The Dentinoenamel Junction

8

Michel Goldberg

Abstract

The dentinoenamel junction (DEJ) is the border where five different structures meet: the cervical enamel, two superficial outer dentin layers (Tomes' granular and Hopewell-Smith hyaline layers), located over the inner circumpulpal dentin, and cementum (afibrillar acellular cementum and fibrillar cellular cementum). The DEJ is a complex scalloped structure associating at least two calcified tissues and preventing the propagation of cracks from enamel to dentin. It constitutes a biomimetic model of a structure uniting dissimilar materials. Its composition includes type I collagen, phosphorylated (SIBLINGs) and non-phosphorylated proteins (e.g., small leucine-rich proteoglycans (SLRPs), and some extracellular matrix molecules taking origin from the blood serum. Enzymes, metalloproteinases, and lipoproteins participate in its formation. Altogether they contribute to the DEJ mineralization, human enamel rod presenting anisotropic and nanotribological properties. Gradient of mineralization influences abfraction formation (Cuy et al. Arch Oral Biol 47:281–91, 2002; He and Swani J Dent 35:431–7, 2007; Imbeni et al. Nat Mater 4:229–32, 2005). The mechanical properties of the cervical zone of the teeth are functions of microstructural orientation of the mineral and organic matrix.

8.1 Cervical Cemento-Dentinal Formation

During initial tooth formation, four layers of the enamel organ (the outer and inner enamel layers associated with the stellate reticulum and stratum intermedium) contribute to the construction of the crown. Building the limits of the coronal part of the tooth, the outer and inner enamel layers merge in the cervical zone and form the so-called epithelial Hertwig's root sheath. During the

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initial root shaping, the epithelial cells influence the recruitment and cytodifferentiation of pulp stem cells, which migrate from the neural crest toward the dental mesenchyme. Later, stem cells move from the central embryonic pulp toward the pulp periphery, where they differentiated into pre-odontoblasts, and then become polarizing odontoblasts. Odontoblasts initiate the synthesis and secretion of predentin, which represent an early stage of dentinogenesis. Predentin matures and some extracellular matrix components are added, contributing to dentin formation and mineralization. At this stage of crown formation, maturing enamel covers primary dentin.

Near the outer dentin surface, the inner layer of the Hertwig's epithelial sheath synthetizes and secretes an acellular afibrillar cementum covering the rudiments of the roots. As an alternative possibility, it was also reported that the cells issued from the dental sac or from the dental follicle migrate through the enlarged intercellular spaces. They migrate between the cells of the disintegrated Hertwig's sheath, cross through the periodontal ligament, and migrate toward the forming root, where they contribute to the acellular cervical cementum formation. At that stage, the initial root elongates but is not yet associated with tooth eruption. During primary eruption, the lengthening of the root occurs in close association with the onset of tooth development.

Therefore, the cervical region involves a complex imbrication of tissues and cells. In the cervical zone, at the enamel margin, two outer dentin layers are found. Depending on the species studied, the Tomes' granular layer and the Hopewell-Smith hyaline zone are involved. The structure formed is also named intermediate cementum layer. Either cementum covers the cemento-dentinal junction of human teeth, or enamel and dentin are contiguous, end-to-end, or there is a gap between enamel and cementum, the dentin outer layer being naked, uncovered by cementum.

According to some authors, the growth of enamel crystals starts from the underlying calcified dentin, promoting the formation of enamel crystals (Arsenault and Robinson 1989). An opposite point of view was developed by Diekwisch et al. (2001). They put emphasis on the fact that enamel crystal formation is totally independent from the dentin crystals. Enamel crystal formation starts near the DEJ; some distance away from the mineralizing dentin, but seem to be not associated to the formation of dentin crystals. These diverging views still need to be elucidated or reconciled, perhaps depending on the species examined (Diekwisch et al, 1995, Baldassarri et al. 2008, Weber et al. 1974, and Hayashi 1992).

8.2 Biomechanical Aspects of the Cervical Zone

8.2.1 Structural Aspects of Cervical Enamel and Dentin

8.2.1.1 Enamel

In the cervical region, the prisms appear to be directed horizontally, passing from the amelodentinal junction to the surface of the tooth, and covered by a thin aprismatic enamel layer. In a few cases, this enamel layer is partially covered in the cervical region by a thin border of aprismatic afibrillar cementum. The tissue nonspecific alkaline phosphatase (TNAP) and blood circulation act as a major source of osteopontin (VandenBos et al. 1999) and they regulate its formation (Fig. 8.1).

Non-carious cervical lesions refer to the loss of tooth structure at the cemento-enamel junction, and they are unrelated to bacterial action (see Chapter 2 of this book). Horizontal scratch marks are characteristics of the abrasion processes. Shallow, grooved, and wedge-shaped cervical lesions were identified. Corrosion induces a smoother appearance. Enamel displays a honeycomb structure, whereas dentin exhibits an undulating or rippled surface. In vitro study notes fracture and chipping of enamel in the cervical region (Nguyen et al. 2008). The AFM nanoindentation was used to probe the mechanical and tribological properties of enamel rods. Microhardness and elastic properties are significantly higher in the head region of the rod rather than in the tail region (Jeng et al. 2011).

Enamel, dentin, and cementum are coupled at the DEJ. The DEJ is a complex structure associating

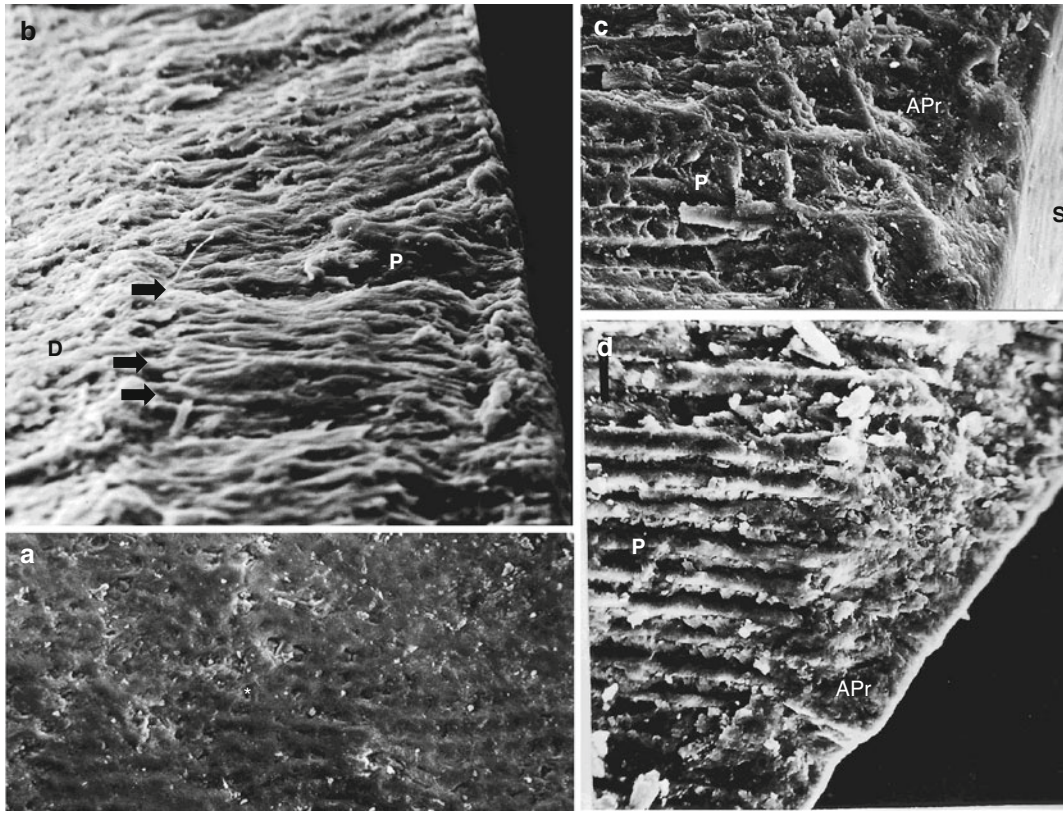

Fig. 8.1 (**a**) Enamel surface near the enamel-cementum junction. Prints or depressions of the proximal zone of ameloblasts form alignments (*asterisk*). (**b**) In the cervical zone, prisms (*P*) are aligned and parallel. The dentinoenamel junction (*black arrows*) separates the cervical enamel from dentin (*D*). In (**c**), the prismatic enamel (*P*) is covered by a thin layer of aprismatic enamel (*Apr*), ending at the enamel surface (*S*). In (**d**), in older enamel, the same distribution is visible

two calcified tissues, preventing the propagation of cracks from enamel to dentin. It is a scalloped structure with convexities toward dentin and concavities toward enamel (Shimizu & Macho 2007, Brauer et al. 2010, Beniash et al 2006) (Figs. 8.2 and 8.3).

8.2.1.2 Dentin

In the superficial part of the outer dentin, two different layers have been identified. The thin (8–15 μm thick) Tomes' granular layer is composed by calcospheritic (oval or round) structures. This zone contains a few minute tubules. The thin curved tubules are scarce and bent around the calcospheritic structures. Between the calcospherites, interglobular spaces are hypomineralized.

The subjacent layer (Hopewell-Smith hyaline layer, 8–15 μm thick) displays a small number of tubules. These tubules are straight, aligned, and at right angles to the tooth surface. The bulk of inner dentin, located around the dental pulp, is implicated in circumpulpal dentin formation. Usually, cells are alive and tubules are filled with odontoblast processes and their lateral branching. The zone of sclerotic dentin is thinner in these areas compared with normal dentin, and the changes occurring near the dentin surface are due to abrasion, abfraction, erosion, and wedge-shaped lesions. The carious decay contributes to the occlusion of the tubules by intratubular mineralizations (whitlockite crystallites, tricalcium phosphate) (Goldberg 2014; Tay and Pashley 2004) (Figs. 8.3 and 8.4).

AFM-based nanoindentations found the DEJ to only be 11.8 μm across. Micro-Raman indicated a DEJ width of 7.0 μm, while dynamic

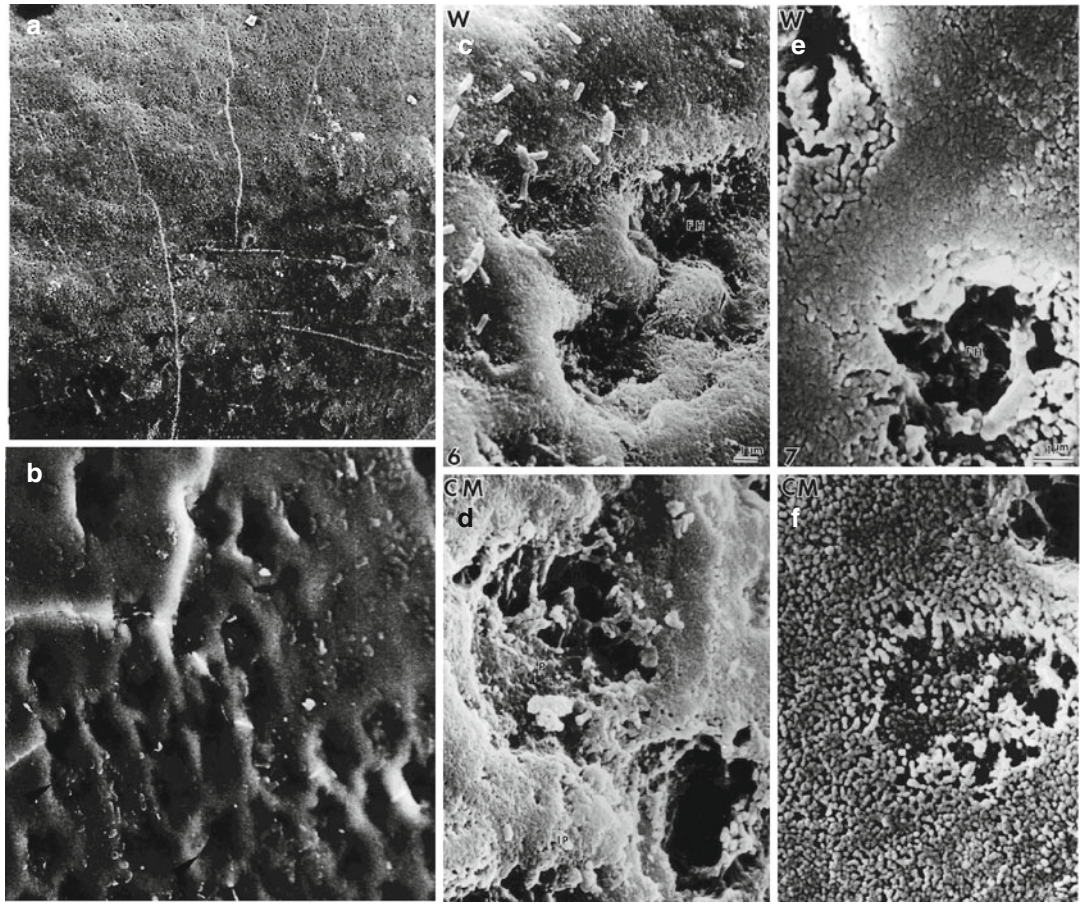

Fig. 8.2 (**a**) Water-treated enamel surface. (**b**) Enamel surface after water treatment. (**c**) Water treatment (**d**) enamel surface treated with chloroform-methanol. (**e**) Water treatment revealed the end of rods. (**f**) Chloroform-methanol treatment of enamel surface. The distal ending of rods displays large porosities, whereas in the interrod enamel, inter-crystallite porosities appear after the removal of extracellular lipids

modulus mapping indicated it was less than 1 μm across. The variations based on enamel-dentin phase intermixing suggest that DEJ is a biomimetic model for interfaces joining dissimilar materials (Marshall et al. 2003).

8.2.1.3 Cervical Lesions

Cracks generated in enamel stop at the DEJ, preventing catastrophic failure of the tooth. The major emerging question concerns the effects of organic matrix and water on the structural organization and how tooth microhardness and fracture toughness are affected. The removal of organic matrix resulted in 23 % increase in microhardness and 46 % decrease in fracture toughness. In contrast, water does not seem to influence these parameters. Moreover, the removal of organic matrix weakened the DEJ, leading to the formation of longer and more numerous cracks. Delamination of dentin and enamel along the DEJ suggests a strong physical bond between dentin and enamel crystals at the interface.

Retzius lines in the human cervical enamel display a staircase configuration. The Retzius lines have a curvilinear configuration, and

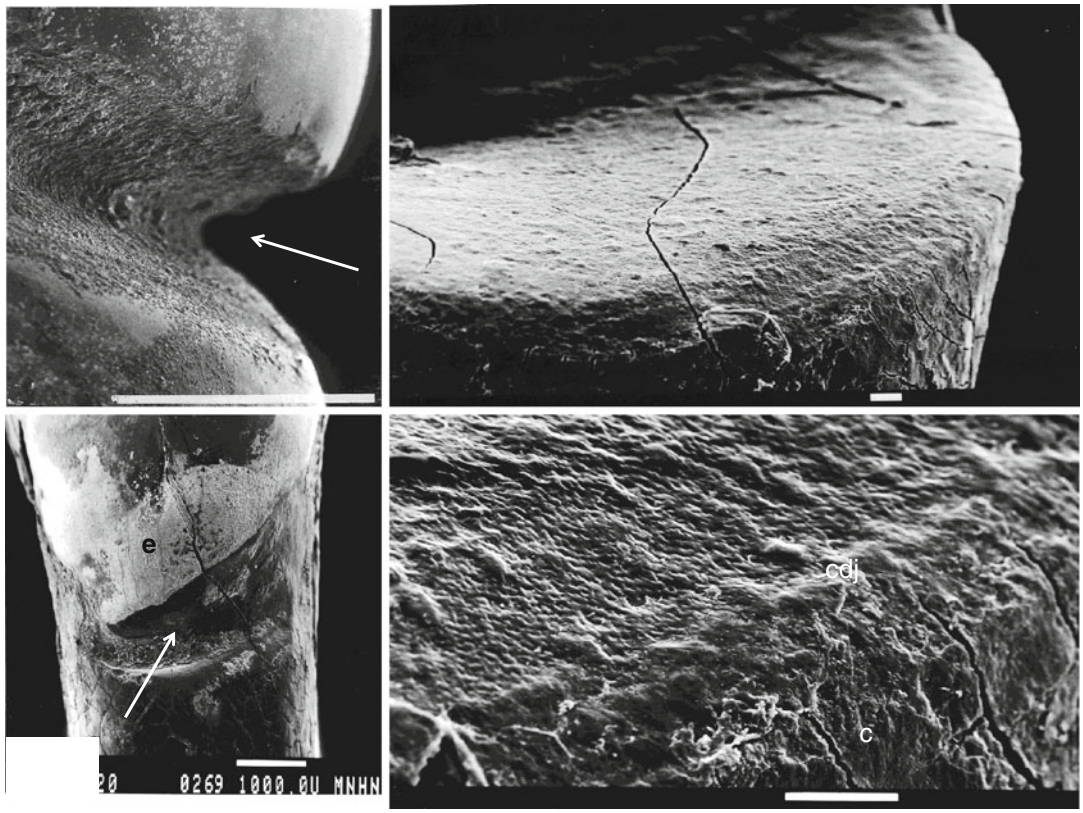

Fig. 8.3 Cervical erosion (*arrows*); *C* cementum, *cdj* cemento-dentinal junction

crystallites are deficient in composition. Finally, direct contact is occurring between lattice fringes of dentin and enamel crystals, fusion being observed between enamel and dentin crystals. The DEJ is weak in mechanical and/or chemical attacks (Fig. 8.3).

8.2.2 Mechanical and Tribological Properties of the DEJ

The DEJ is a complex structure and poorly defined, bridging enamel to the bulk dentin. This structure constitutes a biomimetic model of a structure uniting dissimilar materials. Cracks cannot traverse the DEJ and/or produce cracks in dentin. The fracture toughness values for enamel were evaluated as 0.6–0.9 MPa.m1/2 (Marshall et al. 2001) (Figs. 8.3 and 8.4).

Many reports suggest that abfraction lesion formation is caused by the physical overloading of enamel (Rees and Hammadeh 2004). In the crowns of human teeth, beneath enamel, a 200–300 μm zone of resilient (less mineralized and elastic) dentin has been found in the DEJ (Zaslansky et al. 2006). The strain distribution in the 200 μm thick zone in dentin beneath the DEJ is a structural adaptation for transferring and minimizing stress (Wang and Weiner 1997). Root dentin is highly anisotropic in fracture behavior. Coronal dentin has a typical brittle fracture behavior along peritubular dentin, and this should be taken into consideration that dentin is not homogeneous with respect to fracture properties (Wang 2005).

Peritubular dentin located at some distance from the DEJ gradually thickens with increasing depth in the bulk dentin. A significant reduced stiffness of

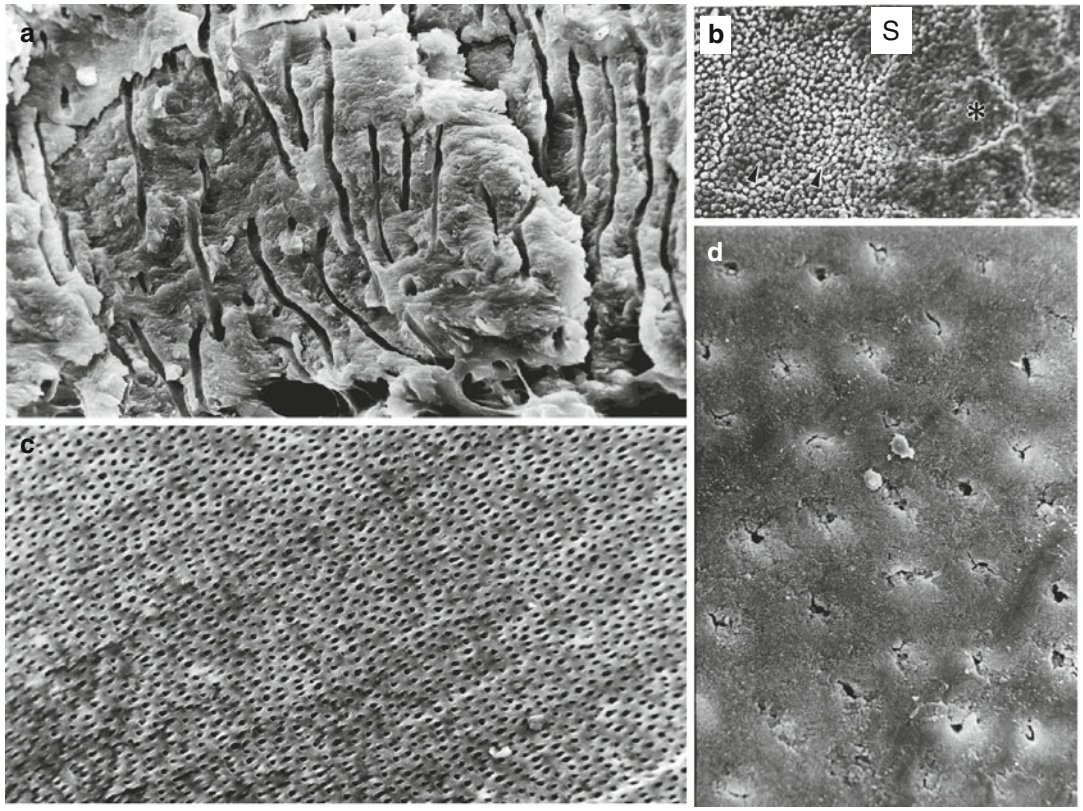

Fig. 8.4 (**a**) Outer globular Tomes' granular structure. (**b**) Cementum covering the root surface (*S*). *Left part of the figure*: acid-etched enamel. (**c**) Circumpulpal dentin. (**d**) Sclerotic dentin

the superficial zone has been reported compared to bulk dentin. In mid-buccal regions of teeth, the average was 3.5GPa, compared with the 9.7 GPa in the mid-lingual regions. It was concluded that the superficial layer behaves as a stress-relieving layer between enamel and bulk dentin.

Microhardness and elastic modulus are higher in the head region of enamel rods. They decrease from the enamel surface toward the DEJ. Head and tail area are questionable structures, and many ultrastructural studies deny the reality of this organization. The mechanical and nanotribological properties of enamel rods depend on HAp orientation inside each rod. The wear rate increases with an increasing distance from the outer enamel surface along the longitudinal axis of the enamel rod reaching the DEJ (Jeng et al. 2011).

The DEJ exhibits a scalloped appearance. The scalloped model has higher maximum tensile stresses than the straight model, but axial pressures would push the two tissue apart, leading to delamination of the DEJ during loading (mastication). There is a direct correlation between prism decussation and scallop magnitude. The scallops are linked with the response to high bite forces. *Exaptation* is used for a function other than what is developed by natural selection.

The posterior teeth showed larger scallops compared to anterior teeth. Molars subjected to higher masticatory loads showed larger and more pronounced scallops (Simmer et al. 2011; Ivancik et al. 2011).

8.2.3 Specific Composition of the DEJ

The enamel layer has a rich content in enamel proteins (amelogenins, enamelin, tuftelin, and other molecules). The proteins are characterized

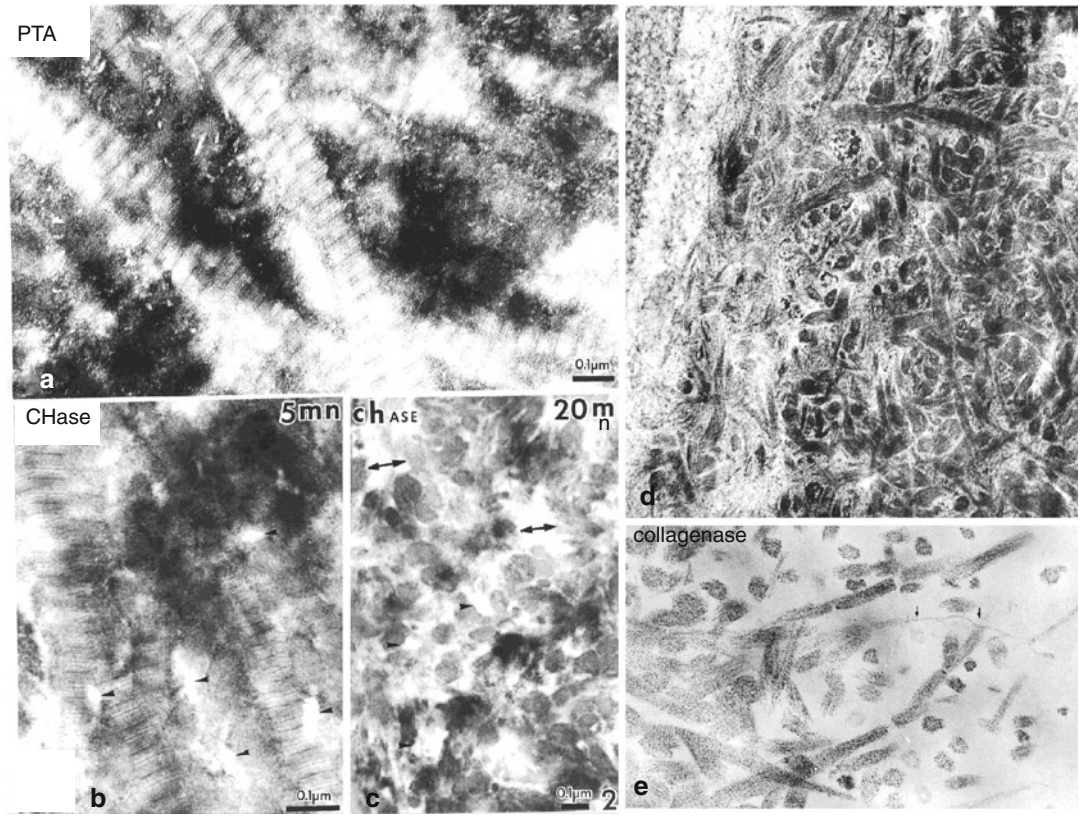

Fig. 8.5 (**a**) Phosphotungstic acid-stained circumpulpal dentin. At the surface of the collagen fibrils (characterized by their periodic banding), PTA (glycoprotein-stained) electro-dense underlines the collagen profile. Electro-dense staining is present in the intercollagen spaces. These phosphorylated glycoproteins (SIBLINGs) contribute to dentin mineralization. (**b**, **c**) Chondroitinase acting for 5 min (**b**) or 20 min (**c**) removes gradually intercollagen fibrils glycosaminoglycans, without destruction of the collagen fibrils. (**d**) In circumpulpal dentin, a dense network of collagen fibrils forms the intertubular dentin, whereas along the tubule (left part of the figure), collagen fibrils are less numerous and display less density. (**e**) Section treatment with a bacterial collagenase removes a large part of the collagen fibrils. Thin fibrils are cut in ¼ and ¾ (*small arrows*), contributing to gelatinase A and B formation

by the presence of high serine, glutamic acid, and glycine content. Components rich in proline and histidine are lost during enamel development and maturation (Glimcher et al. 1964).

These extracellular matrix proteins contribute to initiate the formation of the large and elongated enamel crystallites. By contrast, the ECM dentin molecules are composed by 90 % collagen, namely, type I collagen (Lin et al. 1993). The dentinoenamel junction is a fibril-reinforced bond, which is mineralized to a moderate degree.

Other types of collagen are scarce (type III and V collagens), but actually present. Deposition of dentin crystallites occurs (1) within the collagen gap regions (due to the quarter stagger structure of collagen fibrils), (2) along the collagen fibrils network, and (3) bridging inter-collagen spaces. The nucleation and crystal growth of dentin crystals are promoted and developed by a series of non-collagenous proteins, namely, phosphorylated proteins (SIBLINGs) (Fig. 8.5a, b). Five of them play crucial roles in the mineralization process: the dentin phosphoprotein, dentin matrix protein-1, bone sialoprotein, osteopontin, and MEPE.

Dentin sialoprotein and dentin phosphoprotein have distinct functions related to tooth formation and DEJ formation. Dentin proteins expressed by presecretory ameloblasts contribute to the unique properties of the dentinoenamel

junction. These results support the notion that the dentin proteins expressed by presecretory ameloblasts contribute to the unique properties of the dentinoenamel junction (Paine et al. 2005).

Non-phosphorylated molecules such as γ-carboxyglutamic acid (GLA-rich, osteocalcin) are also found in the developing enamel proteins. They are inhibitors of mineralization.

Proteins issued from the serum, proteoglycans (small leucine-rich proteoglycans (SLRPs)), nucleating enzymes (tissue nonspecific alkaline phosphatases – TNAP), and a series of proteases (MMPs and ADAMs) act either as nucleators or inhibitors of dentin mineralization.

The dentinoenamel junction is not a simple inert interface between two mineralized structures. A less simplistic view suggests that the dentinoenamel junctional complex should also include the inner aprismatic enamel and the mantle dentin. At early stages of enamel formation, fibroblast growth factor (FGF)-2 is stored in and released from the inner aprismatic enamel, possibly under the control of matrix metalloproteinase (MMP)-3 (DenBesten et al. 1989). The concentration peak for MMP-2 and MMP-9 observed in the mantle dentin coincided with a very low labeling for TIMP-1 and TIMP-2 (Fig. 8.5e). This distribution favors the cross talk between mineralizing epithelial and connective structures and as a consequence the translocation of enamel proteins toward odontoblasts and pulp cells. Vice versa, it facilitates the translocation of dentin proteins toward secretory ameloblasts and cells of the enamel organ (Goldberg et al. 2002). Finally, in X-linked hypophosphatemic rickets, large interglobular spaces in the circumpulpal dentin were the major defect induced by the gene alteration, whereas the mantle dentin was constantly unaffected. Altogether, these data plead for the recognition of the dentinoenamel junctional complex as a specific entity bearing its own biological characteristics.

8.2.4 Enzymes

Host MMP-2 may be involved in caries progression and BSP in MMP-2 modulation. Enamelysin (MMP-20)-deficient mouse incisors display delamination of the enamel layer. At early stages of tooth morphogenesis of KO mice (MMP-20 KO mice), the mantle dentin is hypomineralized at the onset of enamel mineralization. Later no difference is found, so the mineralization of mantle dentin is simply postponed but not arrested.

A KLK4-null mouse has a normal thickness and pattern of enamel rods but contains residual enamel proteins. Enamel is less mineralized, and fractures are just above the DEJ. The breakage of enamel is apparently related to the progressive hypomineralization of enamel with depth.

The relationship between deep, middle, and peripheral coronal dentin supports that deep dentin exhibits significantly lower resistance to the initiation and growth of fatigue crack growth compared with the middle and peripheral dentin.

Different types of dentin are observed in human teeth. The primary dentin is formed before any occlusal contact. Secondary dentin is formed during the whole life and constitutes an answer to physiological aging. This dentin corresponds to the gradual narrowing of the pulp chamber. Sclerotic dentin and transparent dentin refer to tertiary dentin. These structures result from caries or irritation processes. Aging, abrasion, and diseases processes are implicated in what has been named reactionary dentin formation. In addition to the peripheral dentin layers (Tomes' granular layer and Hopewell-Smith hyaline layer), the circumpulpal dentin varies between the lingual and labial zones, where dentin is mostly tubular, and the mesial or distal pulp surfaces (proximal dentin surfaces) are mostly formed by fibrodentin structures (Fig. 8.5e).

8.2.5 Abfraction Lesion Formation

In order to understand the microfracture and deformation and the microcrack-microstructure interactions, the influence (effect) of enamel rod orientation was checked by propagating cracks in the occlusal surface. The cracks propagating toward the DEJ were arrested and unable to penetrate dentin. The mechanical properties of teeth are functions of microstructural orientations (Xu et al. 1998).

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