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Structure of periodontal tissues in health and disease*

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The periodontium, defined as those tissues supporting and investing the tooth, comprises root cementum, periodontal ligament, bone lining the tooth socket (alveolar bone), and that part of the gingiva facing the tooth (dentogingival junction). The widespread occurrence of periodontal diseases and the realization that lost tissues can be repaired and, perhaps, regenerated has generated considerable interest in the factors and cells regulating their formation and maintenance. It is important to understand that each of the periodontal components has its very specialized structure and that these structural characteristics directly define function. Indeed, proper functioning of the periodontium is only achieved through structural integrity and interaction between its components.

In recent years, a number of detailed descriptions of the structural and compositional features of periodontal tissues have been published (3, 5–7, 9, 15, 17, 46, 50, 56, 58, 61); we refer the reader to these for a comprehensive description of the development, formation, and structure of periodontal tissues. The present review will focus on structure–function relationships pertinent to understanding periodontal tissue breakdown and the repair/regeneration of affected structures.

Healthy periodontal tissues

Dentogingival junction

The dentogingival junction (gingiva facing the tooth) is an adaptation of the oral mucosa that comprises epithelial and connective tissue components. The epithelium is divided into three functional compartments – *gingival*, *sulcular*, and *junctional epithelium* – and the connective tissue into s*uperficial* and *deep* compartments. The junctional epithelium plays a crucial role since it essentially seals off periodontal tissues from the oral environment. Its integrity is thus essential for maintaining a healthy periodontium. Periodontal disease sets in when the structure of the junctional epithelium starts to fail, an excellent example of how structure determines function.

The junctional epithelium

The junctional epithelium arises from the reduced enamel epithelium as the tooth erupts into the oral cavity. It forms a collar around the cervical portion of the tooth that follows the cementoenamel junction (Fig. 1). The free surface of this collar constitutes the floor of the gingival sulcus. Basically, the junctional epithelium is a nondifferentiated, stratified squamous epithelium with a very high rate of cell turnover. It is thickest near the bottom of the gingival sulcus and tapers to a thickness of a few cells as it descends apically along the tooth surface. This epithelium is made up of flattened cells oriented parallel to the tooth that derive from a layer of cuboidal basal cells situated away from the tooth surface that rest on a basement membrane. Suprabasal cells have a similar ultrastructure and, quite remarkably, maintain the ability to undergo cell division. The cell layer facing the tooth provides the actual attachment of the gingiva to the tooth surface by means of a structural complex called the *epithelial attachment*. This complex consists of a basal lamina-like structure that is adherent to the tooth surface and to which the superficial cell layer is attached by hemidesmosomes. The basal lamina-like structure is a specialized extracellular matrix in which typical basement membrane constituents have not been immunodetected in any significant quantity but which is

*Parts of this article are adapted from Reference 50.

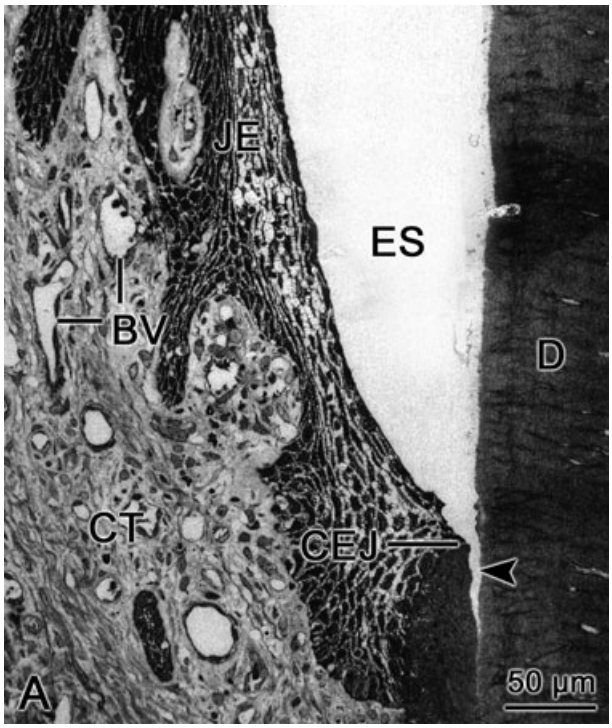

Fig. 1. Backscattered scanning electron micrograph of a decalcified tissue section showing the cervical region of a rat tooth with the junctional epithelium (JE), the enamel space (ES), and the cemento-enamel junction (CEJ). Numerous blood vessels (BV) are present in the connective tissue (CT) of the lamina propria. Note how the enamel space extends between cementum and dentin (arrowhead), a situation which may give the impression that there is an intermediate layer between them. D, dentin.

enriched in glycoconjugates and contains laminin 5. The latter matrix protein mediates cell adhesion and regulates the polarization and migration of keratinocytes (27).

Junctional epithelial cells differ considerably from those of the gingival epithelium. They contain more cytoplasm, rough endoplasmic reticulum, and Golgi bodies. They exhibit fewer tonofilaments and desmosomes, and wider intercellular spaces. The latter fluid-filled spaces normally contain polymorphonuclear leukocytes and monocytes that pass from the subepithelial connective tissue through the junctional epithelium and into the gingival sulcus. The mononuclear cells, together with molecules they secrete and others originating from junctional epithelial cells, blood and tissue fluid represent the first line of defense in the control of the perpetual microbial challenge. Among these molecules are α- and β-defensins, cathelicidin LL-37, interleukin (IL)-8, IL-1α and -1β, tumor necrosis factor-α, intercellular adhesion molecule-1, and lymphocyte function antigen-3.

Connective tissue compartment

The connective tissue supporting the junctional epithelium is structurally different from that supporting the oral gingival epithelium. Even in clinically normal circumstances, it shows an inflammatory cell infiltrate. The gingival connective tissue adjacent to the junctional epithelium contains an extensive vascular plexus. Inflammatory cells such as polymorphonuclear leukocytes and T-lymphocytes continually extravasate from this dense capillary and postcapillary venule network, and migrate across the junctional epithelium into the gingival sulcus and eventually the oral fluid. The vascular distribution in the gingival lamina propria is described in detail in Schroeder & Listgarten (58).

One point of view considers the junctional epithelium as an incompletely developed stratified squamous epithelium. Alternatively, it may be viewed as a structure that evolves along a different pathway and produces the components of the epithelial attachment instead of progressing further into a keratinized epithelium. The special nature of the junctional epithelium is believed to reflect the fact that the connective tissue supporting it is functionally different than that of the sulcular epithelium, a difference with important implications for understanding the progression of periodontal disease and the regeneration of the dentogingival junction after periodontal surgery. The subepithelial connective tissue (lamina propria) is believed to provide instructive signals for the normal progression of stratified squamous epithelia (36, 38). Such signaling presumably is absent from deeper connective tissues so that epithelium in contact with it does not attain the same degree of differentiation.

Thus the sulcular epithelium, in marked distinction to the gingival epithelium, is nonkeratinized, yet both are technically supported by a similar lamina propria. Indeed, this difference in epithelial expression may be attributed to inflammation. Even under normal clinical conditions, the connective tissue associated with the dentogingival junction is slightly inflamed. If the inflammatory process is removed by implementation of a strict regimen of oral hygiene combined with antibiotic coverage in experimental animals, the sulcular epithelium keratinizes (21, 22).

Cementum

Cementum is the hard, avascular connective tissue that coats the roots of teeth and that serves primarily

to invest and attach the principal periodontal ligament fibers. There are basically two varieties of cementum distinguished on the basis of the presence or absence of cells within it and the origin of the collagen fibers of the matrix.

Cementum varieties

Acellular extrinsic fiber cementum (primary cementum or acellular cementum) is found on the cervical half to two thirds of the root (Fig. 2–4). It develops very slowly and is considered to be acellular since the cells that form it remain on its surface. The very high number of principal periodontal ligament fibers inserting into the AEFC (where they are called Sharpey's fibers) points to its important function in tooth attachment. The overall degree of mineralization of AEFC is about 45–60%, but soft X-ray examination reveals that the innermost layer is less mineralized and that the outer layers are characterized by alternating bands of more and less mineral content that run parallel to the root surface.

Cellular intrinsic fiber cementum (secondary cementum, cellular cementum) is distributed along the apical third or half of the root and in furcation areas (Fig. 5). As cellular intrinsic fiber cementum is also produced as a repair tissue that fills resorptive defects and root fractures, it may also be found further coronally. Collagen produced by cementoblasts (intrinsic collagen fibers) and the presence of cementoblasts entrapped in lacunae within the matrix they produce (cementocytes) are the characteristic features of cellular intrinsic fiber cementum. The heterogeneous collagen organization, its rapid speed of formation, and the presence of cells and lacunae may be the reason why this cementum variety is less well mineralized than acellular extrinsic fiber cementum.

Cellular intrinsic fiber cementum constitutes the intrinsic component of cellular mixed stratified

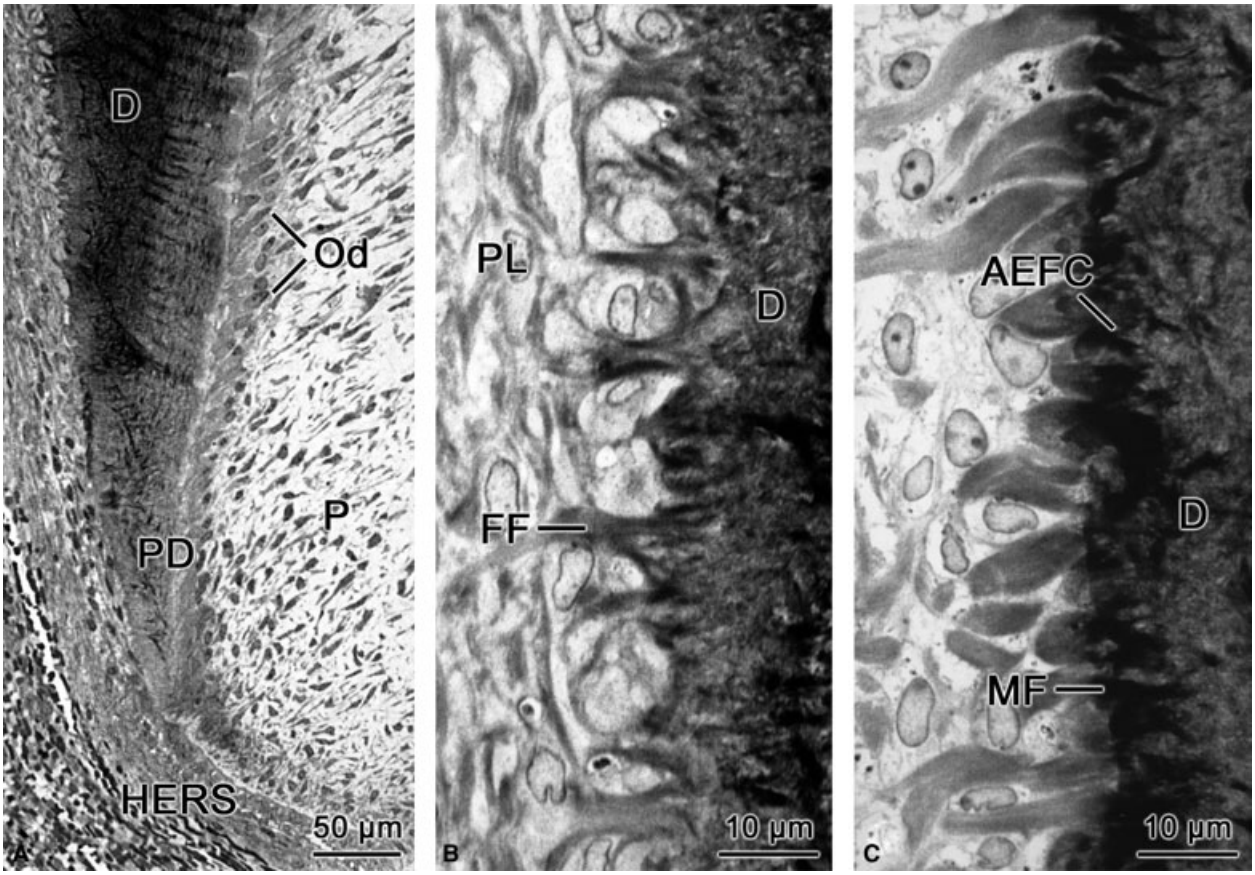

Fig. 2. Backscattered scanning electron micrographs showing the development of acellular extrinsic fiber cementum (AEFC) in a human premolar from apical (A) to cervical (B, C). A) Following disintegration of Hertwig's epithelial root sheath (HERS), cells on the exposed root surface implant a collagenous fiber fringe (FF) into the not yet mineralized dentin matrix (PD = predentin). B) The fiber fringe is oriented perpendicular to the root surface and engulfs the adjacent cells. C) When the cementum layer has attained a thickness of approximately 10 µm, most of the fringe fibers are still short, while others have elongated into the periodontal ligament (PL) space. D, dentin; MF, mineralization front; P, pulp; Od, odontoblasts.

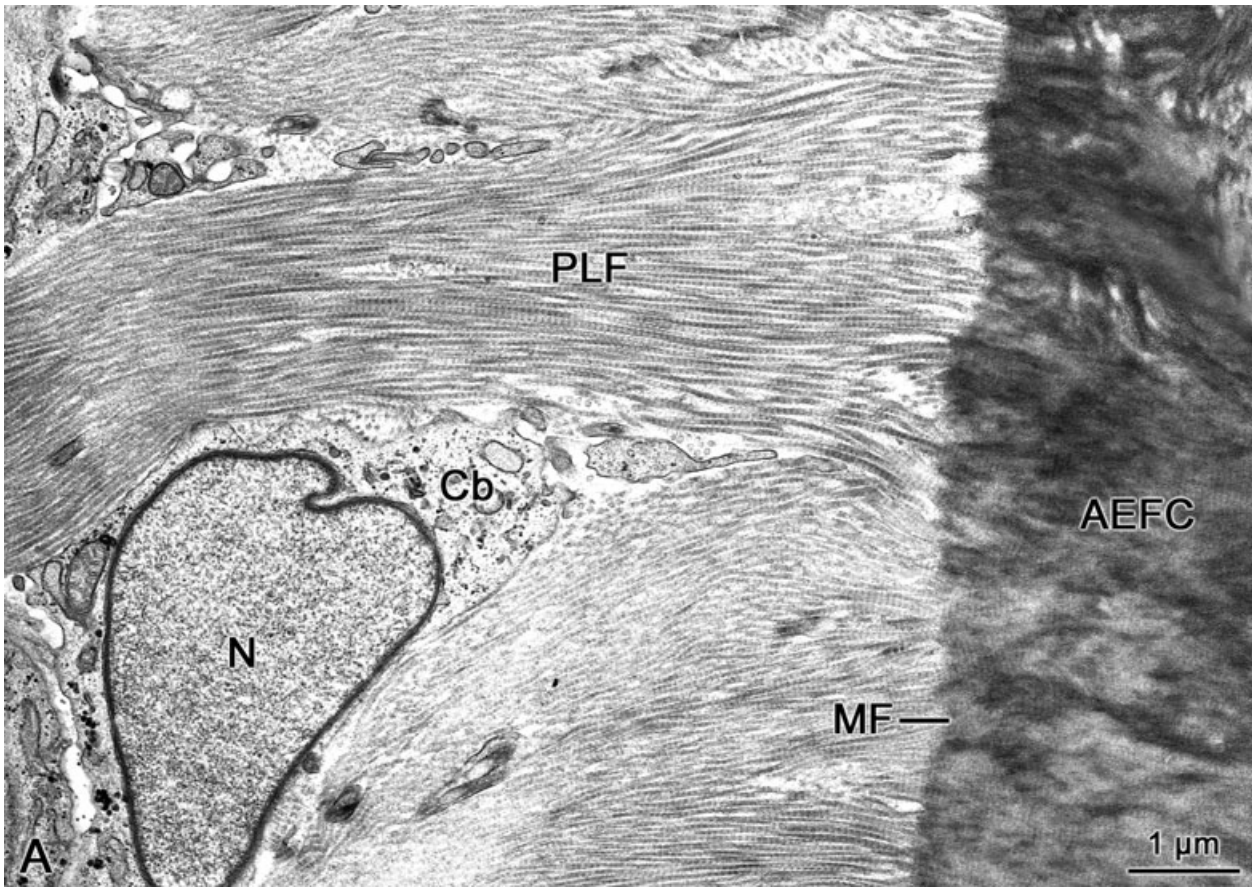

Fig. 3. Transmission electron micro-graph illustrating the cervical root surface of a human tooth. acellular extrinsic fiber cementum (AEFC), which prevails in this root region, is characterized by densely packed periodontal ligament fibers (PLF) that enter the cementum layer at the mineralization front (MF). Cb, cementoblast; N, nucleus.

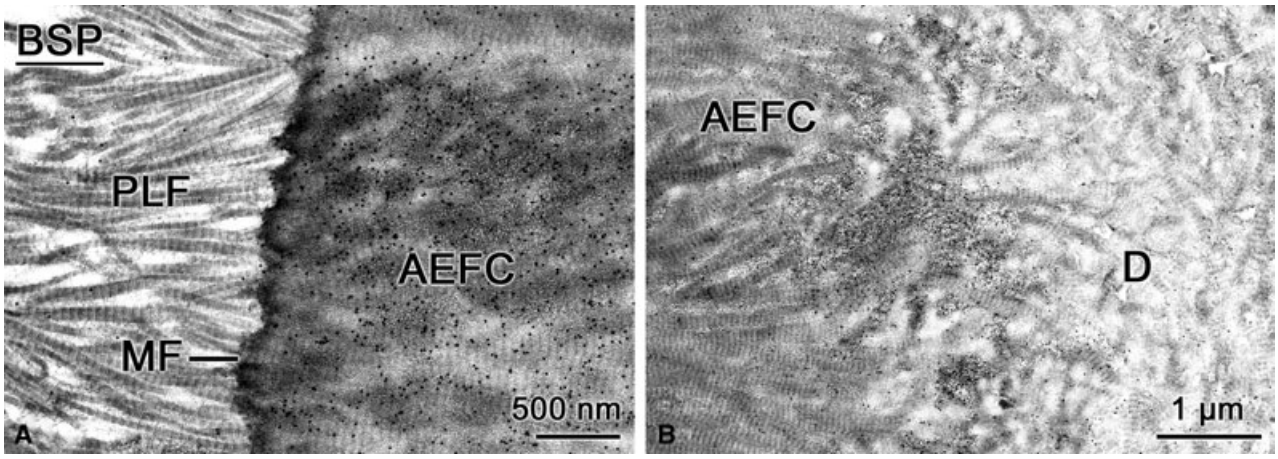

Fig. 4. Electron micrographs of acellular extrinsic fiber cementum (AEFC) from tissue sections following immunogold labeling for bone sialoprotein (BSP). A) Periodontal ligament fibers (PLF) enter the cementum layer at the mineralization front (MF). Labeling predominates over the interfibrillar cementum matrix. B) In the region of the dentino-cemental junction, cemental and dentinal collagen fibrils overlap and interdigitate. noncollagenous proteins like bone sialoprotein fill the wide interfibrillar spaces. D, dentin.

cementum, which possesses a stratification that is derived from consecutively deposited, alternating layers of acellular extrinsic fiber cementum and cellular intrinsic fiber cementum. Cellular mixed stratified cementum is not found in rodent molars but is always present in human teeth. Because the

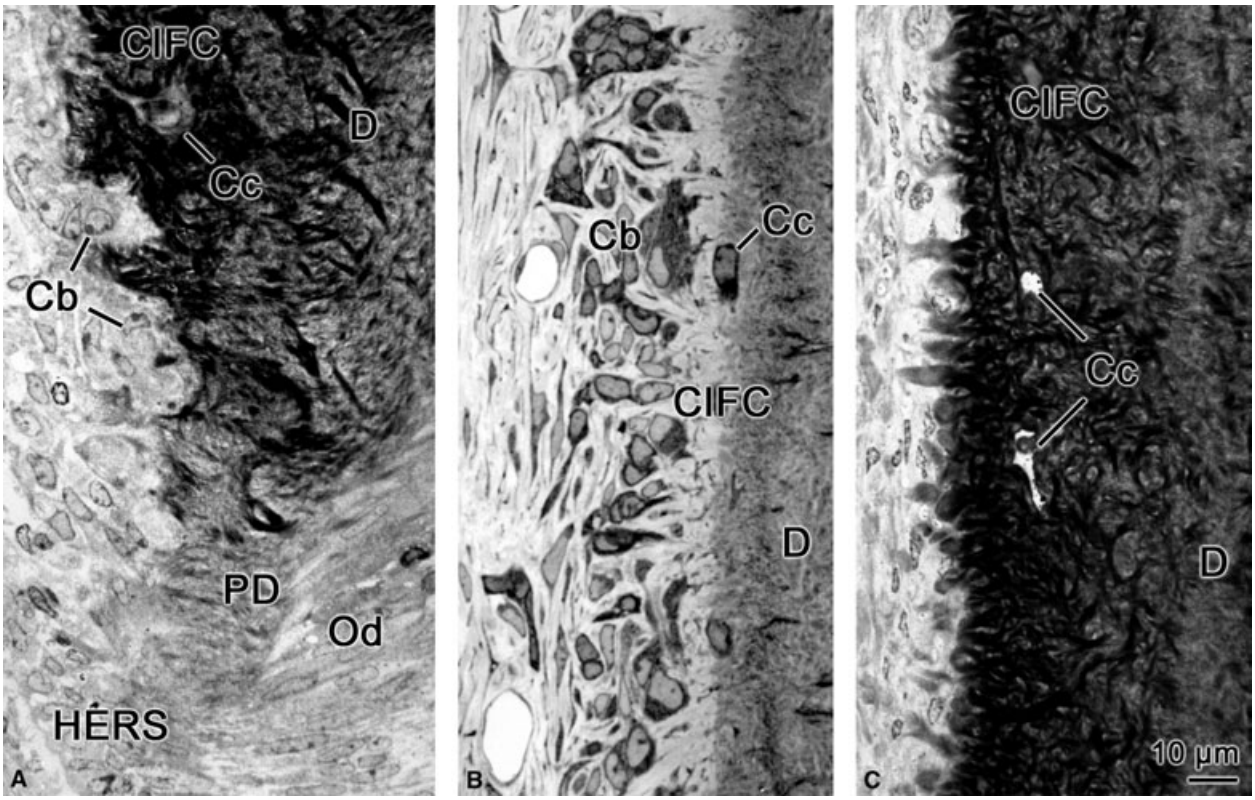

Fig. 5. Backscattered scanning electron micrographs showing the development of cellular intrinsic fiber cementum (CIFC) in a human premolar from apical (A) to a coronal direction (B, C). A) Following disintegration of Hertwig's epithelial root sheath (HERS), cementoblasts (Cb) on the exposed root surface rapidly deposit the cementum matrix onto the not yet mineralized dentin matrix (PD = predentin). B) Some cementoblasts become embedded as cementocytes (Cc) in their own matrix. C) In a more mature, thicker cementum layer, the cementocytes lodge in lacunae. D, dentin; Od, odontoblasts; PGE_2, prostaglandin E_2.

intrinsic cementum variety can be formed very rapidly and focally, it may serve as a means to adjust the tooth position to new requirements.

Biochemical composition of cementum

The composition of cementum resembles that of bone. As a bulk, it contains about 50% mineral (substituted apatite) and 50% organic matrix. Type I collagen is the predominant organic component, constituting up to 90% of the organic matrix. Other collagens associated with cementum include type III, a less cross-linked collagen found in high concentrations during development and repair / regeneration of mineralized tissues, and type XII, a fibril-associated collagen with interrupted triple helices (FACIT) that binds to type I collagen and also to noncollagenous matrix proteins. Trace amounts of other collagens, including type V, VI, and type XIV, are also found in extracts of mature cementum; however, these may be contaminants from the periodontal ligament region associated with fibers inserted into cementum. Almost all noncollagenous matrix proteins identified in cementum are also found in bone (7). These include bone sialoprotein (Fig. 4), dentin matrix protein 1 (DMP-1) (20, 24, 44), dentin sialoprotein (1), fibronectin, osteocalcin, osteonectin, osteopontin, tenascin (47, 69), proteoglycans, proteolipids, and several growth factors including cementum growth factor that appears to be an insulin-like growth factor (IGF)-like molecule. Enamel proteins have also been suggested to be present in cementum. It has been reported that Hertwig's epithelial root sheath (HERS) cells may synthesize amelogenins that accumulate on the forming root surface to form a layer, referred to as intermediate cementum (40–42, 60). To date, however, there is no conclusive evidence that either amelogenins or nonamelogenins accumulate in normal cementum matrix constituents or even form a distinct layer between dentin and cementum. Whenever enamel matrix proteins are found on the root, their presence is limited to a very short, cervical region which likely represents the cervical extremity of the crown onto which cementum is deposited (11, 12). Sporadic expression of enamel proteins has also been reported along the root in porcine teeth

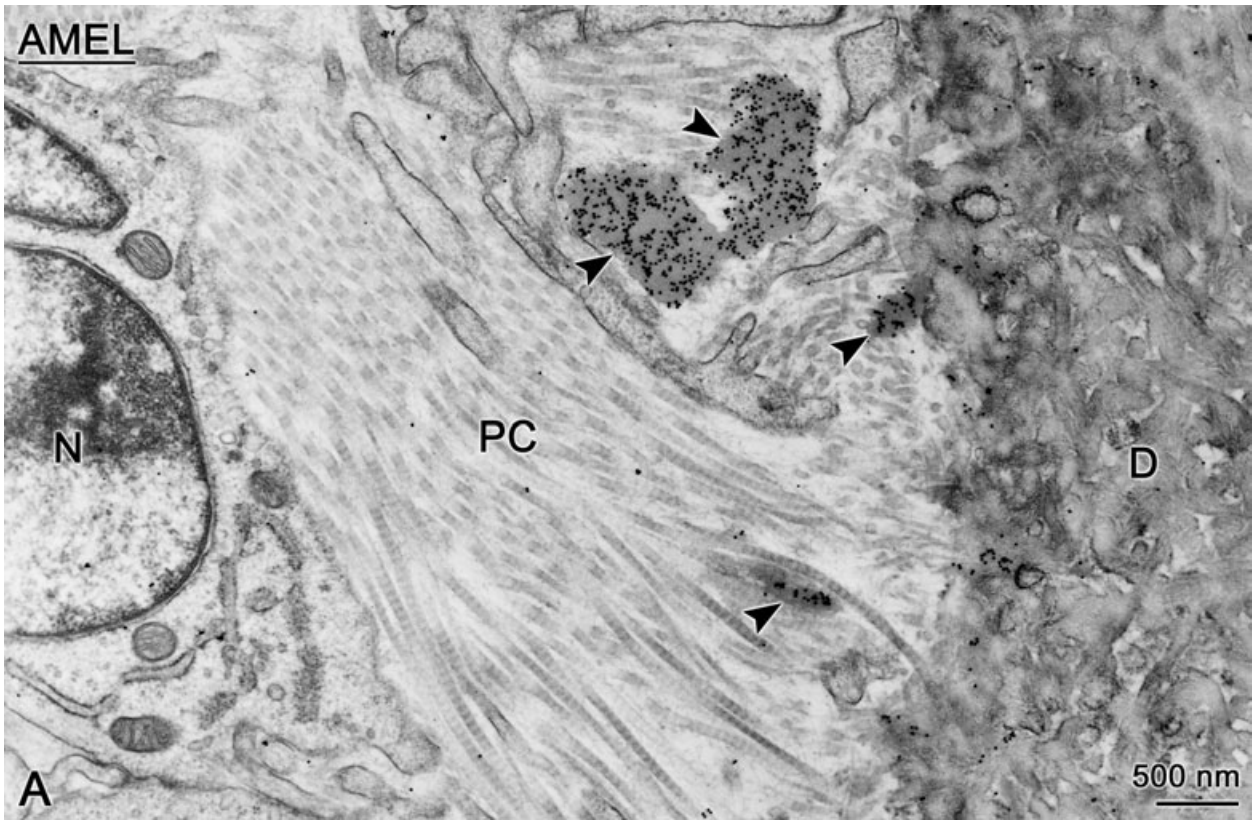

Fig. 6. Transmission electron micro-graph showing the cervical root surface at the beginning of root formation in a porcine tooth; the tissue section was processed for immunogold labeling with anti-amelogenin antibody. Matrix masses containing amelogenin (arrowheads) are sporadically observed along the dentin (D) surface. They co-localize with the collagenous pre-cementum (PC) matrix. AMEL; amelogenin; N, nucleus.

(Fig. 6) (13), and in rodent molars in association with epithelial cells entrapped in cellular intrinsic fiber cementum (12, 25, 26, 63). Finally, an apparently unique cementum attachment protein has also been identified in cementum (64).

Cementum development

Cementum formation takes place along the entire root and during the entire life of the tooth. However, its initiation is limited to the advancing root edge during root formation. At this site, Hertwig's epithelial root sheath, which derives from the apical extension of the inner and outer enamel epithelium, is believed to send an inductive message, possibly by secreting some enamel matrix proteins, to the facing ectomesenchymal pulp cells. These cells differentiate into odontoblasts and produce a layer of predentin. Soon after, HERS becomes fragmented and ectomesenchymal cells from the inner portion of the dental follicle can now come in contact with the predentin. Some cells from the fragmented root sheath form discrete masses surrounded by a basement membrane, known as epithelial rests of Malassez that persist in the mature periodontal ligament. Following these events, cementoblasts will differentiate and deposit cementum matrix onto the forming radicular dentin. The origin of cementoblasts and series of events that culminates in their differentiation is still unresolved and will be discussed below.

Cementum formation

Acellular extrinsic fiber cementum: during root development in human teeth, the first cells that align along the newly formed, but not yet mineralized, mantle dentin surface exhibit fibroblastic characteristics (Fig. 2). These cells deposit collagen within the unmineralized dentin matrix so that fibrils from both matrices interdigitate. Mineralization of the mantle dentin starts internally and does not reach the surface until blending of collagen fibrils from both layers has occurred. It then spreads across into cementum matrix, thereby establishing the dentin–cementum junction. Initial acellular extrinsic fiber cementum thus consists of a thin mineralized layer with a short fringe of collagen fibers implanted perpendicular to the root surface. The cells on the root surface continue to deposit collagen so that the fiber fringe lengthens and thickens. At the same time, they also

secrete noncollagenous matrix proteins that fill in the spaces between the collagen fibers and regulate mineralization of the forming cementum layer (Fig. 4). This activity continues until about 15–20 μm of cementum has been formed, at which time the intrinsic fibrous fringe becomes connected to the developing periodontal ligament fiber bundles (Fig. 3). Thereafter, acellular extrinsic fiber cementum formative cells will be essentially engaged in synthesis of noncollagenous matrix proteins; collagen fibrils that embed in it will be formed by periodontal ligament fibroblasts. No morphologically distinct layer of cementoid, akin to osteoid or predentin, exists on the surface of acellular extrinsic fiber cementum. Although this cementum variety is classified as having extrinsic fibers, one may question whether its initial part should rather be classified as having intrinsic fibers. As described above, the collagenous matrix of the first-formed cementum is the result of cementum-associated cells and is elaborated before the periodontal ligament forms; therefore, the collagen is of local origin and thus of intrinsic derivation.

Cellular intrinsic fiber cementum: after at least half of the root has been formed, cementoblasts start forming a less mineralized variety of cementum that is distinctive in that its constituent collagen fibrils are produced by the cementoblasts themselves (Fig. 5). In all cases, the first collagen is deposited onto the unmineralized dentin surface such that fibrils from both layers intermingle. As for acellular extrinsic fiber cementum, cellular intrinsic fiber cementum-forming cementoblasts also manufacture a number of noncollagenous matrix proteins that fill in the spaces between the collagen fibrils, regulate mineral deposition and impart cohesion to the mineralized layer. A layer of unmineralized matrix, termed cementoid, is established at the surface of the mineralized cementum matrix, with the mineralization front at the interface between the two layers. In contrast to osteoid, cementoid is not as regular and readily discernible. As the process proceeds, some cementoblasts become trapped in the matrix they form. These entrapped cells, with reduced secretory activity, are called *cementocytes* and sit in lacunae. The structural organization of the matrix and the presence of cells in it give cellular intrinsic fiber cementum a bone-like appearance. Collagen fibrils are produced rapidly and deposited haphazardly during the initial phase; however, subsequently the bulk of fibrils organize as bundles oriented mostly parallel to the root surface. When the periodontal ligament becomes organized, cementum may form around some of the periodontal ligament fiber bundles – they are thus incorporated into cementum and become partially mineralized. In human teeth, incorporation of periodontal ligament fibers into cellular intrinsic fiber cementum occurs only rarely, essentially in the acellular extrinsic fiber cementum component of cellular mixed stratified cementum.

How does cementum hold onto dentin?

The attachment mechanism of cementum to dentin is both of biological interest and of clinical relevance, since pathological alterations and clinical interventions may influence the nature of the exposed root surface and hence the quality of the new attachment that forms when repair cementum is deposited. The mechanism by which these hard tissues bind together is essentially the same for acellular extrinsic fiber cementum and cellular intrinsic fiber cementum. Mineralization of the mantle dentin starts internally and does not reach the surface until the collagen fibrils of dentin and cementum have had time to blend together. It then spreads through the surface layer of dentin, across the dentin–cementum junction and into cementum, essentially resulting in an amalgamated mass of mineral. Whereas dentin mineralization is initiated by matrix vesicles, the subsequent spread of mineral deposition is under the regulatory influence of noncollagenous matrix proteins. From a biomechanical perspective, this arrangement appears optimal for a strong union between dentin and cementum. In acellular extrinsic fiber cementum of rodent teeth, cementum is deposited onto mineralized dentin, making amalgamation of dentin and cementum impossible and establishing a weakened interface. Indeed, histological sections of rodent teeth often show a separation between dentin and cementum in the cervical third of the root. Although tissue processing is commonly held responsible for tissue separation in histologic sections, arguments have been raised to question this generalized interpretation (16). Interestingly, repair cementum adheres very well to the root surface if a resorptive phase precedes new matrix deposition (14, 8), implying that odontoclasts favorably precondition the root surface. Chemical preconditioning of the root surface with acids or chelators (Fig. 7B) is an often-applied step in periodontal therapy (43, 45). Following various regenerative procedures, tissue separation between repair cementum and the treated root surface is frequently observed, implying poor attachment quality (8, 16) and suggesting that there

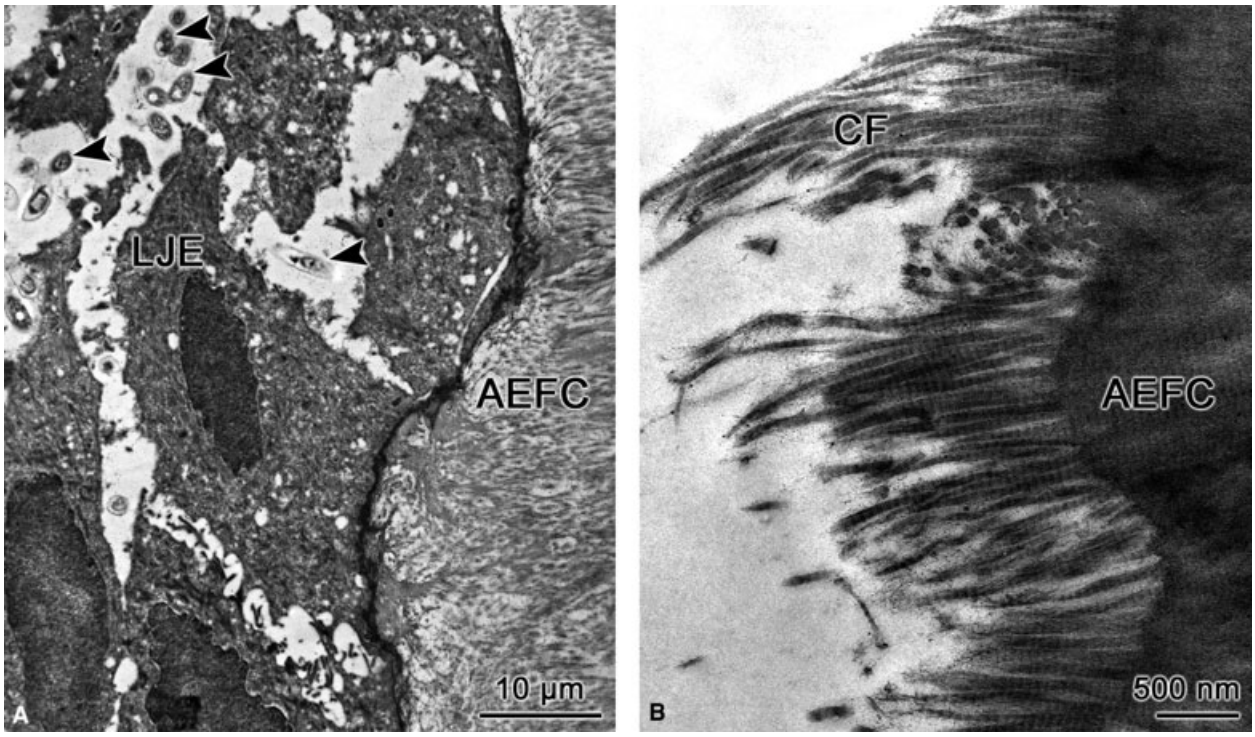

Fig. 7. Transmission electron micrographs of human teeth affected by periodontitis and conservatively treated with root scaling and planing, and root surface demineralization using EDTA. A) A long junctional epithelium (LJE) may establish on the treated root. Note the presence of bacteria (arrowheads) among the epithelial cells. B) The EDTA treatment aims at exposing collagen fibrils (CF). AEFC, acellular extrinsic fiber cementum.

still is room for improvements in chemical root surface conditioning of periodontitis-affected teeth.

Origin of cementoblasts and periodontal ligament fibroblasts

There are still several fundamental issues that need to be resolved and whose clarification is not only essential to understand the process of cementogenesis but, most importantly, to devise targeted therapeutic approaches for the prevention and treatment of periodontal diseases. These include determining the following:

- the precursors of cementoblasts;
- whether cementoblasts are a distinct cell population that expresses unique gene products;
- whether acellular and cellular cementum are distinct tissues;
- what regulates formation and maintenance of periodontal ligament vs. cementum, thus preventing fusion of the root to the alveolar bone (ankylosis).

The long-standing view is that precursors of cementoblasts and periodontal ligament fibroblasts reside in the dental follicle and that factors within the local environment regulate their ability to function as cementoblasts that form root cementum, fibroblasts of the periodontal ligament or osteoblasts forming bone tissue (32). It is widely held that infiltrating dental follicle cells receive a reciprocal inductive signal from the forming dentin and differentiate into cementoblasts. However, there is increasing evidence that HERS cells may undergo epithelial–mesenchymal transformation into cementoblasts during development (7). This is a fundamental process in developmental biology that occurs, among others, as ectodermal cells migrate away from the neural crest and during medial edge fusion of the palatal shelves. Structural and immunocytochemical data support the possibility that cementoblasts derive, at least in part, from transformed epithelial cells of HERS. In rodents, initial formation of acellular cementum takes place in the presence of epithelial cells and it has been shown that enamel organ cells are capable of producing typical mesenchymal products such as type I collagen, bone sialoprotein, and osteopontin (10, 12, 13, 18, 48).

There is still debate as to whether acellular (primary) and cellular (secondary) cementum are produced by distinct populations of cells expressing spatio-temporal behaviors that result in the characteristic histological differences between these tissues. The possibility has been raised that acellular extrinsic

fiber cementum is formed by HERS-derived cells, whereas cellular intrinsic fiber cementum is produced by cells that derive from the dental follicle (68).

Experimental evidence supports the concept that the periodontal ligament is a repository for cells involved in the formation of cementum, periodontal ligament itself, and alveolar bone (37); however, the nature and precise location of progenitor cells remain to be determined. It is also not known whether distinct precursor cell lines exist for each of the three support tissues or whether periodontal ligament fibroblasts, cementoblasts, and osteoblasts arise from a common precursor. The complexity of the periodontal ligament is enhanced by the fact that it contains several cell types (fibroblastic subpopulations, osteoblasts, cementoblasts, endothelial cells, perivascular cells, blood-borne cells, and epithelial cells). In addition, recent findings also suggest the presence of cells with stem cell characteristics (59). A comprehensive review of the literature on cell origin and differentiation has recently been published by Bosshardt (7). This review highlights several lines of evidence that support the concept that cementoblasts producing both acellular extrinsic fiber cementum and cellular intrinsic fiber cementum are unique phenotypes that differ from osteoblasts, and proposes a model that may explain how cell diversity evolves in the periodontal ligament. This new theory proposes that cells derived from HERS play an essential role in tissue development and maintenance, and that periodontal regeneration recapitulates tooth development. Cells descending from HERS may give direct rise to cells that form new cementum and periodontal ligament tissues, or play an indirect role by producing the necessary signaling molecules for cell recruitment and differentiation. Understanding cell origin and cell differentiation mechanisms within the periodontal ligament is mandatory for the development of more effective therapies aimed at achieving true and significant periodontal regeneration.

Molecular factors regulating cementogenesis

Bone morphogenetic proteins: Bone morphogenic proteins are members of the transforming growth factor β (TGF-β) superfamily that act through transmembrane serine/threonine protein kinase receptors. These signaling molecules have a variety of functions during morphogenesis and cell differentiation and, in teeth, they are considered to be part of the network of epithelial–mesenchymal signaling molecules regulating crown development. The roles for bone morphogenic proteins in root development, including whether they are involved in epithelial–mesenchymal signaling, and the signaling pathways and transcription factors involved in modulating their behavior remain to be defined. However, it is known that several of the bone morphogenic proteins, including BMP-2, -4, and -7, promote differentiation of preosteoblasts and putative cementoblast precursor cells. In this context, bone morphogenic proteins have been successfully used to induce periodontal regeneration in a number of experimental models but their clinical use is still lagging behind.

Epithelial factors: The same two populations of cells involved in crown morphogenesis, i.e. enamel epithelium and ectomesenchymal cells, also take part in root formation. It would thus not be surprising if some of the same signaling molecules implicated in crown morphogenesis were also active during development of the root. Prospective candidates include enamel matrix proteins, parathyroid hormone-related protein and basement membrane constituents. In the case of enamel matrix proteins, the debate centers on the fact that they have not been consistently detected along the root, in every species and in all teeth. However, this inconsistency does not rule out their participation in root formation. Some proteins may still be transiently secreted in limited amounts at early stages of root formation by HERS cells to influence odontoblast or cementoblast differentiation; such a limited expression would be difficult to detect.

Matrix proteins: as indicated above, bone sialoprotein and osteopontin are fundamental constituents of cementum matrix, during both its development and repair. Present data suggest that osteopontin is involved in regulating mineral growth, whereas bone sialoprotein promotes mineral formation on the root surface (18, 49). They may also be involved in cellular events through their RGD cell-binding motifs. Since no tooth root developmental anomalies have to date been reported in osteopontin knockout mice, it is likely that other noncollagenous matrix proteins compensate for the absence of osteopontin in these animals.

Bone 'Gla' protein (osteocalcin) is a marker for maturation of osteoblasts, odontoblasts and cementoblasts that may regulate the extent of mineralization. No root development and formation problems have so far been reported in knockout mice.

Transcription factors: Runx-2 (runt-related transcription factor 2), also known as Cbfa1 (core binding factor alpha 1), and osterix, downstream from Cbfa1,

have been identified as master switches for differentiation of osteoblasts. Runx-2 has now been found in dental follicle cells, periodontal ligament cells, cementoblasts, cementocytes, odontoblasts, and ameloblasts. Based on proposed similarities with osteoblasts, they may also be involved in cementoblast differentiation. However, the expression of Runx-2 in fully differentiated cells suggests additional roles. The exact factors triggering expression and/or activation of these key transcription factors are currently being investigated, however, bone genetic proteins have already been identified as factors promoting expression of Runx-2.

Other factors: Other molecules that may have a regulatory function in cementoblast differentiation and activity and that are found within the developing and mature periodontal tissues include alkaline phosphatase, several growth factors (e.g. IGF, TGF-β and platelet-derived growth factor), metalloproteinases, and proteoglycans. The latter are important in the formation of mineralized tissues, although a specific role related to promoting/inhibiting differentiation of the cementoblasts has not been established. An accumulation of proteoglycans has been observed at the dentin–cementum junction and it has been proposed that, together with other noncollagenous matrix proteins such as bone sialoprotein and osteopontin, they may be associated with initial mineralization and fiber attachment (65).

The significance of alkaline phosphatase for cementum formation has long been appreciated, particularly with regard to the potential cellular and formative distinctiveness between acellular extrinsic fiber cementum and cellular intrinsic fiber cementum. In mice null for tissue nonspecific alkaline phosphatase gene or rats treated with bisphosphonates, acellular cementum formation is significantly affected whereas cellular cementum appears to develop normally. This suggests differences in cell types and/or factors controlling the development of these two varieties of cementum. In the human counterpart, hypophosphatasia, characterized by very low levels of alkaline phosphatase, there appears to be limited or no cementum formation. In contrast, mice with mutations in genes that maintain extracellular pyrophosphate levels, such as ank and PC-1, resulting in limited levels of pyrophosphate, exhibit more cellular cementum when compared with wild-type littermates, even at early stages of root development (51). These findings suggest an important role for phosphate in controlling the rate of cementum formation.

Periodontal ligament

The bulk of the periodontal ligament is that soft, specialized connective tissue situated between the cementum covering the root of the tooth and the bone forming the socket wall (alveolo-dental ligament). It ranges in width from 0.15 to 0.38 mm, with its thinnest portion around the middle third of the root, showing a progressive decrease in thickness with age. It is a connective tissue particularly well adapted to its principal function, supporting the teeth in their sockets and at the same time permitting them to withstand the considerable forces of mastication. In addition, the periodontal ligament has the capacity to act as a sensory receptor necessary for the proper positioning of the jaws during mastication and, very importantly, it is a cell reservoir for tissue homeostasis and repair/regeneration.

Periodontal ligament formation

Apart from the recognition that the periodontal ligament forms within the dental follicle region, the exact timing of events associated with the development of an organized periodontal ligament varies among species, with individual tooth families and between primary and permanent teeth. At the beginning, the ligament space is occupied by an unorganized connective tissue extending between bone and cementum. This tissue is then remodeled and the provisional extracellular matrix is converted into a fiber system organized as bundles that extend between the bone and cementum surfaces. The reorganized tissue can now establish continuity across the ligament space and thereby secure the attachment of the tooth to bone. Eruptive tooth movement and the establishment of occlusion then further modify this initial attachment system.

Periodontal ligament cells and extracellular matrix constituents

Similar to all other connective tissues, the periodontal ligament consists of cells and an extracellular compartment comprising collagenous and noncollagenous matrix constituents. The cells include osteoblasts and osteoclasts, fibroblasts, epithelial cell rests of Malassez, monocytes and macrophages, undifferentiated mesenchymal cells, and cementoblasts and odontoclasts. The extracellular compartment consists mainly of well-defined collagen fiber bundles embedded in an amorphous background material, known as ground substance.

Fibroblasts: The principal cells of the periodontal ligament are fibroblasts. Although all fibroblasts look alike microscopically, heterogeneous cell populations exist between different connective tissues and also within the same connective tissue. The fibroblasts of the periodontal ligament are characterized by their rapid turnover of the extracellular compartment, in particular, collagen. Periodontal ligament fibroblasts are large cells with an extensive cytoplasm containing an abundance of organelles associated with protein synthesis and secretion. They have a well-developed cytoskeleton and show frequent adherens and gap junctions, reflecting the functional demands placed on the cells. Ligament fibroblasts are aligned along the general direction of the fiber bundles and extend cytoplasmic processes that wrap around them. The collagen fibrils of the bundles are continuously being remodeled by the fibroblasts, which are capable of simultaneously synthesizing and degrading collagen.

Epithelial cells: The epithelial cells in the periodontal ligament are remnants of HERS and known as the epithelial cell rests of Malassez. They occur close to the cementum as a cluster of cells that form an epithelial network, and seem to be more evident or abundant in furcation areas. The function of these rests is unclear but they could be involved in periodontal repair/regeneration (discussed in [7]).

Undifferentiated mesenchymal cells: An important cellular constituent of the periodontal ligament is the undifferentiated mesenchymal cell, or progenitor cell. The fact that new cells are being produced for the periodontal ligament whereas cells of the ligament are in a steady state means that selective deletion of cells by apoptosis must balance the production of new cells. In periodontal wound healing, the periodontal ligament contributes cells not only for its own repair but also to restore lost bone and cementum (5, 37). Recently, cells with stem cell characteristics have been isolated from the human periodontal ligament (59).

Fibers: The predominant collagens of the periodontal ligament are type I, III, and XII, with individual fibrils having a relatively smaller average diameter than tendon collagen fibrils, a difference believed to reflect the relatively short half-life of ligament collagen, and hence less time for fibrillar assembly. The vast majority of collagen fibrils in the periodontal ligament are arranged in definite and distinct fiber bundles, and these are termed *principal fibers*. Each bundle resembles a spliced rope; individual strands can be continually remodeled while the overall fiber maintains its architecture and function. In this way the fiber bundles are able to adapt to the continual stresses placed on them. The extremities of collagen fiber bundles are embedded in cementum or bone. The embedded portion is referred to as *Sharpey's fibers*. Sharpey's fibers in primary acellular cementum are fully mineralized; those in cellular cementum and bone are generally only partially mineralized at their periphery.

Other fiber bundles (gingival ligament fibers) are found extending from the cervical region of a tooth to that of the adjacent tooth (transseptal ligament fibers), and in the lamina propria of the gingiva. These, together with the main alveolo-dental ligament fibers, constitute the periodontal ligament-fiber system.

Elastic fibers: There are three types of elastic fibers: elastin, oxytalan, and elaunin. Only oxytalan fibers are present within the periodontal ligament; however, elaunin fibers may also be found in association with fiber bundles in the gingival ligament.

Oxytalan fibers are bundles of microfibrils that run more or less vertically from the cementum surface, forming a three-dimensional branching meshwork that surrounds the root and terminates in the apical complex of arteries, veins, and lymphatics. They are also associated with neural elements. Although their function has not been fully determined, they are thought to regulate vascular flow in relation to tooth function. Because they are elastic, they can expand in response to tensional variations, with such variations then registered on the walls of the vascular structures.

Noncollagenous matrix proteins: Several noncollagenous matrix proteins produced locally by resident cells or brought in by the circulation are found in the periodontal ligament, these include alkaline phosphatase (31), proteoglycans (33), and glycoproteins such as undulin, tenascin, and fibronectin (69).

Ground substance: The periodontal ligament ground substance has been estimated to be 70% water and is thought to have a significant effect on the tooth's ability to withstand stress loads. There is an increase in tissue fluids within the amorphous matrix of the ground substance in areas of injury and inflammation.

Periodontal ligament homeostasis and adaptation to functional demand

A remarkable capacity of the periodontal ligament is that it maintains its width more or less over time, despite the fact that it is squeezed in between two

hard tissues. Compelling evidence exists indicating that populations of cells within the periodontal ligament, both during development and during regeneration, secrete molecules that can regulate the extent of mineralization and prevent the fusion of tooth root with surrounding bone, e.g. ankylosis. Among these molecules, balance between the activities of bone sialoprotein and osteopontin may contribute to establishing and maintaining an unmineralized periodontal ligament region. Matrix 'Gla' protein is also present in periodontal tissues; based on its role as an inhibitor of mineralization, it may also act to preserve the periodontal ligament width. At the cell level, it has been reported that Msx2 prevents the osteogenic differentiation of periodontal ligament fibroblasts by repressing Runx2 / Osf2 transcriptional activity (67). Indeed, Msx2 may play a central role in preventing ligaments and tendons, in general, from mineralizing (67). It has also been suggested that glycosaminoglycans (39) or RGD–cementum attachment protein, a collagen-associated protein (52) may also play a role in maintaining the unmineralized state of the periodontal ligament. At this point, the issue of how the periodontal ligament stays uncalcified while being trapped between two calcified tissues remains unresolved and will require more attention.

The periodontal ligament has also the capacity to adapt to functional changes. When the functional demand increases, the width of the periodontal ligament can increase by as much as 50%, and the fiber bundles also increase markedly in thickness. Conversely, a reduction in function leads to narrowing of the ligament and a decrease in number and thickness of the fiber bundles. These functional modifications of the periodontal ligament also implicate corresponding adaptive changes in the bordering cementum and alveolar bone.

Alveolar bone

The alveolar process is that bone of the jaws containing the sockets (alveoli) for the teeth. It consists of outer cortical plates (buccal, lingual, and palatal) of compact bone, a central spongiosa, and bone lining the alveolus (alveolar bone). The cortical plate and bone lining the alveolus meet at the alveolar crest. The bone lining the socket is specifically referred to as *bundle bone* because it provides attachment for the periodontal ligament fiber bundles.

The cortical plates consist of surface layers (lamellae) of fine-fibered bone supported by Haversian systems. They are generally thinner in the maxilla and thickest on the buccal aspect of mandibular premolars and molars. The trabecular (or spongy) bone occupying the central part of the alveolar process also consists of bone disposed in lamellae, with Haversian systems present in the larger trabeculae. Yellow marrow, rich in adipose cells, generally fills the intertrabecular spaces, although sometimes there can also be some red or hematopoietic marrow. Trabecular bone is absent in the region of the anterior teeth and, in this case, the cortical plate and alveolar bone are fused together. The important part of this complex, in terms of tooth support, is the bundle bone, which consists of successive layers of intrinsic fiber bundles running more or less parallel to the socket. Embedded within this bundle bone, almost perpendicular to its surface, are the extremities (Sharpey's fibers) of the extrinsic collagen fiber bundles of the periodontal ligament (which, as in cellular intrinsic fiber cementum / cellular mixed stratified cementum, are mineralized only at their periphery). Because the tooth is constantly making minor movements and alveolar bone must respond to the functional demand placed on it by the forces of mastication, the bone of the socket wall is constantly remodeled and its structural organization varies along the wall (56). The presence of an alveolar bone along the entire tooth socket separates the support bone anatomically and functionally from the periodontal ligament. The organization of the alveolar process is yet another example of structure–function relationship in the periodontium.

Whereas the overall formation and regulatory events in alveolar bone are the same as at other anatomical sites, alveolar bone is distinctive because it turns over very rapidly and it is lost in the absence of a tooth. These two characteristics suggest that local regulatory mechanisms are particularly important in the case of alveolar bone. They also clearly demonstrate the interdependence of the periodontal tissues and underlines the important fact that the periodontal tissues function together as a unit.

The remodeling process of alveolar bone is essentially similar to that of bone in general (56). However, resorption is asynchronous, so that periodontal ligament attachment is lost only focally and for short periods of time. During tooth migration, the distribution of force is such that bone lost by resorption on one surface of the tooth socket is balanced by bone formation along the opposite surface. This bone balance together with the continued deposition of cementum throughout life act to maintain a more or less constant relationship between the root surface and that of the alveolar socket. The factors that

trigger the various events in periodontal homeostasis still need to be ascertained. In elucidating this question, perhaps we could take advantage of events during orthodontic treatment, which essentially represents a circumstance where the limits of the normal physiology are stretched.

As was mentioned above, there are progenitor cells in the periodontal ligament that can differentiate into osteoblasts for the physiological maintenance of alveolar bone and, most likely, for its repair as well. Since the evidence points towards a common precursor residing in the periodontal ligament for cementoblasts, periodontal ligament fibroblasts and bone cells (7), an important issue for devising targeted regenerative therapies is identification of the signals that guide cell differentiation along each of these pathways.

Pathological structure-function alterations of periodontal tissues

Gingivitis and periodontitis (Fig. 7 and 8) are infectious diseases that afflict a high percentage of the population, even at younger ages. In its FY2003 Fact Sheet, the American Association for Dental Research reports that '48% of adults aged 35–44 years of age have inflammation of the gingiva (gingivitis), and 22% destructive periodontal disease – a major cause of tooth loss'. In addition, evidence has been mounting that chronic periodontal diseases are linked with major systemic diseases such as cardiovascular and pulmonary diseases (4, 23, 28, 53). Although bacteria are essential for periodontitis to develop, the fact that it develops to variable degrees in different individuals suggests a multifactorial etiology. All forms of periodontitis, however, appear to have a common series of underlying events leading to tissue breakdown and tooth attachment loss.

The junctional epithelium, by virtue of its structural and functional uniqueness, provides a very efficient barrier against periodontal pathogens and their products. However, periodontal pathogens, in particular *Porphyromonas gingivalis*, may perturb its integrity allowing subgingival spread of bacteria and their antigens (9, 19, 35, 55). The ensuing inflammatory response leads to the degradation of the underlying connective tissue, first around blood vessels and

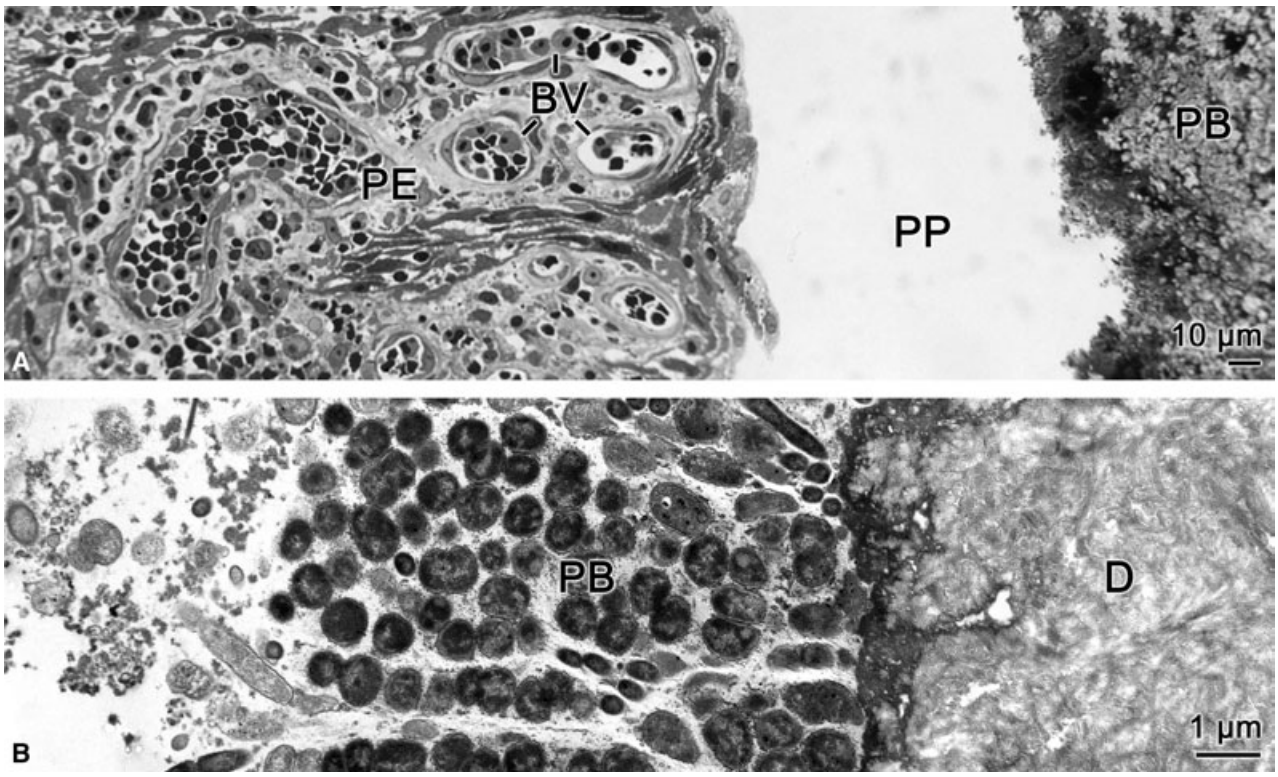

Fig. 8. Light (A) and transmission electron (B) micrographs of human teeth affected by periodontitis. A) The periodontal pocket (PP) occupies the space between the plaque bacteria (PB) adhering to the root surface and the epithelium (PE) lining the pocket space. Note the large number of blood vessels (BV) in the pocket epithelium. (B) After root scaling and planing, periodontal pathogens may re-establish a bacterial biofilm within a few days on the exposed dentin (D) surface.

then spreading into adjacent regions, resulting in the structural and functional disintegration of the gingiva.

One of the first changes of periodontitis is the migration of the junctional epithelium along the root surface and its elongation, resulting in the formation of a long junctional epithelium and a gingival pocket. This structural alteration is accompanied by a number of functional changes. The direction of neutrophil migration and of the crevicular exudate flow across the epithelium changes drastically as the free surface of the epithelium is now displaced from the sulcus bottom to the root surface. Also, the free surface increases in size and is therefore exposed to more bacterial plaque.

Just as the nature of the connective tissue with which the junctional epithelium is in contact is believed to influence its development, one may explain the formation of a long junctional epithelium along the same lines. The junctional epithelium needs a 'certain' connective tissue environment to establish itself whose specific characteristics have not yet been defined but which, in a healthy periodontium, is usually found near the cervical portion of the tooth. When gingivitis sets in, the connective tissue bordering the junctional epithelium is continuously altered by the inflammatory response. Thus, it needs to migrate deeper along the root surface to find a connective tissue structure that is intact enough and capable of signaling the epithelium to stop its downward movement, form a functional epithelial attachment, and attach to the tooth surface.

Bacteria cause tissue destruction indirectly by exacerbating the host's immune response. Recent advances in the acquired immune responses involving B-lymphocytes, T-lymphocytes, and inflammatory mediators in the context of periodontal disease progression have been reviewed by Teng (62) and Yamazaki et al. (66). A number of pro-inflammatory cytokines and growth factors, in particular IL-1 and tumor necrosis factor-α (TNF-α), are known to be associated with bone resorption (Fig. 9). Normal bone remodeling depends on a delicate balance

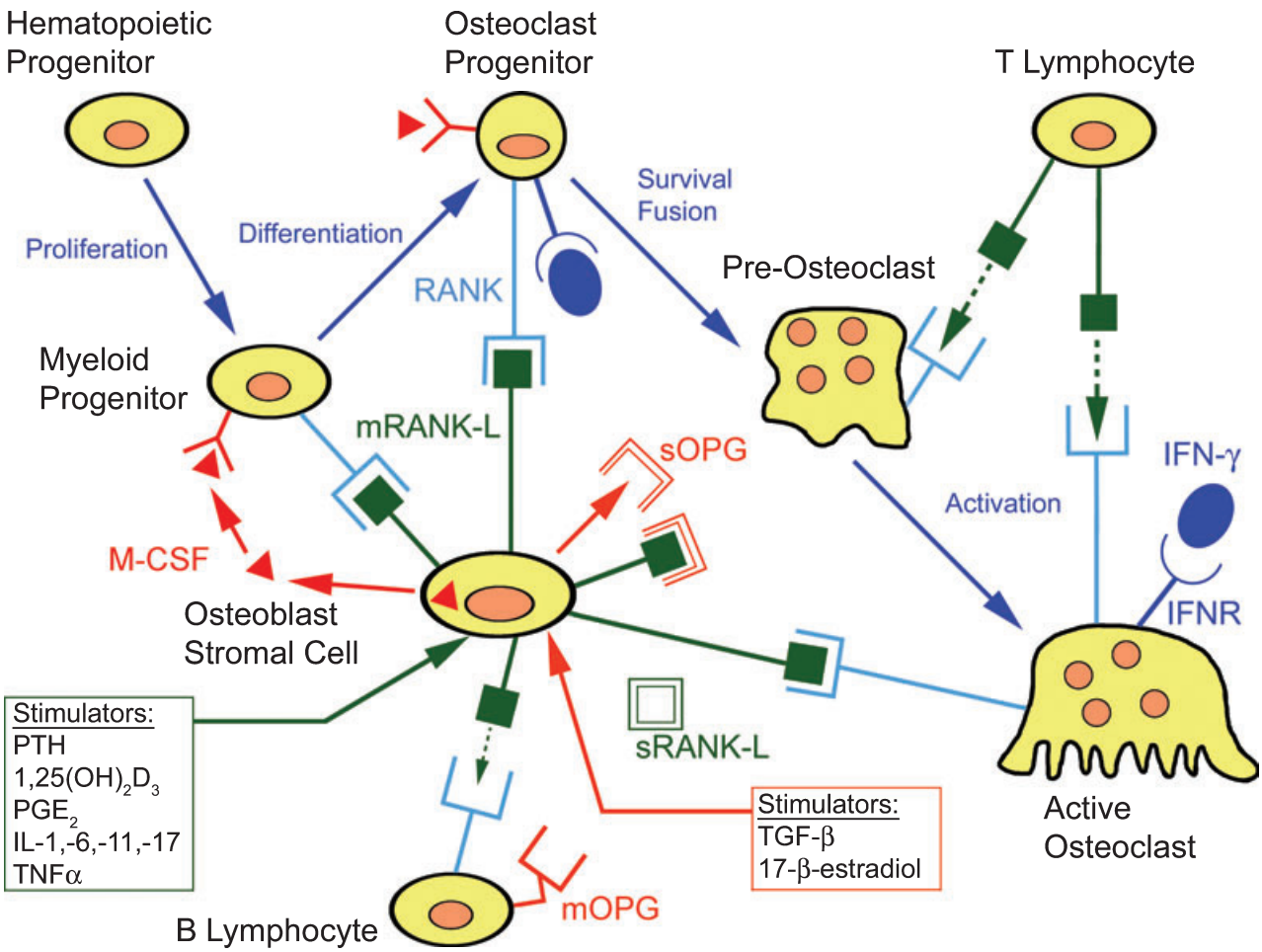

Fig. 9. Schematic illustration of the RANK-RANKL-osteoprotegerin system.

between bone formation and bone resorption. Receptor of nuclear factor-kappa (RANK) and its ligand RANK-L, members of the tumor necrosis factor family of receptors, are directly involved in the differentiation of osteoclast precursors and activation and survival of osteoclasts (Fig. 9). RANK-L is expressed by bone marrow stromal cells, osteoblasts, and fibroblasts, whereas RANK is expressed by osteoclasts precursors and mature osteoclasts. The binding of RANK and RANK-L induces osteoclast differentiation and activity. Osteoprotegerin, which is produced by bone marrow stromal cells, osteoblasts, and periodontal ligament fibroblasts, however, competes for this binding and functions as a soluble decoy receptor for RANK-L. Thus, osteoprotegerin is a natural inhibitor of osteoclast differentiation and activation. Any interference with this system can shift the balance towards increased bone formation or resorption. It has been shown that pro-inflammatory cytokines such as IL-1 and TNF-α, two very important players in periodontal bone loss, regulate the expression of RANK-L and osteoprotegerin. Moreover, T-cells also express RANK-L, which by binding directly to RANK on the cell membrane of osteclast progenitors, preosteoclasts, and osteoclasts, stimulates both cell differentiation and activation of cells of the osteoclast lineage. Thus, the shift of bone homeostasis toward bone resorption in periodontitis may be driven by pro-inflammatory cytokines that regulate the expression of RANK-L on both mesenchymal cells and specific activated T-cells. In this context, it has been demonstrated that there is diminished alveolar bone loss after oral infection with *P. gingivalis* in mice lacking T-lymphocytes (2). The discovery of this regulatory system linking bone biology with immune cell biology (29, 30, 34) has opened new therapeutic possibilities such as the inhibition of RANK–RANK–L interaction through the local application of osteoprotegerin.

Because of the exceptionally high rate of turnover of collagen in the periodontal ligament, any interference with fibroblast function by disease rapidly produces a loss of the tooth's supporting tissue. Importantly, in inflammatory situations, such as those associated with periodontal diseases, there is an increased expression of matrix metalloproteinases that aggressively destroy collagen (54). Thus, attractive therapies for controlling tissue destruction may include host-modulators that have the capacity to inhibit matrix metalloproteinases.

When inflammation reaches the root surface, resorption may occur, resulting in its excavation. It is likely that this destructive process involves some imbalance in the RANK / RANKL / osteoprotegerin system (Fig. 9) (7). Root resorption occurs quite commonly with hyperplastic gingivitis and less frequently adjacent to inflammatory lesions in the periodontal ligament. Fractures and microcracks in the superficial root portion may facilitate invasion of bacteria or diffusion of bacterial products into the root. Damaged cementum and dentin may also serve as a bacterial reservoir from which recolonization of scaled and planed root surfaces can occur. On the positive side, exposure to the oral environment often leads to the establishment of a hypermineralized zone in the superficial cementum layer. The mineral crystals in this superficial layer are very resistant to acid demineralization, slowing down the progression of carious lesions.

Concluding remarks

In the preface of Periodontology 2000 (57), editor Schroeder states the following: 'Insight into the architecture of (human) tissues is necessary to enable a creative mind to ask pertinent biological questions'. Indeed, knowledge of how tissue structure develops and how it relates to function is fundamental for understanding the disease process, and for devising effective therapeutic strategies, particularly in the case where tissue destruction, and hence a concomitant loss of function, ensues. The present chapter has been assembled with this in mind, and with the hope of providing investigators with solid bases for regenerative therapies. One particularly good example of induced tissue repair that may not give optimal functional results is the stimulation of cellular intrinsic fiber cementum formation for root repair. As was discussed, cellular intrinsic fiber cementum deposited onto periodontitis-affected roots that were treated in various ways is not a major medium for tooth attachment and, when deposited onto a mineralized surface, it does not interface with the preexisting calcified matrix and is thus subject to detachment. This may, however, be related to inappropriate root surface properties.

The first line of defense is the junctional epithelium. Its structural alteration is clearly the first step towards the progression of disease. Comparatively little attention has been given to understanding what triggers its formation and the composition of the basal lamina-like structure that mediates its attachment to the tooth surface. Could the constituents of this adhesive layer be used to slow down the downgrowth of the junctional epithelium and its

detachment from the tooth surface? Once disease has progressed beyond the epithelial seal and spreads to the connective tissue elements of the periodontium, regeneration is complicated by the fact that three tissues are now involved – cementum, the periodontal ligament, and bone. Although any of these can be in principle rebuilt, one must remember that tooth attachment function requires that the architecture of all three tissues be restored to corresponding degrees. In the case of cementum, in addition to quantity, the type of cementum will also be critical.

We still do not know whether the precursor to periodontal cells is ectodermal or ectomesenchymal, or whether there is more than one precursor. This may be an issue of semantics, however, since ectomesenchymal cells are also of ectodermal origin. The fact that current periodontal therapies mainly stimulate cellular intrinsic fiber cementum formation and that acellular extrinsic fiber cementum and cellular intrinsic fiber cementum can be distinctively affected by disease or experimental conditions, certainly suggests that specific cell pathways can be elicited and that different signaling pathways exist. For effective and targeted periodontal regeneration, it is thus most important to recognize this.

Over the past few years, enamel matrix proteins have generated much attention for periodontal repair and shown promising clinical results. Variability in clinical outcomes and the benefit with respect to open-flap debridement or guided tissue regeneration are still issues that need to be resolved. Relatively close clinical results can be obtained with very distinct therapeutic approaches, suggesting that these enamel matrix proteins may function indirectly. Indeed, there is still no evidence that they really are major players in root formation. Certainly, no major root defect has yet been reported in amelogenin and ameloblastin knockout and transgenic mice. This is not to say that enamel matrix proteins should not be used for periodontal treatment but rather that if we understood better the mechanism by which they exert their influence, we could use them or other proteins more efficiently.

References

1. Baba O, Qin C, Brunn JC, Jones JE, Wygant JN, Mcintyre BW, Butler WT. Detection of dentin sialoprotein in rat periodontium. *Eur J Oral Sci* 2004: **112**: 163–170.
2. Baker PJ, Howe L, Garneau J, Roopenian DC. T cell knockout mice have diminished alveolar bone loss after oral infection with *Porphyromonas gingivalis*. *FEMS Immunol Med Microbiol* 2002: **34**: 45–50.
3. Bartold PM, Walsh LJ, Narayanan S. Molecular cell biology of the gingiva. *Periodontol 2000* 2000: **24**: 28–55.
4. Beck JD, Slade G, Offenbacher S. Oral disease, cardiovascular disease and systemic inflammation. *Periodontol 2000* 2000: **23**: 110–120.
5. Beertsen W, Van Den Bos T, Everts V. Continuous growth of acellular extrinsic fiber cementum: a review. *Acta Med Dent Helv* 1997: **2**: 103–115.
6. Berkovitz BK. Periodontal ligament: structural and clinical correlates. *Dent Update* 2004: **31**: 46–50.
7. Bosshardt DD. Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? *J Dent Res* 2005: **84**: 390–406.
8. Bosshardt DD, Degen T, Lang NP. Sequence of protein expression of bone sialoprotein and osteopontin at the developing interface between repair cementum and dentin in human deciduous teeth. *Cell Tissue Res* 2005: **320**: 399–407.
9. Bosshardt DD, Lang NP. The junctional epithelium: from health to disease. *J Dent Res* 2005: **84**: 9–20.
10. Bosshardt DD, Nanci A. Immunodetection of enamel-, cementum-related (bone) proteins at the enamel-free area and cervical portion of the tooth in rat molars. *J Bone Miner Res* 1997: **12**: 367–379.
11. Bosshardt DD, Nanci A. Immunolocalization of epithelial and mesenchymal matrix constituents in association with inner enamel epithelial cells. *J Histochem Cytochem* 1998: **46**: 135–142.
12. Bosshardt DD, Nanci A. Immunocharacterization of cementicles and ectopic enamel. *J Dent Res (Spec Iss)* 2000: **79**: 563.
13. Bosshardt DD, Nanci A. Hertwig's epithelial root sheath enamel matrix proteins and initiation of cementogenesis in porcine teeth. *J Clin Periodontol* 2004: **31**: 184–192.
14. Bosshardt DD, Schroeder HE. How repair cementum becomes attached to the resorbed roots of human permanent teeth. *Acta Anat (Basel)* 1994: **150**: 253–266.
15. Bosshardt DD, Schroeder HE. Cementogenesis reviewed: a comparison between human premolars and rodent molars. *Anat Rec* 1996: **245**: 267–292.
16. Bosshardt DD, Sculean A, Windisch P, Pjetursson BE, Lang NP. Effects of enamel matrix proteins on tissue formation along the roots of human teeth. *J Periodontal Res* 2005: **40**: 158–167.
17. Bosshardt DD, Selvig KA. Dental cementum: the dynamic tissue covering of the root. *Periodontol 2000* 1997: **13**: 41–75.
18. Bosshardt DD, Zalzal S, Mckee MD, Nanci A. Developmental appearance, distribution of bone sialoprotein, osteopontin in human and rat cementum. *Anat Rec* 1998: **250**: 1–21.
19. Brien-Simpson NM, Veith PD, Dashper SG, Reynolds EC. *Porphyromonas gingivalis* gingipains: the molecular teeth of a microbial vampire. *Curr Protein Pept Sci* 2003: **4**: 409–426.
20. Butler WT, Brunn JC, Chunlin C, Mckee MD. Cell differentiation (cost-wg3); extracellular matrix proteins, the dynamics of dentin formation. *Connect Tissue Res* 2002: **43**: 301–307.

21. Bye FI, Caffesse RG, Nasjleti CE. The effect of different plaque control modalities on the keratinizing potential of the sulcular epithelium in monkeys. *J Periodontol* 1980: **51**: 632–641.
22. Caffesse RG, Kornman KS, Nasjleti CE. The effect of intensive antibacterial therapy on the sulcular environment in monkeys. Part II. Inflammation, mitotic activity and keratinization of the sulcular epithelium. *J Periodontol* 1980: **51**: 155–161.
23. Dave S, Batista ELJ, Van Dyke TE. Cardiovascular disease and periodontal diseases: commonality and causation. *Compend Contin Educ Dent* 2005: **25**: 26–37.
24. Feng JQ, Huang H, Lu Y, Ye L, Xie Y, Tsutsui TW, Kunieda T, Castranio T, Scott G, Bonewald LB, Mishina Y. The dentin matrix protein 1 (dmp1) is specifically expressed in mineralized, butnotsoft tissues during development. *J Dent Res* 2003: **82**: 776–780.
25. Fong CD, Hammarstrom L. Expression of amelin and amelogenin in epithelial root sheath remnants of fully formed rat molars. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000: **90**: 218–223.
26. Fong CD, Hammarström L, Lundmark C, Wurtz T, Slaby I. Expression patterns of RNAs for amelin and amelogenin in developing rat molars and incisors. *Adv Dent Res* 1996: **10**: 195–200.
27. Frank DE, Carter WG. Laminin 5 deposition regulates keratinocyte polarization and persistent migration. *J Cell Sci* 2004: **117**: 1351–1363.
28. Garcia RI, Henshaw MM, Krall EA. Relationship between periodontal disease and systemic health. *Periodontol 2000* 2001: **25**: 21–36.
29. Goldring SR. Inflammatory mediators as essential elements in bone remodeling. *Calcif Tissue Int* 2003: **73**: 97–100.
30. Grcevic D, Katavic V, Lukic IK, Kovacic N, Lorenzo JA, Marusic A. Cellular and molecular interactions between immune system and bone. *Croat Med J* 2001: **42**: 384–392.
31. Groeneveld MC, Van Den Bos T, Everts V, Beertsen W. Cell-bound and extracellular matrix-associated alkaline phosphatase activity in rat periodontal ligament. *J Periodontal Res* 1996: **31**: 73–79.
32. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. *Crit Rev Oral Biol Med* 2002: **13**: 474–484.
33. Hakkinen L, Oksala O, Salo T, Rahemtulla F, Larjava H. Immunohistochemical localization of proteoglycans in human periodontium. *J Histochem Cytochem* 1993: **41**: 1689–1699.
34. Hofbauer LC, Heufelder AE. Role of receptor activator of nuclear factor-κB ligand and osteoprotegerin in bone cell biology. *J Mol Med* 2001: **79**: 243–253.
35. Kadowaki T, Yamamoto K. Suppression of virulence of *Porphyromonas gingivalis* by potent inhibitors specific for gingipains. *Curr Protein Pept Sci* 2003: **4**: 451–458.
36. Karring T, Lang NP, Loe H. The role of gingival connective tissue in determining epithelial differentiation. *J Periodontal Res* 1975: **10**: 1–11.
37. Karring T, Nyman S, Gottlow J, Laurell L. Development of the biological concept of guided tissue regeneration – animal and human studies. *Periodontol 2000* 1993: **1**: 26–35.
38. Karring T, Ostergaard E, Loe H. Conservation of tissue specificity after heterotopic transplantation of gingiva and alveolar mucosa. *J Periodontal Res* 1971: **6**: 282–293.
39. Kirkham J, Brookes SJ, Shore RC, Bonass WA, Robinson C. The effect of glycosylaminoglycans on the mineralization of sheep periodontal ligament *in vitro*. *Connect Tissue Res* 1995: **33**: 23–29.
40. Lindskog S. Formation of intermediate cementum. I. Early mineralization of aprismatic enamel and intermediate cementum in monkey. *J Craniofac Genet Dev Biol* 1982: **2**: 147–160.
41. Lindskog S. Formation of intermediate cementum. II. A scanning electron microscopic study of the epithelial rooth sheath of Hertwig in monkey. *J Craniofac Genet Dev Biol* 1982: **2**: 161–169.
42. Lindskog S, Hammarström L. Formation of intermediate cementum. III: ^{3}H-tryptophan and ^{3}H-proline uptake into the epithelial root sheath of Hertwig *in vitro*. *J Craniofac Genet Dev Biol* 1982: **2**: 171–177.
43. Lowenguth RA, Blieden TM. Periodontal regeneration: root surface demineralization. *Periodontol 2000* 1993: **1**: 54–68.
44. Macdougall M, Gu TT, Luan XG, Simmons D, Chen JK. Identification of a novel isoform of mouse dentin matrix protein. 1. Spatial expression in mineralized tissues. *J Bone Miner Res* 1998: **13**: 422–431.
45. Mariotti A. Efficacy of chemical root surface modifiers in the treatment of periodontal disease: a systematic review. *Ann Periodontol* 2003: **8**: 205–226.
46. Marks SCJR, Mckee MD, Zalzal S, Nanci A. The epithelial attachment and the dental junctional epithelium: ultrastructural features in porcine molars. *Anat Rec* 1994: **238**: 1–14.
47. Matias MA, Li H, Young WG, Bartold PM. Immunohistochemical localisation of extracellular matrix proteins in the periodontium during cementogenesis in the rat molar. *Arch Oral Biol* 2003: **48**: 709–716.
48. Mizuno N, Shiba H, Mouri Y, Xu W, Kudoh S, Kawaguchi H, Kurihara H. Characterization of epithelial cells derived from periodontal ligament by gene expression patterns of bone-related and enamel proteins. *Cell Biol Int* 2005: **29**: 111–117.
49. Nanci A. Content and distribution of noncollagenous matrix proteins in bone and cementum: relationship to speed of formation and collagen packing density. *J Struct Biol* 1999: **126**: 256–269.
50. Nanci A, Somerman M. The periodontium. In: Nanci A, ed. *Ten Cate's Oral histology: development, structure, and function.* St. Louis: Harcourt Health Sciences, 2003.
51. Nociti FH, Berry JE, Foster BI, Gurley KA, Kingsley DM, Takata T, Miyauchi M, Somerman MJ. Cementum: a phosphate-sensitive tissue. *J Dent Res* 2002: **81**: 817–821.
52. Ohno S, Doi T, Fujimoto K, Ijuin C, Tanaka N, Tanimoto K, Honda K, Nakahara M, Kato Y, Tanne K. Rgd-Cap (Beta Ig-H3) exerts a negative regulatory function on mineralization in the human periodontal ligament. *J Dent Res* 2002: **81**: 822–825.
53. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontol 2000* 1997: **14**: 216–248.
54. Potempa J, Banbula A, Travis J. Role of bacterial proteinases in matrix destruction and modulation of host responses. *Periodontol 2000* 2000: **24**: 153–192.
55. Potempa J, Travis J. *Porphyromonas gingivalis* proteinases in periodontitis: a review. *Acta Biochim Pol* 1996: **43**: 455–465.

56. Saffar JI, Lasfargues JJ, Cherruau M. Alveolar bone and the alveolar process: the socket that is never stable. *Periodontol 2000* 1997: **13**: 76–90.
57. Schroeder HE. Biological structure of the normal and diseased periodontium – preface. *Periodontol 2000* 1997: **13**: 7–7.
58. Schroeder HE, Listgarten MA. The gingival tissues: the architecture of periodontal protection. *Periodontol 2000* 1997: **13**: 91–120.
59. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004: **364**: 149–155.
60. Slavkin HC, Bessem C, Fincham AG, Bringas PJR, Santos V, Snead MI, Zeichner-David M. Human and mouse cementum proteins immunologically related to enamel proteins. *Biochim Biophys Acta* 1989: **991**: 12–18.
61. Sodek J, Ganss B, Mckee MD. Osteopontin. *Crit Rev Oral Biol Med* 2000: **11**: 279–303.
62. Teng YT. The role of acquired immunity and periodontal disease progression. *Crit Rev Oral Biol Med* 2003: **14**: 237–252.
63. Thomas CH, Mcfarland CD, Jenkins MI, Rezania A, Steele JG, Healy KE. The role of vitronectin in the attachment and spatial distribution of bone-derived cells on materials with patterned surface chemistry. *J Biomed Mater Res* 1997: **37**: 81–93.
64. Wu DY, Ikezawa K, Parker T, Saito M, Narayanan AS. Characterization of a collagenous cementum-derived attachment protein. *J Bone Miner Res* 1996: **11**: 686–692.
65. Yamamoto T, Domon T, Takahashi S, Arambawatta AKS, Wakita M. Immunolocalization of proteoglycans and bone-related noncollagenous glycoproteins in developing acellular cementum of rat molars. *Cell Tissue Res* 2004: **317**: 299–312.
66. Yamazaki K, Yoshie H, Seymour GJ. T cell regulation of the immune response to infection in periodontal diseases. *Histol Histopathol* 2003: **18**: 889–896.
67. Yoshizawa T, Takizawa F, Iizawa F, Ishibashi O, Kawashima H, Matsuda A, Endo N, Kawashima H. Homeobox protein Msx2 acts as a molecular defense mechanism for preventing ossification in ligament fibroblasts. *Mol Cell Biol* 2004: **24**: 3460–3472.
68. Zeichner-David M, Oishi K, Su Z, Zakartchenko V, Chen LS, Arzate H, Bringas P Jr. Role of Hertwig's epithelial root sheath cells in tooth root development. *Dev Dyn* 2003: **228**: 651–663.
69. Zhang X, Schuppan D, Becker J, Reichart P, Gelderblom HR. Distribution of undulin, tenascin, and fibronectin in the human periodontal ligament and cementum: comparative immunoelectron microscopy with ultra-thin cryosections. *J Histochem Cytochem* 1993: **41**: 245–251.