2 Dentin Basic Structure, Composition, and Function

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Abstract

Dentin is the largest structural component of the human tooth. Dentin provides support to enamel, preventing enamel fractures during occlusal loading. It also protects the pulp from microbial and other potentially harmful stimuli. As vital tissue, dentin is not only a passive mechanical barrier between the oral environment and the pulp tissue but, in many ways, participates in the overall protection of the continuum of the hard and soft tissue often referred as the dentin-pulp complex. For example, dentin contains several growth factors that may be liberated during wear or caries and participate in the regulation of the defensive reaction at the dentin-pulp border or the pulp proper. Odontoblasts project their cell processes into dentinal tubules, and also therefore the division of the "vital" pulp and "dead" mineralized dentin is artificial. Different parts of the dentin in a particular tooth may also qualitatively differ from each other, which enables it to meet the requirements of that specific location.

2.1 Introduction

Dentin can be described in various ways, based on the composition, structure, or type of dentin. Usually, dentin is described as extracellular organic matrix that has been mineralized, much like the bone. As a matter of fact, dentin is nanocrystalline-reinforced biocomposite, which gives it its unique properties. About 70% (55% in volume) and 20% (30% in volume) in weight are minerals and organic components, respectively, the rest being water. However, due to the tubular nature of dentin and the occlusion of tubules by peritubular (intratubular) dentin with age and as defensive reaction, these values are only average.

The structure of dentin can also be divided into intertubular and peritubular dentin. Vast majority of the organic component is located in the intertubular dentin formed by the odontoblasts at the dentin-pulp border. Due to the tubular occlusion, the amount of minerals and division between inter- and peritubular dentin can vary significantly between the different parts of the tooth, as the peritubular dentin slowly occupies the tubular lumen. At the same time, water content in these areas is, respectively, reduced. And finally, dentin

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is frequently classified by its phase of formation: dentin-enamel junction, mantle dentin, primary dentin, secondary dentin, and tertiary dentin. Tertiary dentin formation is part of the dentin-pulp complex defensive reaction aiming to protect the pulp and can further be divided into reactionary and reparative dentin, depending on the structure and cells forming the dentin (primary or replacement odontoblasts, respectively) [1].

2.2 Dentin Formation

2.2.1 Odontoblasts

Dentin is almost exclusively formed by the odontoblasts that are derived from embryonic connective ectomesenchymal cells from the cranial neural crest [2]. The differentiating odontoblasts start the secretion of the predentinal proteins, followed by the initiation of enamel matrix secretion by the differentiating ameloblasts, at the site where the dentin-enamel junction (DEJ) is formed. During and right after the differentiation, the odontoblasts organize into a distinguished odontoblast cell layer, and the mineralization of organic matrix completes the formation of the first layer of dentin, mantle dentin [2].

In the coronal part of the tooth, odontoblasts are tall cells, and their morphological and cell membrane polarization [3] is unique in the group of collagen-synthesizing cells. Odontoblasts form a single layer of cells between dentin and pulp, with the cell body located on a pulpal wall of dentin and odontoblast processes inserted into dentinal tubules (Figs. 2.1 and 2.2a). The cell bodies are 20–40 μm tall, depending on dentinogenic activity. The odontoblast process is a cytoplasmic process which penetrates into mineralized dentin tubules. The process has the 0.5–1 μm main trunk and thinner lateral branches [4].

One of the longest controversies in dentin-pulp complex research has been the extent of odontoblast processes into dentinal tubules. This is caused by the conflicting results obtained with different research methods and by the possible differences between the species [4]. In human teeth, most studies indicate that the odontoblast cell processes would not extend far from the dentin-pulp border (200–700 μm) (Fig. 2.2b).

2.2.2 Predentin and Mineralization Front

The 10–30 μm layer of unmineralized predentin is located between odontoblasts and mineralized dentin (Figs. 2.1 and 2.2a). This is where the dentin organic matrix is organized before the controlled mineralization at the mineralization front

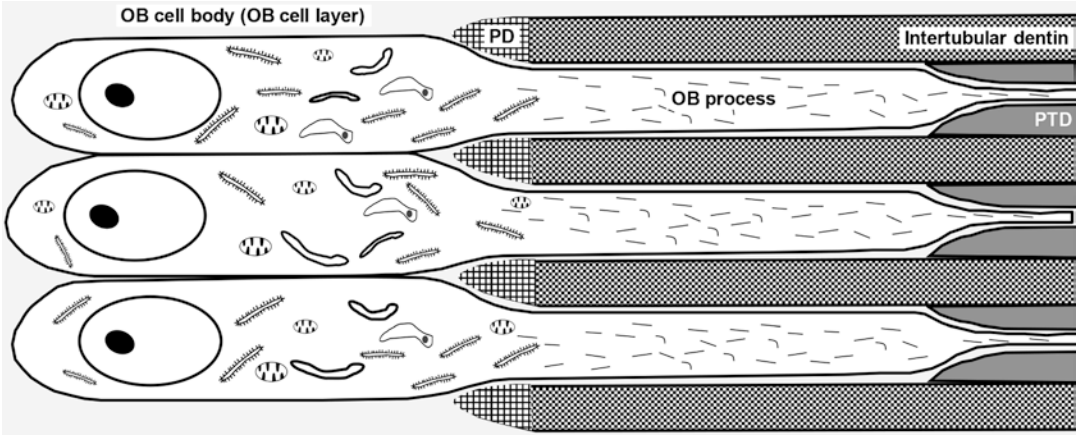

Fig. 2.1 Drawing of the dentin-pulp border. Odontoblast (OB) cell bodies with large nucleus at the pulpal terminus of the cell and cytoplasmic organelles form a tight cell layer separated from the mineralized dentin by predentin (PD) where the intertubular dentin organic matrix is organized before mineralization. Odontoblast processes penetrate into mineralized intertubular dentin in dentinal tubules. The cell processes are devoid of cytoplasmic organelles. Peritubular dentin (PTD) formation starts some distance from the mineralization front (modified from Tjäderhane and Haapasalo [4], with permission)

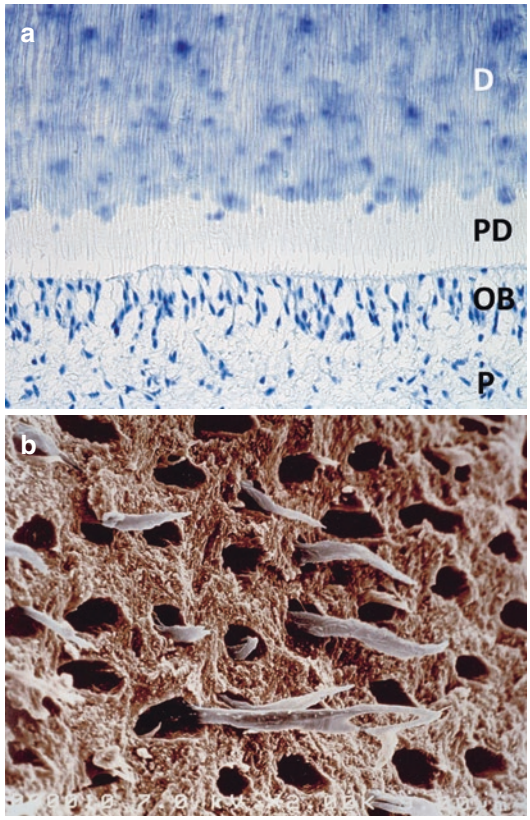

Fig. 2.2 (**a**) Dentin-pulp complex interface, displaying odontoblasts (OB), predentin (PD), tubular dentin (D), and pulp proper (P) beneath the odontoblasts. Note that instead of being straight, the mineralization front is irregular, presenting the mineralization in the form of calcospherites. Demineralized section, toluidine blue. Magnification 20×. (**b**) Odontoblast processes protruding from the dentin fracture site exposed at 0.4 mm from the pulp. Magnification 2000× (from Goracci et al. [47], with permission)

to form intertubular dentin. The backbone of the organic matrix is type I collagen, whereas non-collagenous proteins—glycoproteins, proteoglycans, and enzymes—control the matrix maturation and mineralization. The mineralization front is often considered to be linear, but actually mineralized globular protrusions called calcospherites are common (Fig. 2.2a) [4].

2.3 Dentin-Enamel Junction

The dentin-enamel junction (DEJ) is not just an inactive interface between enamel and dentin but seems to be much more complex and interactive structure than previously believed [1]. Phylogenetic, developmental, structural, and biological characteristics have led to the suggestions that instead of the DEJ, this structure should be termed the dentin-enamel junctional complex [5].

The DEJ of human tooth is wavy, scalloped line between two mineralized structures. Laser-induced autofluorescence and emission spectroscopy demonstrate the DEJ as 7–15-μm-wide structure, which is distinct from both enamel and dentin [6]. The "primary" scallop size varies from 25 to 50 μm. They contain smaller (0.25–2 μm) "secondary scallops" and intermingling sub-micrometer-sized ridges of dentin and enamel [7, 8]. The scalloped form of the interface is thought to improve the mechanical attachment of enamel to dentin [1]. However, human is among the very few species in which the scalloping DEJ has been demonstrated, questioning the role of this wavy structure to the enamel-dentin attachment. Instead of the scalloped form of the DEJ—or in addition to it—hydroxyapatite crystals extending through the DEJ into both structure [9–11] and dentinal collagen fibrils reaching into enamel [12] may contribute to the durability and toughness of the DEJ under occlusal forces [1].

2.4 Mantle Dentin

The mantle dentin is 5–30-μm-thick layer of the outermost dentin that in many aspects is different from the rest of the dentin. This is due to the different process of formation. The mantle dentin organic matrix is laid down during and immediately after the terminal differentiation of the odontoblasts and before their spatial organization into distinct cell layer. It also contains the remnant components of dental papilla, and the mechanisms of mineralization are different from what occurs at the mineralization front [4]. It is devoid of large tubules; instead, multiple small ramifications of each tubule are present in the mantle dentin. The organic matrix in mantle dentin is less regular and contains so-called von Korff fibers consisting mainly of type III collagen [13]. The mineral content of mantle dentin has also been thought to be lower than in circumpulpal dentin,

but the differences may be very minor [14], and the change of the mineralization rate toward the pulp may be more gradual [15, 16].

Although mantle dentin has traditionally been considered to provide the elastic properties of dentin necessary to withstand high occlusal forces without enamel or dentin fractures, the actual "resilience zone" may be much wider [1], even up to 500 μm [17]. This may be contributed to the changes in tubular direction [17], changes in collagen fibril direction [18], and gradual increase in mineralization from the DEJ toward the pulp [15, 16].

2.5 Circumpulpal Dentin

2.5.1 Primary and Secondary Dentin

Primary dentin is formed fast during the formation and growth of the tooth and forms the main portion of dentin. After completion of primary dentinogenesis, dentin formation continues as secondary dentin at much slower rate (approximately 1/10) [1]. The exact timing of the "end" of primary dentin formation has not been convincingly demonstrated, and animal experiments have indicated that primary dentin formation slows down gradually [19]. It is often difficult to distinguish secondary dentin from primary dentin even in histological or electron microscopy images, and in clinical conditions it is not possible at all.

2.5.2 Composition of Dentin Extracellular Matrix

Dentin organic matrix is in many ways similar to that in bone; in other ways, it is quite unique. The absence of type I collagen and high level of collagen cross-linking is typical features to mineralized tissue. About 90% of dentinal organic matrix is type I collagen, the rest being non-collagenous proteins such as proteoglycans and other proteins, growth factors and enzymes, and small amount of lipids [1]. However, mature human odontoblasts produce type III collagen, and it is present in dentinal tubules [20]. Type III collagen is also found in *dentinogenesis imperfecta* [21] and in reparative dentin under carious lesions [22, 23].

2.5.3 Dentinal Tubules

Tubularity is an important characteristic of dentin, contributing, e.g., to the mechanical properties and behavior in dentin bonding. Although the tubules are generally believed to extend from the DEJ at right angles and run slightly S-shaped course through the dentin, the direction may be different immediately beneath enamel [17]. There may also be differences in tubule orientation between the dental arches [17], which may reflect the response to loading of teeth under occlusal forces [1, 17]. Tubular density is highest, and the direction is straighter under the cuspal area [24], where the odontoblast processes and dense nerve innervation have also been suggested to penetrate deeper into the tubules [1]. These features may be related to the sensing of external irritation and regulation of dentin-pulp complex defensive reactions, since the cusp tips are the first to be worn in mastication [1].

2.5.4 Peritubular Dentin and Dentin Sclerosis

Peritubular dentin is highly mineralized circular cuff forming to the inner walls of dentinal tubules (Fig. 2.3). The name "peritubular" is, strictly speaking, incorrect, since "peri" ("around," "surrounding," "enclosing") would indicate something that is formed around the tubules. A more correct phrase would be intratubular, but since "peritubular" has been and is still used extensively, this phrase will also be used here. Peritubular dentin formation causes an age-related reduction in tubular lumen even in intact dentin, best seen in the increased dentinal transparency advancing from the tip of the root toward the crown with age [25]. In case of extensive

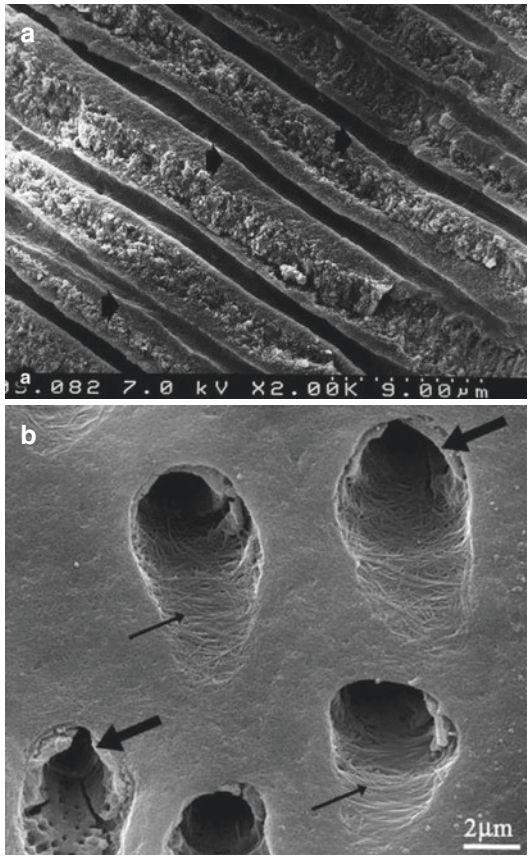

Fig. 2.3 (**a**) Scanning electron micrograph of fractured dentin about 1 mm from the dentin-pulp border. Peritubular dentin (arrows) is present already this close to the pulp. Magnification 2000× (from Goracci et al. [47], with permission). (**b**) SEM image of dentin surface. In the majority of tubules, peritubular dentin has fractured and was lost during the polishing. Two tubules retain the peritubular dentin (thick arrows) which appears non-fibrillar and porous/perforated. The internal surfaces of tubule walls exposed by the fragmentation of the peritubular dentin contain intertubular collagen fibrils (thin arrows) (from Gotliv et al. [26], with permission)

wear or caries, the tubules may also be occluded by mineral crystals formed due to reprecipitation of minerals or from the mineral ions delivered from the pulpal side via dentinal fluid. This phenomenon is—confusingly—also called dentin sclerosis, although "reactive (dentin) sclerosis" might be the more appropriate term [1].

Peritubular dentin is a separate phase from intertubular dentin, forming a distinct annulus within each tubule instead of intertubular dentin matrix-mediated crystallization (Fig. 2.3b). However, peritubular dentin is often heterogeneous, and several separate or connected mechanisms may occur at the same time [1, 4]. Peritubular dentin is perforated by tubular branches but also by several small fenestrations [26] (Fig. 2.3b), which allow tubular fluid and its components pass back and forth across the peritubular dentin. Peritubular dentin may thus not act only as a passive blockage of dentinal tubules but also contribute to the vitality and possibly even remodeling of mineralized dentin as a whole.

2.6 Tertiary Dentin

Tertiary dentin formation is a response to external irritation, such as wear, erosion, trauma, caries, or cavity preparation. The growth factors present in mineralized dentin and liberated during caries or wear are believed to initiate and control the tertiary dentin formation and structure [27]. Tertiary dentin increases the mineralized barrier thickness between oral microbes and other irritants and pulp tissue, aiming to retain the pulp tissue vital and noninfected. The form and regularity of tertiary dentin depend on the intensity and duration of the stimulus. There are two kinds of tertiary dentin, namely, reactionary dentin, formed by original odontoblasts, and reparative dentin, formed by newly differentiated replacement odontoblasts (Fig. 2.4) [2, 4]. Reactionary dentin is tubular and relatively similar to secondary dentin in structure, while reparative dentin is usually atubular (or poorly tubularized) and may present variable forms (Fig. 2.5). Reparative dentin is believed to be relatively impermeable, forming a barrier between tubular dentin and pulp tissue.

2.7 Root Dentin

Root dentin bears strong resemblance but also certain distinct differences to coronal dentin. The outermost layer of root dentin, the granular layer of Tomes, is located right beneath the root cemen-

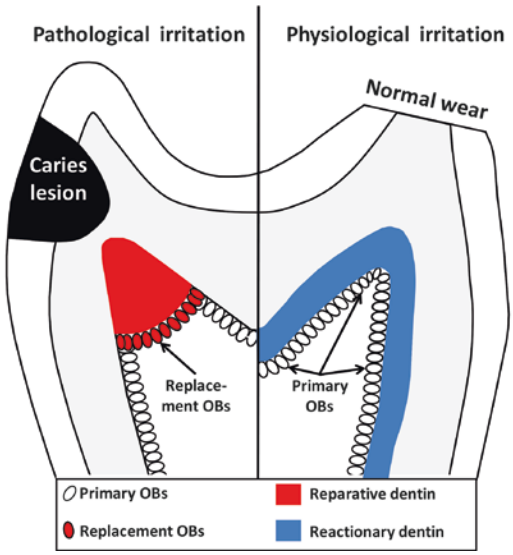

Fig. 2.4 A model of the fate of odontoblasts under pathological and physiological irritation. Intensive pathological irritation (deep and/or active caries, extensive wear, trauma), bacteria, and/or dentinal growth factors induce local odontoblast death. At the same time, the pulp stem cells migrate and differentiate into replacement odontoblasts and start the local synthesis of reparative dentin, usually distinctly different from primary dentin (lack of dentinal tubules, lamellar osteodentin-type calcification, etc.). In teeth with physiological wear or other mild irritation, slow but continuous dentin formation (either physiological or reactionary) by primary odontoblasts leads to decrease in pulpal space (modified from Mitsiadis et al. [2], and Tjäderhane and Haapasalo [4], with permissions)

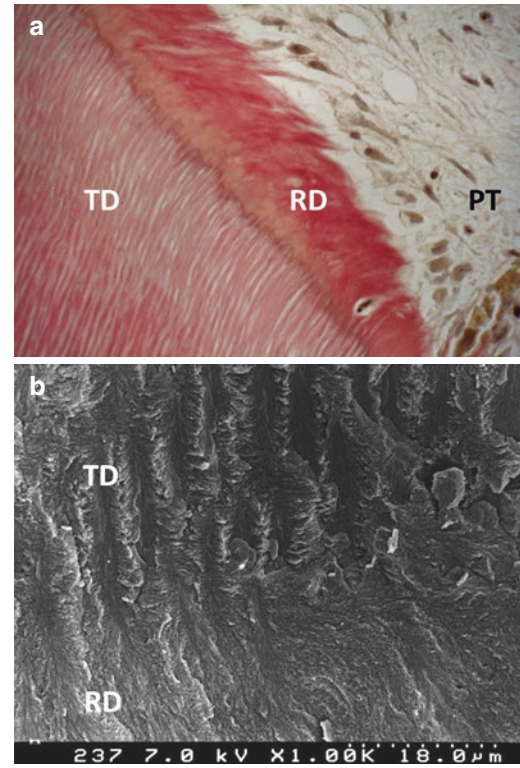

Fig. 2.5 (**a**) Histological view at the dentin-pulp border with physiological tubular dentin (TD) and reparative dentin (RD) lacking tubular continuity. Also note the poorly organized odontoblast-like cell layer. PT: pulp tissue. Light microscopy, magnification 250×. (**b**) SEM image of the borderline between normal tubular dentin (TD) and atubular reparative dentin (RD). Magnification 1000× (both images from Goracci et al. [47] with permission)

tum. It is thought to represent the mantle dentin with thin canaliculi and poorly fused globules which perhaps represent the mineralization pattern in the early stages of root dentin/cementum formation [1]. The tubular density in root dentin is at least moderately [24, 28] or even drastically [29] lower than in coronal dentin, especially in the most apical part [24, 28, 30, 31]. The apical portion of human dentin has also other structural variations, such as relatively large number of accessory root canals, transient and repaired surface resorption, and cementum-like lining the apical root canal wall [1]. Interestingly, age-related root tubular sclerosis starts from the apical region and advances coronally [25, 32], and it may be the main factor influencing permeability of root dentin [33, 34] (Fig. 2.6a). Root dentin has also other regional differences in permeability, as buccal/lingual root canal dentin has patent tubules, while the mesial/distal dentin-pulp borders may be completely occluded with minerals [33, 34] (Fig. 2.6b). This kind of patterns of tubule patency may correspond to local stress distributions of the roots under occlusal loading [1].

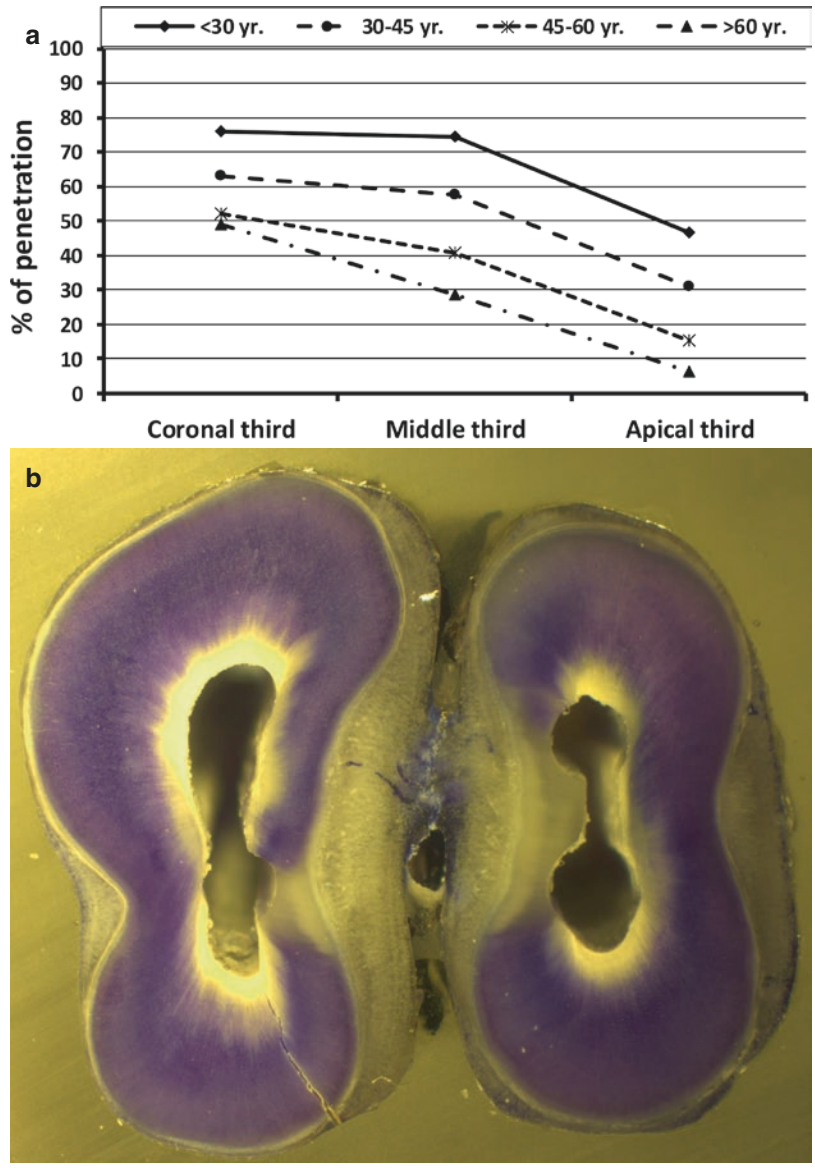

Fig. 2.6 (**a**) Relative mean dye penetration (in percentage of complete dentin area) after 2-month methylene blue incubation in instrumented root canals (data from Thaler et al. [34]). (**b**) The patency of tubules demonstrated in lower molar roots with the removal of methylene blue stain with 5.25% NaOCl irrigation. Buccal and lingual curvatures of canals demonstrate clear penetration, while in approximal and especially furcal sides, the effect is less pronounced. At furcal sites, the lack of tubular patency is also seen with the lack of methylene blue staining. Reflective light microscope, 10× magnification

2.8 Physiological and Pathological Changes in Dentin

2.8.1 Age-Related Changes

The best known—and the most important in terms of clinical endodontology—age-induced changes in human dentin-pulp complex are the obliteration of the pulp chamber and root canals even in intact teeth, due to physiological slow-rate secondary dentin formation. In incisors, canines, and premolars, the physiological age-related obliteration usually advances from the coronal direction, while in molars the dentin in the pulp chamber floor may also grow toward the roof, contributing to the pulp chamber occlusion. The clinical relevance of these phenomena is

discussed in more details in other chapters of this book.

The effects of aging on dentin mechanical properties are less known and have been a subject of debate for decades. However, the more recent studies strongly indicate that mineralized dentin may not be as stable as previously indicated, and the aging induces changes that should be taken into consideration in clinical work. Perhaps the most important aspect is the increased mineralization—or more precisely, increased mineral-to-collagen ratio—in aged dentin that increases the hardness especially in outer dentin [35]. This is mostly due to the peritubular dentin occlusion of dentinal tubules [35, 36]. At the same time, the mechanical properties of dentin change: the fatigue crack growth exponent is about 40% lower [37], the endurance strength about 48% lower [38], and the fatigue crack propagation over 100 times faster [37] in old than in young dentin. As a result, dentin flexure strength has been calculated to reduce approximately 20 MPa/decade [36, 38], and this reduction correlates well with the occlusion of tubules with age (Fig. 2.7). Reduction of the lumen diameter and increase in mineral content may not be the only factors contributing to the changes in mechanical behavior of human dentin with age, as changes in the organic components have also been speculated to contribute to the structural response [38]. While the potential age-related changes in, e.g., dentin collagen cross-link remain to be shown, loss of matrix-degrading enzymes has already been demonstrated [39–41] and may implicate also changes in their substrates, including collagen and non-collagenous proteins.

2.8.2 Caries-Affected Dentin

The concept of *minimally invasive dentistry* limits the cavity preparation to the removal of caries-infected dentin, leaving the restoration to be adhesive-bonded to caries-affected dentin. The immediate bond strengths to caries-affected dentin are commonly 20–50% lower than to sound dentin and even lower with caries-infected dentin [42]. Caries-affected dentin has lower mineral content, increased porosity, and altered structure and distribution of dentin collagen and non-collagenous proteins [43]. These changes increase dentin wetness and significantly reduce dentin mechanical properties, such as hardness, stiffness, tensile strength, modulus of elasticity, and shrinkage during drying [42] (Fig. 2.8), which make the dentin in and under the hybrid layer more prone to cohesive failures due to the polymerization shrinkage (Fig. 2.9) and under occlusal forces. *In vitro* experiments have shown

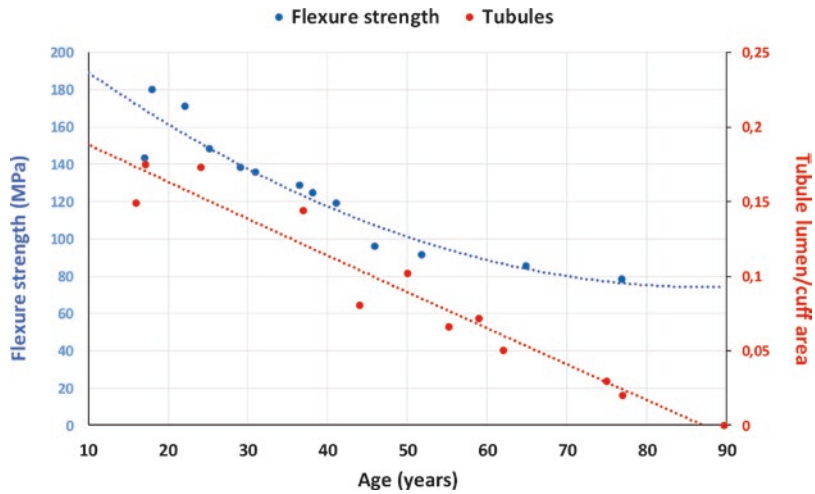

Fig. 2.7 The change in average dentin tubular lumen dimensions (red) and the influence of age on the strength of coronal dentin (blue) in adult human third molars. "Cuff" indicates peritubular dentin. The average reduction in strength over the adult age span is 20 MPa per decade at least until approximately 50 years of age (data adapted from Arola et al. [36])

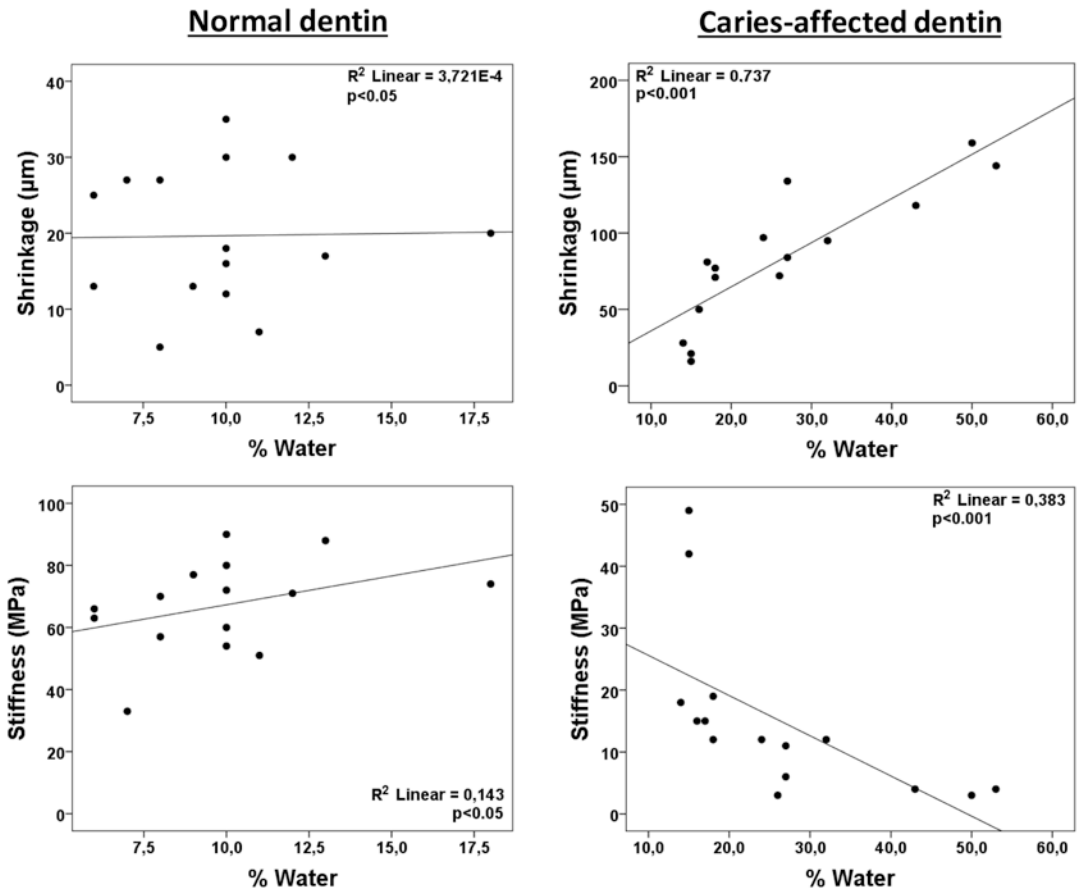

Fig. 2.8 Shrinkage and stiffness of normal (intact) and caries-affected dentin. Note the differences in both shrinkage and stiffness scales (*Y*-axis) and water content (*X*-axis) between the normal and caries-affected dentin (data adapted from Ito et al. [48])

that even short exposure of dentin to lactic acid (the acid produced by *S. mutans* and mainly responsible for caries demineralization) at pH 5 significantly reduces dentin fatigue strength, increases the rate of crack extension, and reduces the fatigue crack growth resistance [44, 45] in a way that is not prevented by sealing the tubular lumens with adhesive resin [45]. Since fatigue crack and its growth are precursors to unstable fracture, lactic acid exposure, which has occurred in caries-affected dentin and may again occur, e.g., in secondary caries, substantially increases the likelihood of restored tooth failure by fracture at lower mastication forces [45]. And finally, deep restorations (typically present in endodontically treated teeth) are more prone to cracks and fractures, not only because of the weaker structure due to loss of tooth tissue but also because of the incremental crack extension with significantly lower cyclic stresses in deep vs. superficial dentin [46].

Taken together, the age- and caries-related changes in dentin composition and structure that may have deleterious effects on dentin mechanical cannot be avoided. However, the dramatic consequences, such as catastrophic tooth fractures, can be avoided if the restorative procedures are performed not only to repair and limit the damage from caries but also to protect and preserve the tooth structure.

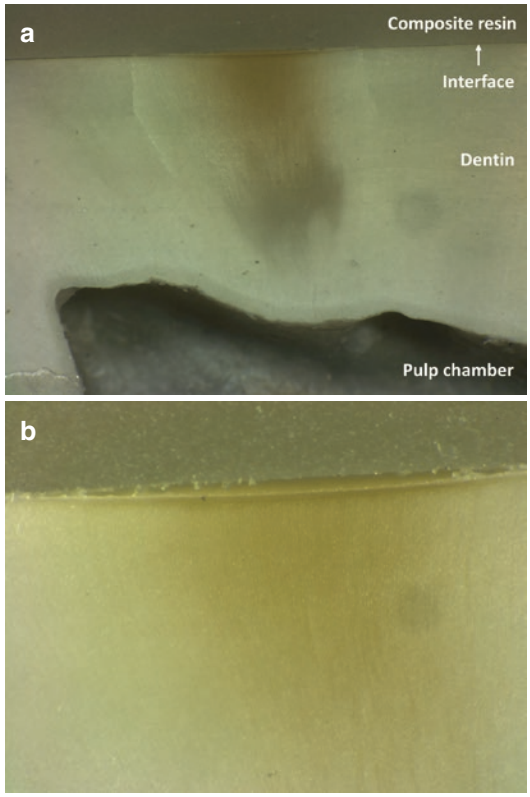

Fig. 2.9 (**a**) Dentin bonding over caries-affected dentin that clinically appeared sound without apparent discoloration. The interface between intact dentin and composite resin is tight and intact, while over caries-affected dentin the interface is clearly defective. Due to polymerization shrinkage stress and shrinkage of the less mineralized caries-affected dentin, cohesive fracture lines can be seen on both sides of the caries-affected dentin. Reflected light microscope, 20× magnification. (**b**) Higher magnification of the interface at the caries-affected dentin site, clearly demonstrating gap formation at the resin-dentin interface. Reflected light microscope, 64× magnification

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