the matrix of acellular extrinsic fiber cementum. Note that there is no reversal line seen interfacing the new fibrillar junction. g. Large cementoblasts attach a thin layer of cementoid on the resorption lacuna. Note that there is no reversal line seen at the new junction (arrowheads). h. A fine granular and electron-dense material progressively obscures the fibrillar junction when the initial repair matrix attachment is established on the resorbed root surface. Sources: d-f: Bosshardt (35); h: Bosshardt & Schroeder (36) with permission from the publisher. Original magnification: a, d: $\times 130$; b, c: $\times 440$; e: $\times 10,000$; f: $\times 3000$; g: $\times 4000$; h: $\times 17,500$.

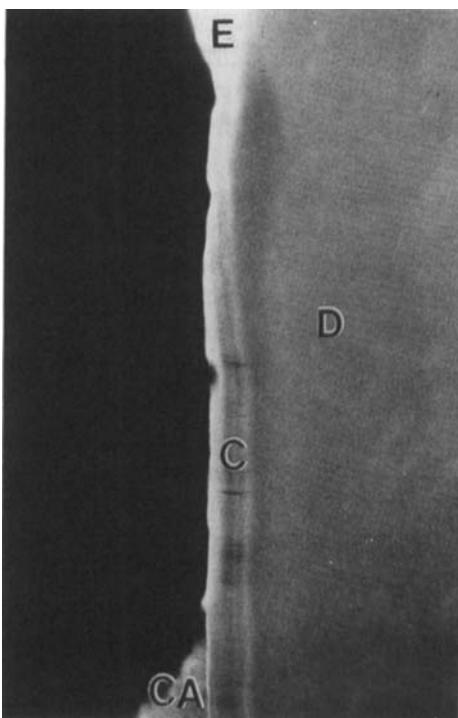


Fig. 19. Microradiograph of a longitudinal section through a periodontally diseased tooth showing hypermineralized cementum. The highly radiopaque surface zone decreases in thickness apically and disappears underneath the calcified concrement. C: cementum; CA: calculus; D: dentin; E: enamel. Original magnification $\times 100$.

which seem to develop frequently in the exposed cementum. Early observations of histological alterations of periodontally involved cementum included the appearance of pathological granules or vacuoles (3, 4, 13, 20) and the presence of lipid granules or strong periodic acid-Schiff staining, polysaccharide staining extending 3–12 μm into the surface of cementum from overlying plaque (55). Armitage & Christie (4) and Armitage (5) concluded that the cemental granules represented areas of denatured cemental collagen which have picked up unidentified substances from the oral environment. This hypothesis is supported by the subsequent observation that similar granules were present near the dentinoemental junction in teeth with heavily infected root canals (6).

Bacterial invasion into cementum and root dentin is a common sequela to chronic periodontal disease (55, 235). Adriaens et al. (1) were able to grow anaerobic bacteria from samples of root dentin from 87% of periodontally diseased teeth and suggested that cementum and root dentin may serve as reservoirs from which recolonization of mechanically debrided root surfaces can occur, as

well as infection of the dental pulp. More recently, several fatty acids indicating the presence of bacterial lipopolysaccharide have been detected in the 40- to 70- μm -deep surface layer of periodontally diseased roots (118). Experimental studies *in vitro*, albeit of short duration, seem to confirm that endotoxin does penetrate into cementum but that its binding to the cementum surface is weak (138).

Hypermineralization. The presence of a highly mineralized surface layer in the cementum following exposure to the external environment has frequently been detected by microradiography (68, 73, 76, 171, 177, 224), chemical analysis (136, 197, 198, 221), electron microprobe analysis (178) and nuclear resonance reaction analysis (52, 160) (Fig. 19). Some studies have failed to demonstrate a hypermineralized surface zone (12, 50). The development of a hypermineralized zone apparently depends on the ionic concentration of inorganic elements in the local environment. Thus, this zone may be more or less generally present on the exposed root surface or may be completely absent (171). Furseth (75) found that healthy cementum experimentally exposed to the oral environment by a gingivectomy procedure acquired a hypermineralized surface zone within 21 days. This process could be greatly enhanced by treating the root surface with a 2% solution of sodium fluoride for 10 minutes. Similarly, if the original surface layer of cementum is removed by root planning, a hypermineralized zone may be re-established within 4 to 8 weeks (177).

Ultrastructurally, the hypermineralized surface zone is characterized by the presence of large, atypical crystals, irregular crystal orientation indicative of demineralization and remineralization processes, and loss of characteristic collagen cross banding (76, 177, 178, 222).

The cementum of periodontally involved teeth, and in particular the hypermineralized surface zone, is also characterized by an increased fluoride content (160, 178, 200, 221, 230). This may explain why the mineral crystals in this zone are extremely resistant to acid demineralization *in vitro* (76). Presumably, the high fluoride content of the surface layer also contributes to the subsurface and undermining character of the demineralization process in cementum caries. Moreover, translocation of mineral ions during the caries process may result in the development of a more densely mineralized surface zone in the early cementum caries lesion than in the adjacent exposed, noncarious cementum surface (73).

Conclusion

The periodontal tissues form a functional unit designed to maintain tooth support and protection. In particular, cementum, by virtue of its structural and dynamic qualities, provides tooth attachment and maintenance of occlusal relationship. These multiple functions are fulfilled by the biological activity and reactivity of cementoblasts, which deposit two collagen-containing varieties of cementum with completely different properties. Acellular extrinsic fiber cementum, which grows very slowly on the cervical and middle portions of the root, mainly participates in tooth anchorage. Cellular intrinsic fiber cementum, on the other hand, may be found either alone as a radicular repair tissue at various locations, or in combination with acellular extrinsic fiber cementum as cellular mixed stratified cementum on the apical root portion and in root grooves and fusions. The ability of cellular intrinsic fiber cementum to be rapidly deposited in thick layers demonstrates its important function as an adaptive and reparative tissue. Unless disturbed, the cementum covering of the root increases in thickness throughout life, albeit at a faster rate apically than cervically. Other parameters that less consistently may influence shape and chemical composition of the root surface over time include changes in tooth position, resorption and repair, surface exposure to the environment, bacterial invasion and contamination, and root caries. The dynamic features of cementum are particularly highlighted by its repair potential. Minor, nonpathological resorption defects on the root surface are generally reversible and heal by reparative cementum formation, which obviously recapitulates developmental cementogenesis. Irreversible damage may occur when the cementum surface becomes exposed to the environment of a periodontal pocket and the oral cavity. Surgical procedures aiming to restore periodontal attachment loss have been only partially successful. In this context, the discovery of a variety of noncollagenous proteins in cementum has opened a new research area of great therapeutic potential. Although most of these matrix proteins, which are also found in bone and dentin, seem to regulate mineralization, some may display multifunctional properties. Other matrix proteins appear to be cementum-specific. Conceivably, the future application of cementum-derived growth and/or attachment factors may result in accelerated wound healing and in controlled neocementogenesis following periodontal regenerative surgery.

References

1. Adriaens PA, De Boever JA, Loesche WJ. Bacterial invasion in root cementum and radicular dentin of periodontally diseased teeth in humans – a reservoir of periodontopathogenic bacteria. *J Periodontol* 1988; **59**: 222–230.
2. Anneroth G, Bergman G, Welander E. Microradiographic study of dental tissues using soft and ultrasoft X-rays. *Odontol Revy* 1966; **17**: 131–148.
3. Armitage GC, Christie TM. Structural changes in exposed human cementum. I. Light microscopic observations. *J Periodont Res* 1973; **8**: 343–355.
4. Armitage GC, Christie TM. Structural changes in exposed human cementum. II. Electron microscopic observations. *J Periodont Res* 1973; **8**: 356–365.
5. Armitage GC. Alterations in exposed human cementum. *J West Soc Periodontics Periodont Abstr* 1977; **25**: 60–68.
6. Armitage GC, Ryder MI, Wilcox SE. Chemical changes in teeth with heavily infected root caries. *J Endod* 1983; **9**: 127–130.
7. Aukhil I. The potential contributions of cell and molecular biology to periodontal regeneration. *Curr Opin Dent* 1992; **2**: 91–96.
8. Avery JK. Development of root and supporting structures. In: Avery JK, ed. *Oral development and histology*. Baltimore: Williams & Wilkins, 1987: 93–103.
9. Azaz B, Ulmansky M, Moshev R, Sela J. Correlation between age and thickness of cementum in impacted teeth. *Oral Surg Oral Med Oral Pathol* 1974; **38**: 691–694.
10. Baron R, Vignery A, Tran Van P. The significance of lacunar erosion without osteoclasts: studies on the reversal phase of the remodeling sequence. *Metab Bone Dis Rel Res* 1980; **2S**: 35–40.
11. Bartold PM, Miki Y, Narayanan AS, Page RC. Glycosaminoglycans of human cementum. *J Periodont Res* 1988; **23**: 13–17.
12. Barton NS, Van Swol RL. Periodontally diseased vs. normal roots as evaluated by scanning electron microscopy and electron probe analysis. *J Periodontol* 1987; **58**: 634–638.
13. Bass CC. A previously undescribed demonstrable pathologic condition in exposed cementum and the underlying dentine. *Oral Surg Oral Med Oral Pathol* 1951; **4**: 641–652.
14. Becker J, Schuppan D, Rabanus JP, Rauch R, Niechoy U, Gelderblom HR. Immunoelectron microscopic localization of collagens Type I, V, VI and of procollagen Type III in human periodontal ligament and cementum. *J Histochem Cytochem* 1991; **39**: 103–110.
15. Beertsen W, Van den Bos T. Calcification of dentinal collagen by cultured rabbit periosteum: the role of alkaline phosphatase. *Matrix* 1989; **69**: 159–171.
16. Beertsen W, Everts V. Formation of acellular root cementum in relation to dental and non-dental hard tissues in the rat. *J Dent Res* 1990; **69**: 1669–1673.
17. Beertsen W, Van den Bos T. 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* 1991; **70**: 176–181.
18. Beertsen W, Van den Bos T. Alkaline phosphatase induces the mineralization of sheets of collagen implanted subcutaneously in the rat. *J Clin Invest* 1992; **89**: 1974–1980.
19. Bencze L. Befunde an der Dentinzenmentgrenze. *Z Stomatol* 1927; **5**: 877–896.

20. Benson LA. A study of a pathologic condition in exposed cementum. *Oral Surg Oral Med Oral Pathol* 1963; **16**: 1137–1144.
21. Berkovitz BKB, Shore RC. Cells of the periodontal ligament. In: Berkovitz BKB, Moxham BJ, Newman HN, ed. *The periodontal ligament in health and disease*. Oxford: Pergamon Press, 1982: 25–50.
22. Birk DE, Trelstad RL. Extracellular compartments in matrix morphogenesis: collagen fibril, bundle, and lamellar formation by corneal fibroblasts. *J Cell Biol* 1984; **99**: 2024–2033.
23. Birk DE, Trelstad RL. Extracellular compartments in tendon morphogenesis: collagen fibril, bundle, and macroaggregate formation. *J Cell Biol* 1986; **103**: 231–240.
24. Birk DE, Zycband EI, Winkelmann DA, Trelstad RL. Collagen fibrillogenesis *in situ*: discontinuous segmental assembly in extracellular compartments. *Ann N Y Acad Sci* 1990; **580**: 176–195.
25. Birk DE, Zycband E. Assembly of the tendon extracellular matrix during development. *J Anat* 1994; **184**: 457–463.
26. Birkedal-Hansen H, Butler WT, Taylor RE. Isolation and preliminary characterization of bovine cementum collagen. *Calcif Tissue Res* 1974; **15**: 325–328.
27. Birkedal-Hansen H, Butler WT, Taylor RE. Proteins of the periodontium. Characterization of the insoluble collagens of bovine dental cementum. *Calcif Tissue Res* 1977; **23**: 39–44.
28. Bosshardt DD, Luder HU, Schroeder HE. Rate and growth pattern of cementum apposition as compared to dentine and root formation in a fluorochrome-labelled monkey (*Macaca fascicularis*). *J Biol Buccale* 1989; **17**: 3–14.
29. Bosshardt DD, Schroeder HE. Evidence for rapid multipolar and slow unipolar production of cellular and acellular cementum matrix with intrinsic fibers. *J Clin Periodontol* 1990; **17**: 663–668.
30. Bosshardt DD, Schroeder HE. Initiation of acellular extrinsic fiber cementum on human teeth. A light- and electron-microscopic study. *Cell Tissue Res* 1991; **263**: 311–324.
31. Bosshardt DD, Schroeder HE. Establishment of acellular extrinsic fiber cementum on human teeth. A light- and electron-microscopic study. *Cell Tissue Res* 1991; **263**: 325–336.
32. Bosshardt DD, Schroeder HE. Initial formation of cellular intrinsic fiber cementum in developing human teeth. A light- and electron-microscopic study. *Cell Tissue Res* 1992; **267**: 321–335.
33. Bosshardt DD. Morphologische, morphodynamische und autoradiographische Untersuchung der Zementogenese an menschlichen Zähnen. Thesis, Hamburg: Verlag Dr. Kovac, 1993.
34. Bosshardt DD, Schroeder HE. Attempts to label matrix synthesis of human root cementum *in vitro*. *Cell Tissue Res* 1993; **274**: 343–352.
35. Bosshardt DD. Formation and attachment of new cementum matrix following root resorption in human teeth: a light and electron microscopic study. In: Davidovitch Z, ed. *The biological mechanisms of tooth eruption, resorption and replacement by implants*. Boston: Harvard Society for the Advancement of Orthodontics, 1994: 617–630.
36. Bosshardt DD, Schroeder HE. How repair cementum becomes attached to the resorbed roots of human permanent teeth. *Acta Anat (Basel)* 1994; **150**: 253–266.
37. Bosshardt DD, Zalzal S, McKee MD, Nanci A. Immunocytochemical and lectin-gold characterization of human and porcine cementum. *J Dent Res* 1995; **74**: 24 (abstr).
38. Bosshardt DD, Schroeder HE. Cementogenesis reviewed: a comparison between human premolars and rodent molars. *Anat Rec* 1996; **245**: 267–292.
39. Bronckers ALJJ, Farach-Carson MC, Van Waveren E, Butler WT. Immunolocalization of osteopontin, osteocalcin, and dentin sialoprotein during dental root formation and early cementogenesis in the rat. *J Bone Miner Res* 1994; **9**: 833–841.
40. Brudevold F, Steadman LT, Smith FA. Inorganic and organic components of tooth structure. *Ann N Y Acad Sci* 1960; **85**: 110–132.
41. Bruszt P. Über die netzartige Anordnung des parodontalen Epithels. *Z Stomatol* 1932; **30**: 679–682.
42. Butler WT. The nature and significance of osteopontin. *Connect Tissue Res* 1989; **23**: 123–126.
43. Cho MI, Garant PR. Sequential events in the formation of collagen secretion granules with special reference to the development of segment-long-spacing-like aggregates. *Anat Rec* 1981; **199**: 309–320.
44. Cho MI, Garant PR. Role of microtubules in the organization of the Golgi complex and the secretion of collagen secretory granules by periodontal ligament fibroblasts. *Anat Rec* 1981; **199**: 459–471.
45. Cho MI, Garant PR. Mirror symmetry of newly divided rat periodontal ligament fibroblasts *in situ* and its relationship to cell migration. *J Periodont Res* 1985; **20**: 185–200.
46. Cho MI, Garant PR. Ultrastructural evidence of directed cell migration during initial cementoblast differentiation in root formation. *J Periodont Res* 1988; **23**: 268–276.
47. Cho MI, Garant PR. Radioautographic study of [³H]mannose utilization during cementoblast differentiation, formation of acellular cementum, and development of periodontal ligament principal fibers. *Anat Rec* 1989; **223**: 209–222.
48. Christner P, Robinson P, Clark CC. A preliminary characterization of human cementum collagen. *Calcif Tissue Res* 1977; **23**: 147–149.
49. Christoffersen J, Landis WJ. A contribution with review to the description of mineralization of bone and other calcified tissues *in vivo*. *Anat Rec* 1991; **230**: 435–450.
50. Cohen M, Garnick JJ, Ringle RD, Hanes PJ, Thompson WO. Calcium and phosphorus content of roots exposed to the oral environment. *J Clin Periodontol* 1992; **19**: 268–273.
51. Cohn SA. Development of the molar teeth in the albino mouse. *Am J Anat* 1957; **101**: 295–319.
52. Crawford AW, Sampson WJ, de Bruin HJ. Shallow fluorine depth profiles of cementum in periodontal disease – a pilot study. *J Dent Res* 1983; **62**: 806–810.
53. Cutress TW, Crigger M. Cell rests of the sheep periodontium. *N Z Dent J* 1974; **70**: 39–49.
54. Daly CG, Kieser JB, Corbet EF, Seymour GJ. Cementum involved in periodontal disease: a review of its features and clinical management. *J Dent* 1979; **7**: 185–192.
55. Daly CG, Seymour GJ, Kieser JB, Corbet EF. Histological assessment of periodontally involved cementum. *J Clin Periodontol* 1982; **9**: 266–274.
56. Dastmalchi R, Polson A, Bouwsma O, Proskin H. Cementum thickness and mesial drift. *J Clin Periodontol* 1990; **17**: 709–713.

57. Deporter DA, Ten Cate AR. Collagen resorption by periodontal ligament fibroblasts at the hard tissue-ligament interfaces of the mouse periodontium. *J Periodontol* 1980; **51**: 429–432.
58. Diab MA, Stallard, RE. A study of the relationship between epithelial root sheath and root development. *Periodontics* 1965; **3**: 10–14.
59. Diamond M, Applebaum E. The epithelial sheath: histogenesis and function. *J Dent Res* 1942; **21**: 303–411.
60. Doi Y, Okuda R, Takezawa Y, Shibata S, Moriwaki Y, Wakamatsu N, Shimizu N, Moriyama K, Shimokawa H. Osteonectin inhibiting de novo formation of apatite in the presence of collagen. *Calcif Tissue Int* 1989; **44**: 200–208.
61. Dreyfuss F, Frank R. Microradiographie et microscopie électronique du cément humain. *Bull Group Int Rech Sci Stomatol Odontol* 1964; **7**: 167–181.
62. Ecarot-Charrier B, Bouchard F, Delloye C. Bone sialoprotein II synthesized by cultured osteoblasts contains tyrosine sulfate. *J Biol Chem* 1989; **264**: 20049–20053.
63. Erasquin J, Muruzábal M. Necrosis of cementum induced by root canal treatments in the molar teeth of rats. *Arch Oral Biol* 1967; **12**: 1123–1132.
64. Fisher LW, Termine JD. Noncollagenous proteins influencing the local mechanisms of calcification. *Clin Orthop* 1985; **200**: 362–385.
65. Fisher LW. Structure/function studies of the sialoglycoproteins and proteoglycans of bone: it is still the early days. In: Slavkin H, Price P, ed. *Chemistry and biology of mineralized tissues*. Amsterdam: Elsevier, 1992: 177–186.
66. Fitchett JE, Hay ED. Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. *Dev Biol* 1989; **131**: 455–474.
67. Formicola AJ, Krampf JI, Witte ET. Cementogenesis in developing rat molars. *J Periodontol* 1971; **42**: 766–773.
68. Forsberg A, Lagergren K, Lönnérblad T. Dental calculus. *Oral Surg Oral Med Oral Pathol* 1960; **13**: 1051–1060.
69. Frank RM, Fellinger E, Steuer P. Ultrastructure du ligament alvéolo-dentaire du rat. *J Biol Buccale* 1976; **4**: 295–313.
70. Franzen A, Heinegård D. Isolation and characterization of two sialoproteins present only in bone calcified matrix. *Biochem J* 1985; **232**: 715–724.
71. Freeman E, Ten Cate AR, Dickson J. Development of a gomphosis by tooth germ implants in the parietal bone of the mouse. *Arch Oral Biol* 1975; **20**: 139–140.
72. Furseth R. A microradiographic and electron microscopic study of the cementum of human deciduous teeth. *Acta Odontol Scand* 1967; **25**: 613–645.
73. Furseth R, Johansen E. A microradiographic comparison of sound and carious human dental cementum. *Arch Oral Biol* 1968; **13**: 1197–1206.
74. Furseth R. The fine structure of the cellular cementum of young human teeth. *Arch Oral Biol* 1969; **14**: 1147–1158.
75. Furseth R. A study of experimentally exposed and fluoride treated dental cementum in pigs. *Acta Odontol Scand* 1970; **28**: 833–850.
76. Furseth R. Further observations on the fine structure of orally exposed and carious human dental cementum. *Arch Oral Biol* 1971; **16**: 71–85.
77. Garant PR. An electron microscopic study of the periodontal tissue of germfree rats and rats monoinfected with *Actinomyces naeslundii*. *J Periodont Res* 1976; **11**(suppl 15): 9–79.
78. Garant PR. Collagen resorption by fibroblasts. A theory of fibroblastic maintenance of the periodontal ligament. *J Periodontol* 1976; **47**: 380–390.
79. Garant PR, Cho MI. Cytoplasmic polarization of periodontal ligament fibroblasts. Implication for cell migration and collagen secretion. *J Periodont Res* 1979; **14**: 95–106.
80. Garrett JS. Cementum in periodontal disease. *J West Soc Periodontics Periodont Abstr* 1975; **23**: 6–12.
81. Gedalia I, Yardeni J. Fluoride in teeth of Israeli children in relation to fluorine in domestic waters and to dental caries prevalence. *J Dent Res* 1957; **36**: 203–207.
82. Gedalia I, Nathan H, Schapira J, Haas N, Feldman J. Fluoride concentration of surface enamel, cementum, lamina dura, and subperiosteal bone from the mandibular angle of Hebrews. *J Dent Res* 1965; **44**: 452.
83. Geerts A, Schuppan D, Lazeroms S, de Zanger R, de Wilde A, Wisse E. Presence of hybrid interstitial fibrils containing type I and III collagen in the space of Disse of the rat liver. *Hepatology* 1990; **12**: 233–241.
84. Geppert EG, Müller KH. Die Wurzelzentmentapposition als messbarer Ausdruck der Kaudruckbelastung des Zahnes. *Dtsch Zahn Mund Kieferheilkd* 1974; **15**: 30–48, 97–119.
85. Gottlieb B. Biology of the cementum. *J Periodontol* 1942; **13**: 13–17.
86. Grant DA, Bernick S. A possible continuity between epithelial rests and epithelial attachment in miniature swine. *J Periodontol* 1969; **40**: 87–95.
87. Griffith CM, Hay ED. Epithelial-mesenchymal transformation during palatal fusion: carboxyfluorescein traces cells at light and electron microscopic levels. *Development* 1992; **116**: 1087–1099.
88. Groeneveld MC, Everts V, Beertsen W. Alkaline phosphatase activity in the periodontal ligament and gingiva of the rat molar: its relation to cementum formation. *J Dent Res* 1995; **74**: 1374–1381.
89. Groeneveld MC, Van den Bos T, Everts V, Beertsen W. Cell-bound and extracellular matrix-associated alkaline phosphatase activity in rat periodontal ligament. *J Periodont Res* 1996; **31**: 73–79.
90. Grupe HE, Ten Cate AR, Zander HA. A histochemical and radiobiological study of *in vitro* and *in vivo* human epithelial cell rest proliferation. *Arch Oral Biol* 1967; **12**: 1321–1329.
91. Hals E, Selvig KA. Correlated electron probe microanalysis and microradiography of carious and normal dental cementum. *Caries Res* 1977; **11**: 62–75.
92. Hauschka PV, Wians FH. Osteocalcin-hydroxyapatite interaction in the extracellular organic matrix of bone. *Anat Rec* 1989; **224**: 180–188.
93. Hay ED. Collagen and other matrix glycoproteins in embryogenesis. In: Hay ED, ed. *Cell biology of extracellular matrix*. New York: Plenum Press, 1991: 437–444.
94. Henkel W, Glanville RW. Covalent crosslinking between molecules of type I and type III collagen. The involvement of the *N*-terminal, nonhelical regions of the $\alpha 1(I)$ and $\alpha 1(III)$ chains in the formation of intermolecular crosslinks. *Eur J Biochem* 1982; **122**: 205–213.
95. Hoffman MM, Schour BS. Quantitative studies in the development of the rat molar. II. Alveolar bone, cementum, and eruption (from birth to 500 days). *Am J Orthod* 1940; **26**: 854–874.
96. Hoffman RL. Formation of periodontal tissues around

- subcutaneously transplanted hamster molars. *J Dent Res* 1960; **39**: 781–798.
97. Huang YH, Ohsaki Y, Kurisu K. Distribution of type I and type III collagen in the developing periodontal ligament of mice. *Matrix* 1991; **11**: 25–35.
 98. Hynes RO, Yamada KM. Fibronectins: multifunctional modular glycoproteins. *J Cell Biol* 1982; **95**: 369–377.
 99. Imgrüth W. Polarisationsoptische Untersuchung des Zementes entkalkter retinierter Zähne. *Acta Anat (Basel)* 1959; **38**: 368–379.
 100. Jackson D, Weidman SM. The relationship between age and fluorine content of human dentine and enamel: a regional survey. *Br Dent J* 1959; **107**: 303–306.
 101. Keene DR, Sakai LY, Bachinger HP, Burgeson RE. Type III collagen can be present on banded collagen fibrils regardless of fibril diameter. *J Cell Biol* 1987; **105**: 2393–2402.
 102. Keller H. Polarisationsoptische Untersuchung der Faserstruktur im Zement entkalkter menschlicher Milchzähne. *Acta Anat (Basel)* 1964; **57**: 326–337.
 103. Kellner E. Histologische Befunde an antagonistischen Zähnen. *Z Stomatol* 1928; **26**: 271–283.
 104. Kellner E. Das Verhältnis der Zement- und Periodontalbreiten zur funktionellen Beanspruchung der Zähne. *Z Stomatol* 1931; **29**: 44–62.
 105. Kronfeld R. Die Zementhyperplasien an nicht-funktionsierenden Zähnen. *Z Stomatol* 1927; **25**: 1218–1228.
 106. Kronfeld R. The biology of cementum. *J Am Dent Assoc* 1938; **25**: 1451–1461.
 107. Kvam E, Gilhuus-Moe O. Uptake of ³H-thymidine by an epithelial rest in the periodontal membrane. A preliminary report. *Acta Odontol Scand* 1970; **28**: 142–146.
 108. Lapière CM, Nusgens B, Pierard GE. Interaction between collagen type I and III in conditioning bundles organization. *Connect Tissue Res* 1977; **5**: 21–29.
 109. Leblond CP. Synthesis and secretion of collagen by cells of connective tissue, bone, and dentin. *Anat Rec* 1989; **224**: 123–138.
 110. Le Douarin N. The neural crest. Cambridge: Cambridge University Press, 1982.
 111. Lesot H, Osman M, Ruch JV. Immunofluorescent localization of collagen, fibronectin, and laminin during terminal differentiation of odontoblasts. *Dev Biol* 1981; **82**: 182–192.
 112. Lester KS. The incorporation of epithelial cells by cementum. *J Ultrastruct Res* 1969; **27**: 63–87.
 113. Lester KS. The unusual nature of root formation in molar teeth of the laboratory rat. *J Ultrastruct Res* 1969; **28**: 481–506.
 114. Lindskog S, Hammarström L. Evidence in favor of an anti-invasion factor in cementum or periodontal membrane of human teeth. *Scand J Dent Res* 1980; **88**: 161–163.
 115. Lindskog S, Blomlöf L, Hammarström L. Comparative effects of parathyroid hormone on osteoblasts and cementoblasts. *J Clin Periodontol* 1987; **14**: 386–389.
 116. Löe H, Waerhaug J. Experimental replantation of teeth in dogs and monkeys. *Arch Oral Biol* 1961; **3**: 176–184.
 117. Lukinmaa PL, Mackie EJ, Thesleff I. Immunohistochemical localization of the matrix glycoproteins – tenascin and the ED-sequence-containing form of cellular fibronectin – in human permanent teeth and periodontal ligament. *J Dent Res* 1991; **70**: 19–26.
 118. Lygre H, Solheim E, Gjerdet NR, Skaug N. Fatty acids of healthy and periodontally diseased root substance in human teeth. *J Dent Res* 1992; **71**: 43–46.
 119. MacNeil RL, Somerman MJ. Molecular factors regulating development and regeneration of cementum. *J Periodont Res* 1993; **28**: 550–559.
 120. MacNeil RL, Thomas HF. Development of the murine periodontium. I. Role of basement membrane in formation of a mineralized tissue on the developing root dentin surface. *J Periodontol* 1993; **64**: 95–102.
 121. MacNeil RL, Thomas HF. Development of the murine periodontium. II. Role of the epithelial root sheath in formation of the periodontal attachment. *J Periodontol* 1993; **64**: 285–291.
 122. MacNeil RL, Sheng N, Strayhorn C, Fisher LW, Somerman MJ. Bone sialoprotein is localized to the root surface during cementogenesis. *J Bone Miner Res* 1994; **9**: 1597–1606.
 123. Malassez ML. Sur l'existence de masses épithéliales dans le ligament alvéolodentaire. *C R Soc Biol* 1884; **36**: 241.
 124. Marchi F. Secretory granules in cells producing fibrillar collagen. In: Davidovitch Z, ed. *The biological mechanisms of tooth eruption and root resorption*. Birmingham, AL: EBSCO Media Publisher, 1988: 53–59.
 125. Mariotti A. The extracellular matrix of the periodontium: dynamic and interactive tissues. *Periodontol 2000* 1993; **3**: 39–63.
 126. Markwald RR, Runyan RB, Kitten GT, Funderburg FM, Bernanke DH, Brauer PR. Use of collagen gel culture to study heart development: proteoglycan and glycoprotein interactions during the formation of endocardial cushion tissue. In: Trelstad RL, ed. *The role of extracellular matrix in development*. New York: Alan R. Liss, 1984: 323–350.
 127. Massler M, Malone AJ. Root resorption in human permanent teeth. A roentgenographic study. *Am J Orthod* 1954; **40**: 619–633.
 128. McAllister B, Narayanan AS, Miki Y, Page RC. Isolation of a fibroblast attachment protein from cementum. *J Periodont Res* 1990; **25**: 99–105.
 129. McCulloch CAG. Basic considerations in periodontal wound healing to achieve regeneration. *Periodontol 2000* 1993; **1**: 16–25.
 130. McKee MD, Nanci A. Osteopontin and the bone remodeling sequence. Colloidal-cold immunocytochemistry of an interfacial extracellular matrix protein. *Ann N Y Acad Sci* 1995; **760**: 177–189.
 131. Meyer JM, Lesot H, Stäubli A, Ruch JV. Immunoperoxidase localization of fibronectin during odontoblast differentiation. An ultrastructural study. *Biol Struct Morphogen* 1989; **2**: 19–24.
 132. Murakami T, Nakagaki H, Sakakibara Y, Weatherell JA, Robinson C. The distribution pattern of fluoride concentrations in human cementum. *Arch Oral Biol* 1987; **32**: 567–572.
 133. Nagata T, Goldberg HA, Zhang Q, Domenicucci C, Sodek J. Biosynthesis of bone proteins by fetal porcine calvariae *in vitro*. Rapid association of sulfated sialoproteins (secreted phosphoprotein-1 and bone sialoprotein) and chondroitin sulfate proteoglycan (CS-PGII) with bone mineral. *Matrix* 1991; **11**: 86–100.
 134. Nakagaki H, Weatherell JA, Strong M, Robinson C. Distribution of fluoride in human cementum. *Arch Oral Biol* 1985; **30**: 101–104.
 135. Nakagaki H, Koyama Y, Sakakibara Y, Weatherell JA, Robinson C. Distribution of fluoride across human dental

- enamel, dentine and cementum. *Arch Oral Biol* 1987; **32**: 651–654.
136. Nakata TM, Stepnick RJ, Zipkin I. Chemistry of human dental cementum: the effect of age and fluoride exposure on the concentration of ash, fluoride, calcium, phosphorus and magnesium. *J Periodontol* 1972; **43**: 115–124.
 137. Nakayasu K, Tanaka M, Konomi H, Hayashi T. Distribution of types I, II, III, IV and V collagen in normal and keratoconus corneas. *Ophthalmic Res* 1986; **18**: 1–10.
 138. Nakib NM, Bissada NF, Simmelink JW, Goldstine SN. Endotoxin penetration into cementum of periodontally healthy and diseased human teeth. *J Periodontol* 1982; **53**: 368–378.
 139. Neiders ME, Eick JD, Miller WA, Leitner JW. Electron probe microanalysis of cementum and underlying dentin in young permanent teeth. *J Dent Res* 1972; **51**: 122–130.
 140. O'Brien C, Bhaskar SN, Brodie AG. Eruptive mechanism and movement in the first molar of the rat. *J Dent Res* 1958; **37**: 467–484.
 141. Olive M, Ruch JV. Does the basement membrane control the mitotic activity of the inner dental epithelium of the embryonic mouse first lower molar? *Dev Biol* 1982; **93**: 301–307.
 142. Olson S, Arzate H, Narayanan AS, Page RC. Cell attachment activity of cementum proteins and mechanism of endotoxin inhibition. *J Dent Res* 1991; **70**: 1266–1271.
 143. Orban B. The epithelial network in the periodontal membrane. *J Am Dent Assoc* 1952; **44**: 632–635.
 144. Owens PDA. A light and electron microscopic study of the early stages of root surface formation in molar teeth in the rat. *Arch Oral Biol* 1980; **24**: 901–907.
 145. Palmer RM, Lumsden AS. Development of periodontal ligament and alveolar bone in homografted recombinations of enamel organs and papillary, pulpal and follicular mesenchyme in the mouse. *Arch Oral Biol* 1987; **32**: 281–289.
 146. Paynter KJ, Pudy G. A study of the structure, chemical nature, and development of cementum in the rat. *Anat Rec* 1958; **131**: 233–251.
 147. Pitaru S, McCulloch CAG, Narayanan SA. Cellular origins and differentiation control mechanisms during periodontal development and wound healing. *J Periodont Res* 1994; **29**: 81–94.
 148. Pitaru S, Narayanan SA, Olson S, Savion N, Hekmati H, Alt I, Metzger Z. Specific cementum attachment protein enhances selectively the attachment and migration of periodontal cells to root surfaces. *J Periodont Res* 1995; **30**: 360–368.
 149. Polson A, Caton J, Polson AP, Nyman S, Novak J, Reed B. Periodontal response after tooth movement into intrabony defects. *J Periodontol* 1984; **55**: 197–202.
 150. Prince CW, Oosawa T, Butler WT, Tomana M, Bhowmik AS, Bhowmik M, Schrohenloher RE. Isolation, characterization and biosynthesis of a phosphorylated glycoprotein from rat bone. *J Biol Chem* 1986; **262**: 2900–2907.
 151. Reeve MR, Wentz FM. The prevalence, morphology, and distribution of epithelial rests in the human periodontal ligament. *Oral Surg Oral Med Oral Pathol* 1962; **15**: 785–793.
 152. Reichborn-Kjennerud I. Dento-alveolar resorption in periodontal disorders. In: Sognnaes RF, ed. *Mechanisms of hard tissue destruction*. Washington, DC: Am Assoc Adv Sci, 1963: 297–319.
 153. Reichert T, Störkel S, Becker K, Fisher LW. The role of osteonectin in human tooth development: an immunohistochemical study. *Calcif Tissue Int* 1992; **50**: 468–472.
 154. Reitan K. Behavior of Malassez' epithelial rests during orthodontic tooth movement. *Acta Odontol Scand* 1961; **19**: 443–468.
 155. Röckert H. Variation in the calcification of cementum and dentin as seen by the use of microradiographic technique. *Experientia* 1956; **12**: 16–18.
 156. Röckert H. A quantitative X-ray microscopical study of calcium in the cementum of teeth. *Acta Odontol Scand* 1958; **16**(suppl 25): 1–68.
 157. Romberg RW, Werness PG, Lollar P, Riggs BL, Mann KG. Isolation and characterization of native adult osteonectin. *J Biol Chem* 1985; **260**: 2728–2736.
 158. Ruch JV, Lesot H, Karcher-Djuricic V, Meyer JM, Olive M. Facts and hypothesis concerning the control of odontoblast differentiation. *Differentiation* 1982; **21**: 7–12.
 159. Ruch JV. Odontoblast differentiation and the formation of the odontoblast layer. *J Dent Res* 1985; **64**: 489–498.
 160. Sampson WJ, Crawford AW. Fluorine concentration changes in human periodontally diseased tooth roots following several treatment times with citric acid. *Calcif Tissue Int* 1985; **37**: 381–385.
 161. Sasano Y, Kaji Y, Nakamura M, Kindaichi K, Slavkin HC, Kagayama M. Distribution of glycoconjugates localized by peanut and *Maclura pomifera* agglutinins during mouse molar root development. *Acta Anat (Basel)* 1992; **145**: 149–155.
 162. Schellens JPM, Everts V, Beertsen W. Quantitative analysis of connective tissue resorption in the supra-alveolar region of the mouse incisor ligament. *J Periodont Res* 1982; **17**: 407–422.
 163. Schmid P. Polarisationsmikroskopische Untersuchungen über den Faserverlauf des Zahnezementes des Menschen. *Z Zellforsch* 1951; **36**: 319–332.
 164. Schonfeld SE. Demonstration of an alloimmune response to embryonic enamel matrix proteins. *J Dent Res* 1975; **54**: 72–77.
 165. Schour I, Massler M. Studies in tooth development: the growth pattern of human teeth. *J Am Dent Assoc* 1940; **27**: 1778–1793.
 166. Schroeder HE, Listgarten MA. Fine structure of the developing epithelial attachment of human teeth. *Monogr Dev Biol* 1977; **2**(2nd edn.): 1–146.
 167. Schroeder HE. The periodontium. In: Oksche A, Vollrath L, ed. *Handbook of microscopic anatomy*. Vol. V/5. Berlin: Springer, 1986: 23–129.
 168. Schroeder HE. Biological problems of regenerative cementogenesis: synthesis and attachment of collagenous matrices on growing and established root surfaces. *Int Rev Cytol* 1992; **142**: 1–59.
 169. Schroeder HE. Human cellular mixed stratified cementum: a tissue with alternating layers of acellular extrinsic and cellular intrinsic fiber cementum. *Schweiz Monatsschr Zahnmed* 1993; **103**: 550–560.
 170. Selvig KA, Selvig SK. Mineral content of human and seal cementum. *J Dent Res* 1962; **41**: 624–632.
 171. Selvig KA, Zander HA. Chemical analysis and microradiography of cementum and dentin from periodontally diseased human teeth. *J Periodontol* 1962; **33**: 303–310.
 172. Selvig KA. Electron microscopy of Hertwig's epithelial sheath and of early dentin and cementum formation in

- the mouse incisor. *Acta Odontol Scand* 1963; **21**: 175–186.
173. Selvig KA. The fine structure of human cementum. *Acta Odontol Scand* 1965; **23**: 423–441.
 174. Selvig KA. Ultrastructural changes in cementum and adjacent connective tissue in periodontal disease. *Acta Odontol Scand* 1966; **24**: 459–500.
 175. Selvig KA. Nonbanded fibrils of collagenous nature in human periodontal connective tissue. *J Periodont Res* 1968; **3**: 169–179.
 176. Selvig KA. På hvilke måter skiller tannsementen seg fra bennev. *Nor Tannlaegeforen Tid* 1968; **78**: 71–86.
 177. Selvig KA. Biological changes at the tooth-saliva interface in periodontal disease. *J Dent Res* 1969; **48**: 846–855.
 178. Selvig KA, Hals E. Periodontally diseased cementum studied by correlated microradiography, electron probe analysis and electron microscopy. *J Periodont Res* 1977; **12**: 419–429.
 179. Sequeira P, Bosshardt DD, Schroeder HE. Growth of acellular extrinsic fiber cementum (AEFC) and density of inserting fibers in human premolars of adolescents. *J Periodont Res* 1992; **27**: 134–142.
 180. Shore RC, Berkovitz BKB. An ultrastructural study of periodontal ligament fibroblasts in relation to their possible role in tooth eruption and in intracellular collagen degradation in the rat. *Arch Oral Biol* 1979; **24**: 155–164.
 181. Sicher H, Bhaskar SN. *Orban's oral histology and embryology*. 7th edn. St. Louis: Mosby, 1972.
 182. Simpson HE. The apoxestic microscopy of the periodontal membrane. *J Periodontol* 1964; **35**: 293–295.
 183. Simpson HE. The degeneration of the rests of Malassez with age as observed by the apoxestic technique. *J Periodontol* 1965; **36**: 288–291.
 184. Simpson HE. A three-dimensional approach to the microscopy of the periodontal membrane. *Proc R Soc Med* 1967; **60**: 537–542.
 185. Singer L, Armstrong WD. Comparison of fluoride contents of human dental and skeletal tissues. *J Dent Res* 1962; **41**: 154–157.
 186. Slavkin HC, Boyde A. Cementum: an epithelial secretory product? *J Dent Res* 1974; **53**: 157 (abstr).
 187. Slavkin HC. Towards a cellular and molecular understanding of periodontics: cementogenesis revisited. *J Periodontol* 1976; **47**: 249–255.
 188. Slavkin HC, Bessem C, Fincham AG, Bringas P, Santos V, Snead ML, Zeichner-David M. Human and mouse cementum proteins immunologically related to enamel proteins. *Biochim Biophys Acta* 1989; **991**: 12–18.
 189. Sodek J, Chen J, Kasugai S, Nagata T, Zhang Q, McKee MD, Nanci A. Elucidating the functions of bone sialoprotein and osteopontin in bone formation. In: Slavkin H, Price P, ed. *Chemistry and biology of mineralized tissues*. Amsterdam: Elsevier, 1992: 297–307.
 190. Solheim T. Dental cementum apposition as an indicator of age. *Scand J Dent Res* 1990; **98**: 510–519.
 191. Solursh M, Fisher M, Meier S, Singley CT. The role of extracellular matrix in the formation of sclerotome. *J Embryol Exp Morph* 1979; **54**: 75–98.
 192. Somerman MJ, Shroff B, Argraves WS, Morrison G, Craig AM, Denhardt DT, Foster RA, Sauk JJ. Expression of attachment proteins during cementogenesis. *J Biol Buccale* 1990; **18**: 207–214.
 193. Somerman MJ, Sauk JJ, Foster RA, Norris K, Dickerson K, Argraves WS. Cell attachment activity of cementum: bone sialoprotein II identified in cementum. *J Periodont Res* 1991; **26**: 10–16.
 194. Somerman MJ, Shroff B, Foster RA, Butler WT, Sauk JJ. Mineral-associated adhesion proteins are linked to root formation. *Proc Finn Dent Soc* 1992; **88(suppl I)**: 451–461.
 195. Soni NN, van Huysen G, Swenson HM. A microradiographic and X-ray densitometric study of cementum. *J Periodontol* 1962; **33**: 372–378.
 196. Spouge JD. A new look at the rests of Malassez. A review of their embryological origin, anatomy, and possible role in periodontal health and disease. *J Periodontol* 1980; **51**: 437–444.
 197. Stepnick RJ, Gentleman L. Microhardness of human apical cementum related to fluoride exposure and age. *J Dent Res* 1975; **54**: 905.
 198. Stepnick RJ, Nakata TM, Zipkin I. The effects of age and fluoride exposure on fluoride, citrate and carbonate content of human cementum. *J Periodontol* 1975; **46**: 45–50.
 199. Sterrett JD, Lindhe J, Berglundh T. Epithelial remnants in the crestal periodontium of the dog. *J Clin Periodontol* 1993; **20**: 109–116.
 200. Sugihara N, Nakagaki H, Ito F, Noguchi T, Weatherell JA, Robinson C. Distribution of fluoride in sound and periodontally diseased human cementum. *Arch Oral Biol* 1991; **36**: 383–387.
 201. Ten Cate AR, Mills C, Solomon G. The development of the periodontium. A transplantation and autoradiographic study. *Anat Rec* 1971; **170**: 365–380.
 202. Ten Cate AR. Cell division and periodontal ligament formation in the mouse. *Arch Oral Biol* 1972; **17**: 1781–1784.
 203. Ten Cate AR. Morphological studies of fibrocytes in connective tissue undergoing rapid remodeling. *J Anat* 1972; **112**: 401–414.
 204. Ten Cate AR, Mills C. The development of the periodontium: the origin of alveolar bone. *Anat Rec* 1972; **173**: 69–78.
 205. Ten Cate AR, Deporter DA. The role of the fibroblast in collagen turnover in the functioning periodontal ligament of the mouse. *Arch Oral Biol* 1974; **19**: 339–340.
 206. Ten Cate AR, Deporter DA, Freeman E. The role of fibroblasts in the remodeling of periodontal ligament during physiological tooth movement. *Am J Orthod* 1976; **69**: 155–168.
 207. Tenorio D, Cruchley A, Hughes FJ. Immunocytochemical investigation of the rat cementoblast phenotype. *J Periodont Res* 1993; **28**: 411–419.
 208. Termine JD, Kleinman HK, Whitson SW, Conn KM, McGarvey ML, Martin GR. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell* 1981; **26**: 99–105.
 209. Thesleff I. *In vitro* development of oral tissues. In: Neubert D, Merker H-J, Kwasigroch TE, ed. *Methods in prenatal toxicology*. Stuttgart: Thieme, 1977: 252–262.
 210. Thesleff I, Stenman S, Vaheri A, Timpl R. Changes in the matrix proteins, fibronectin and collagen, during differentiation of mouse tooth germ. *Dev Biol* 1979; **70**: 116–126.
 211. Thesleff I, Barrach HJ, Foidart JM, Vaheri A, Pratt RM, Martin GR. Changes in the distribution of type IV collagen, laminin, proteoglycan and fibronectin during mouse tooth development. *Dev Biol* 1981; **81**: 182–192.
 212. Thesleff I, Hurmerinta K. Tissue interactions in tooth development. *Differentiation* 1981; **18**: 75–88.
 213. Thesleff I, Mackie E, Vainio S, Chiquet-Ehrismann R.

- Changes in the distribution of tenascin during tooth development. *Development* 1987; **101**: 289–296.
214. Thomas HF, Kollar EJ. Tissue interactions in normal murine root development. In: Davidovitch Z, ed. *The biological mechanisms of tooth eruption and root resorption*. Birmingham, AL: EBSCO Media Publisher, 1988: 145–151.
215. Thomas HF, Kollar EJ. Differentiation of odontoblasts in grafted recombinations of murine epithelial root sheath and dental mesenchyme. *Arch Oral Biol* 1989; **34**: 27–35.
216. Thomas HF. Root formation. *Int J Dev Biol* 1995; **39**: 231–237.
217. Waerhaug J. Effect of C-avitaminosis on the supporting structures of the teeth. *J Periodontol* 1958; **29**: 87–97.
218. Wang HM, Nanda V, Rao LG, Melcher AH, Heersche JNM, Sodek J. Specific immunohistochemical localization of type III collagen in porcine periodontal tissues using the peroxidase-antiperoxidase method. *J Histochem Cytochem* 1980; **28**: 1215–1223.
219. Weatherell JA, Robinson C. Fluoride in teeth and bone. In: Ekstrand J, Fejerskov O, Silverstone LM, ed. *Fluoride in dentistry*. Copenhagen: Munksgaard, 1988: 28–59.
220. Wentz FM, Weinmann JP, Schour I. The prevalence, distribution, and morphologic changes of the epithelial remnants in the molar region of the rat. *J Dent Res* 1950; **29**: 637–646.
221. Wirthlin MR, Pedersen ED, Hancock EB, Lamberts BL, Leonard EP. The hypermineralization of diseased root surfaces. *J Periodontol* 1979; **50**: 125–127.
222. Yamada N. Fine structure of exposed cementum in periodontal disease. *Bull Tokyo Med Dent Univ* 1968; **15**: 409–434.
223. Yamamoto H, Masuda M, Toeda K, Suzuki K. Microradiographic and histopathological study of the cementum. *Bull Tokyo Med Dent Univ* 1962; **9**: 141–150.
224. Yamamoto H, Sugahara N, Yamada N. Histopathological and microradiographic study of the exposed cementum from periodontally diseased human teeth. *Bull Tokyo Med Dent Univ* 1966; **13**: 407–421.
225. Yamamoto T. The innermost layer of cementum in rat molars: its ultrastructure, development, and calcification. *Arch Histol Jpn* 1986; **49**: 459–481.
226. Yamamoto T, Wakita M. Initial attachment of principal fibers to the root dentin surface in rat molars. *J Periodont Res* 1990; **25**: 113–119.
227. Yamamoto T, Wakita M. The development and structure of principal fibers and cellular cementum in rat molars. *J Periodont Res* 1991; **26**: 129–137.
228. Yamamoto T, Hinrichsen KV. The development of cellular cementum in rat molars, with special reference to the fiber arrangement. *Anat Embryol* 1993; **188**: 537–549.
229. Yamasaki A, Rose GG, Mahan CJ. Collagen degradation by human gingival fibroblasts. I. *In vivo* phagocytosis. *J Periodont Res* 1981; **16**: 309–322.
230. Yardeni J, Gedalia I, Kohn M. Fluoride concentration of dental calculus, surface enamel and cementum. *Arch Oral Biol* 1963; **8**: 697–701.
231. Yonemura K, Narayanan AS, Miki Y, Page RC, Okada H. Isolation and partial characterization of a growth factor from human cementum. *Bone Miner* 1992; **18**: 187–198.
232. Yoon SH, Brudevold F, Gardner DE, Smith FA, Soni NN. Distribution of fluorine in teeth and alveolar bone. *J Am Dent Assoc* 1960; **61**: 565–570.
233. Yoon SH, Brudevold F, Gardner DE, Smith FA. Distribution of fluoride in teeth from areas with different levels of fluorine in the water supply. *J Dent Res* 1960; **39**: 845–856.
234. Yoshikawa DK, Kollar EJ. Recombination experiments on the odontogenic roles of mouse dental papilla and dental sac tissues in ocular grafts. *Arch Oral Biol* 1981; **26**: 303–307.
235. Zander HA. The attachment of calculus to root surfaces. *J Periodontol* 1953; **24**: 16–19.
236. Zander HA, Hürzeler B. Continuous cementum apposition. *J Dent Res* 1958; **37**: 1035–1044.
237. Zhou H, Cherneky R, Davies JE. Deposition of cement at reversal lines in rat femoral bone. *J Bone Miner Res* 1994; **9**: 367–374.