



## Differentiation of the structure of dentinal tubules and dentinal canaliculi in human teeth of different ages

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The dentinal tubules are designed to protect the odontoblast processes, which leave behind a cytoplasmic process during tooth development. Around the cytoplasmic process, a dentin matrix is deposited, which eventually mineralizes. However, the question of the role and structure of the cytoplasmic process of the odontoblast in each dentinal tubule after dentin has completely finished the formation process remains an unresolved issue. We investigated the dentinal tubules of 20 permanent teeth (premolars and molars) of people of different ages using a scanning electron microscope. The occlusal surfaces and longitudinal fracture planes of both decalcified and non-decalcified native tooth preparations were examined. In longitudinally split teeth at the periphery of the pulp chamber of a young human premolar, odontoblasts can be seen forming a continuous layer adjacent to the parapulpal dentin. Each of the odontoblasts has a separate process that penetrates into the dentinal tubule. More peripheral parts of the dentinal tubules were either empty or contained cylindrical structures that were better visualized after acid etching on longitudinally split samples. Etched split dentinal tubules in the outer dentin more often contained cylindrical tubular structures. In each individual dentinal tubule, the odontoblast processes are arranged in the form of thin-walled tubules, which, with the help of a supporting fibrillar framework, occupy a central position. On the occlusal surface of a mature human molar, the intertubular dentin has the appearance of a smooth-surfaced structure. Practically all dentinal tubules contain more or less preserved dentinal canaliculi, indicating their fairly pronounced resistance to adverse factors. Inside the lumen of the dentinal tubules, three different types of structures are observed: odontoblast processes, cylindrical structures, and collagen fibers. Odontoblast processes are visualized both along the entire length of the dentinal tubules and only in the parapulpal parts of the dentinal tubules. Their peripheral parts were either empty or contained cylindrical structures that likely correspond to the laminae limitantes. Collagen fibers often form a fibrillar network that on one side intertwines into the dentinal canaliculus, and on the other side is connected to the walls of the dentinal tubules due to perpendicular microtubules for anchoring fibrils. Collagen fibers are most numerous in the parapulpal parts of the dentinal tubules. This study only partially explained certain aspects of the dentin microstructure, but further in-depth studies of dentin ultrastructure are necessary to more fully understand the pathology of hard tooth tissues in people of different ages and the possibilities for its treatment and prevention.

**Keywords:** dentinal tubules; odontoblast processes; dentinal canals; fibrillar network.

### Introduction

Dentin is a hard, mineralized connective tissue permeated by numerous fine tubules, which extend from the pulp of the tooth to the dentino-enamel junction (Berkovitz et al., 1992; Arana-Chavez & Massa, 2004; Farci & Soni, 2023). Back in the 19th century, John Tomes (1857) used a light microscope to identify peritubular dentin and thin unmyelinated nerves in the peripheral pulp, laying the foundation for the study of dentin sensitivity in teeth. The dentinal tubules are formed to protect the odontoblast processes, which retract centripetally during tooth development (Nanci, 2016; Scott & Pilloud, 2018). In this process, the dentinal tubules leave behind a cytoplasmic process, around which the dentin matrix is deposited and subsequently mineralized (Basandi et al., 2015; Khurshid et al., 2024). An unresolved question is the fate of the odontoblast cytoplasmic process in each dentinal tubule after dentin is fully formed (Ye et al., 2015). Numerous studies have aimed to determine the contents of dentinal tubules, but data from the scientific literature are often contradictory. Many researchers (Lo Giudice et al., 2015; Wang et al., 2018; Weerakoon et al., 2022) have used both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to address this issue. The majority of researchers have concluded that the odontoblast cytoplasmic process is confined to the parapulpal portion of the dentinal tubule. Other studies claim that odontoblast processes are observed in peripheral

dentin (Bertassoni et al., 2012; Weerakoon et al., 2022). The authors attribute to inadequate tissue fixation the inability of other researchers to prepare an adequate specimen that reflects all structures of the dentinal tubule (Pagavino et al., 1991; Charadram et al., 2013; Khurshid et al., 2024).

A possible explanation for the discrepancies in interpretation is that the structures observed in the outer portion of the dentinal tubules may not be odontoblast processes, but rather collagen fibers or cylindrical structures composed of extracellular organic matrix called laminae limitantes (Bertassoni et al., 2012). The laminae limitantes have been described by several authors (Garcés-Ortíz et al., 2015; Lo Giudice et al., 2015; Nijkowski et al., 2023) as relatively homogeneous structures present in the lumen of the dentinal tubule from the predentin-pulp to the dentinoenamel junction. These structures form the innermost hypomineralized layer of peritubular dentin and are responsible for the permeability of various substances in dentin (Charadram et al., 2013; Lin et al., 2019; Wang et al., 2024). In our study, different types of structures were observed inside the dentinal tubules of tooth roots, and the possible nature of these structures is discussed in detail.

### Materials and methods

The research was approved by the Bioethics Commission of I. Horbachevsky Ternopil National Medical University (protocol No. 75 dated

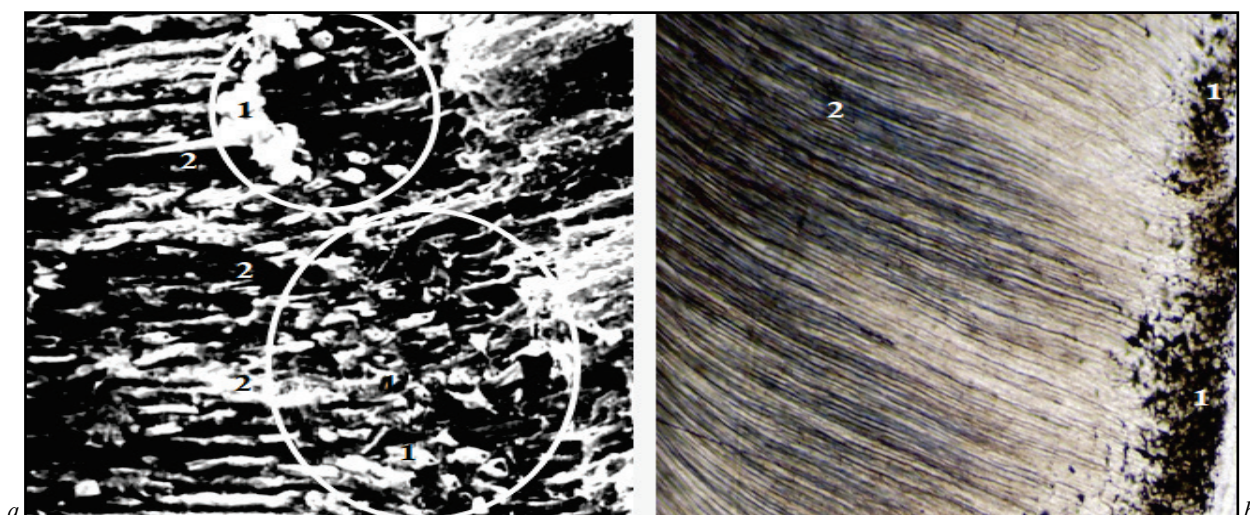
November 1, 2023) and conducted in accordance with the written consent of participants and the principles of bioethics set in the Helsinki Declaration "Ethical Principles for Medical Research Involving Human Subjects" and the "Universal Declaration on Bioethics and Human Rights (UNESCO)".

20 permanent human teeth (premolars and molars) aged 12 to 60 years, extracted as indicated, were used to study dentinal tubules using scanning electron microscopy (SEM). To establish the relationship between odontoblasts and dentin, tooth sections were obtained on a grinding machine with 0.25  $\mu\text{m}$  diamond grinding discs. The obtained sections were impregnated with a 0.10% aqueous solution of 0.25% silver nitrate and examined under a MICROMed Evolution ES-4140 light optical microscope with a 5 MP digital camera ("Mikromed", Ukraine, 2021). For SEM studies, extracted teeth were stored in a 0.5% aqueous neutral formalin solution at 4  $^{\circ}\text{C}$ . Of the examined teeth, 8 were used for observation of their occlusal surface. The mesial half of each fractured tooth was left intact, while the distal half was etched with 20% orthophosphoric acid for 20 s. In the fracture plane, longitudinally opened dentinal tubules were revealed, which could be observed along their entire length from predentin to pulp to the dentinoenamel junction. All teeth were additionally processed for SEM evaluation according to the Perdigao et al. (1995) method. In the remaining 12 teeth (upper or lower premolars), after extraction and fixation, the crowns were separated from the roots in the vestibulo-lingual direction, so that the fracture plane passed through the apices of the pulp chamber cavity. For this, a groove was made with a tungsten carbide bur and high-speed handpiece at the cemento-enamel junction with water cooling. Splitting was performed using a dental spatula and hammer. The crowns were split in the mesiodistal direction into two halves and im-

mediately immersed for 24 hours in Karnovsky's fixative solution at 4  $^{\circ}\text{C}$ . Then the samples were rinsed in cacodylate buffer (pH = 7.4) and demineralized in a 5% aqueous solution of nitric acid. After drying the tooth tissue by freeze-drying in liquid  $\text{CO}_2$  using the critical point transition method, the samples were prepared. The samples prepared in this way were glued with conductive adhesive onto aluminum stubs and coated with a 20 nm layer of chemically pure aluminum (grade 999) by sputtering in a VUP-5M device (VO "Selmi", Sumy, Ukraine) and examined in a JEOL-25M-T220A scanning electron microscope (Japan, 2006) in the modes of absorbed and reflected electrons with an accelerating voltage of 25 kV. The qualitative and quantitative composition of peritubular dentin for the content of essential chemical elements was studied using an energy dispersive X-ray microanalyzer with an accelerating voltage of 20 kV in the energy range from 960 to 19600 eV, with which the electron microscope was equipped. The architectonics of tooth dentin were evaluated using recently developed recommendations for adults (Nanci, 2016).

## Results

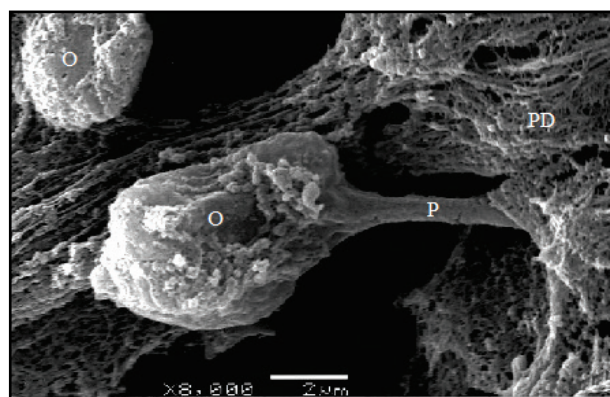
In longitudinally fractured teeth at the periphery of the pulp chamber of the premolar, odontoblasts can be seen forming a continuous layer adjacent to the parapulpal dentin (Fig. 1). Dentin is structurally and functionally closely related to the tooth pulp, as it is formed by odontoblasts – cells located in the peripheral layer of the pulp. Odontoblasts are arranged in a palisade pattern from the underlying pulp, and their processes extend into the dentinal tubules, providing dentin sensitivity in response to physiological and pathological stimuli upon destruction of the protective enamel layer.



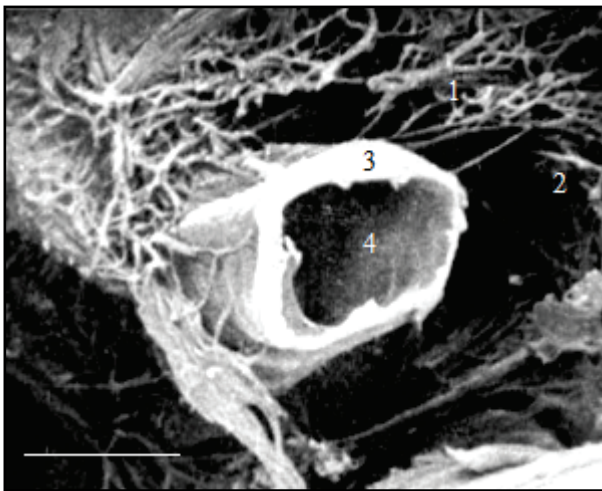
**Fig. 1.** General view of the relationship between odontoblasts (1) and dentin (2) in a 12-year-old child's premolar:  
*a* – freeze-sublimation by the critical point drying method (layer of odontoblasts outlined in white oval) (scanning electron microscopy;  $\times 750$ );  
*b* – ground section, impregnation with silver nitrate solution (light microscopy) (approx.  $\times 7$ , obj.  $\times 40$ )

Each of the odontoblasts had a separate process penetrating into the dentinal tubule (Fig. 2). The diameter of the process was approximately 0.5  $\mu\text{m}$ . Only processes connected to the cell body were observed in the narrow zone at the pulp chamber border. More peripheral portions of the dentinal tubules were empty or contained cylindrical structures. These structures were better revealed after acid etching on longitudinally fractured samples. Etched fractured dentinal tubules in the outer dentin more often contained cylindrical tubular structures.

The peritubular dentin was usually dissolved and not detected on the samples. However, densely interwoven collagen fibers were attached to the walls of the dentinal tubules and formed a framework fibrillar network to support the dentinal tubule (Fig. 3). Dentinal canaliculi penetrate the dentin from the pulp to the enamel-dentin junction in the crown of the tooth and to the cementum-dentin junction in the root portion of the tooth. The space between the dentinal canaliculus wall and the odontoblast processes – the periodontoblastic space – serves as a pathway for the transfer of various substances from the pulp to the enamel-dentin junction.

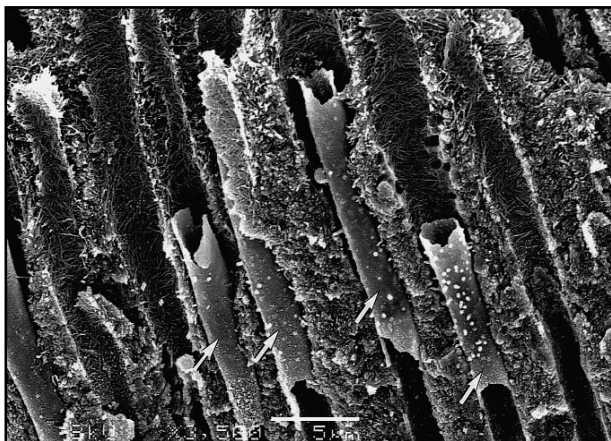


**Fig. 2.** General view of the odontoblast body (O) and its process (P) penetrating into the parapulpal dentin (PD): scanning electron microscopy; accelerating voltage 15 kV; scale bar = 2  $\mu\text{m}$



**Fig. 3.** General view of the framework structure of the fibrillar network (1), which is attached to the wall of the dentinal tubule (2), to support the central position of the dentinal tubule (3), in which a wide cavity is observed (4): scanning electron microscopy, critical point drying sublimation; accelerating voltage 10 kV, scale bar = 1  $\mu\text{m}$

Numerous dentinal tubules pass through the dentin matrix, in which the odontoblast processes are located. In some dentinal tubules, odontoblast processes in the form of thin-walled canaliculi were located within the supporting framework fibrillar network (Fig. 4). Each dentinal canaliculus is surrounded by a layer of peritubular dentin, which forms the walls for the dentinal tubules. Odontoblast processes extend along the entire length of the dentinal canaliculi, providing trophic support to the dentin.



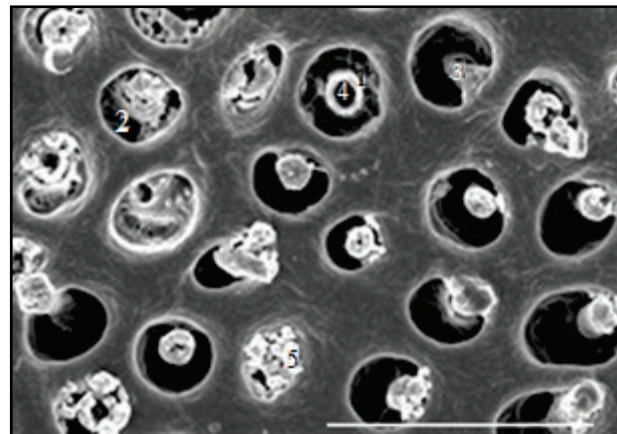
**Fig. 4.** General view of odontoblast processes in the form of thin-walled tubules (arrows), each located in a separate dentinal tubule: scanning electron microscopy; accelerating voltage 8 kV, scale bar = 5  $\mu\text{m}$

On the occlusal surface, the inter-tubular dentin appeared as a smooth surface structure (Fig. 5). Practically all dentinal tubules contained more or less preserved dentinal tubules, or their derivatives, indicating their pronounced resistance to mechanical impact. This is why such dentin retains high sensitivity to external stimuli. The odontoblast processes perform a receptor function, perceiving stimuli and transmitting it to nerve fibers in the dentinal canaliculi or the peripheral regions of the pulp.

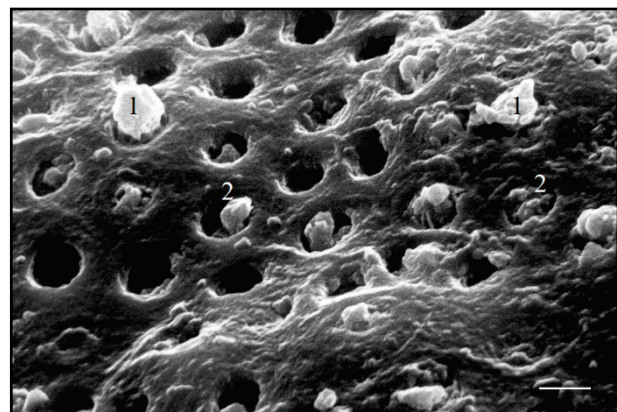
The diameter of most dentinal tubules on the occlusal surface of the molar was 4-5  $\mu\text{m}$ , while the diameter of the dentinal tubule was only 1  $\mu\text{m}$ . To maintain its central position in all dentinal tubules, there was a clearly expressed framework fibrillar network between the tubule wall and the tubule. The lumen of some dentinal tubules was free, with a clear cavity, without contents. In other dentinal tubules, the lumen was closed by an amorphous substance. In some cases, the dentinal tubule contacted the wall of the dentinal tubule on one side or simply tightly adhered to the wall.

In an electron micrograph of another tooth specimen (Fig. 6) on the occlusal surface of a molar with pathological wear, the lumen of the dentinal tubules was "sealed" with an amorphous or crystalline substance.

However, a larger number of "empty" dentinal tubules were revealed, which we believe is due to the particular brittleness of biological membranes obtained by the freeze sublimation critical point drying method.

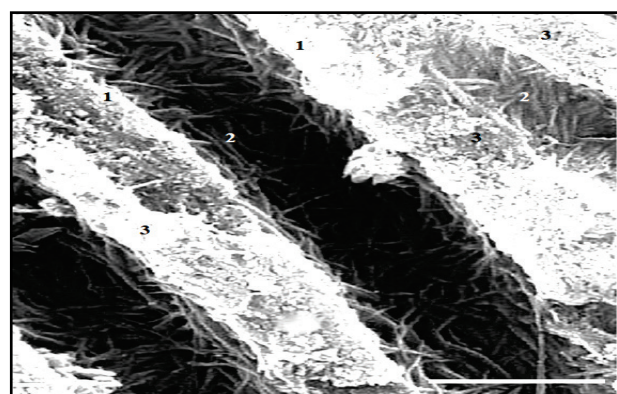


**Fig. 5.** General view of peripheral dentin on the occlusal surface of a 60-year-old human molar: 1 – fibrillar network in the dentinal tubule, 2 – dentinal tubule, 3 – deformed dentinal tubule, 4 – lumen of dentinal tubule, 5 – amorphous substance; scanning electron microscopy; accelerating voltage 8 kV, scale bar = 10  $\mu\text{m}$



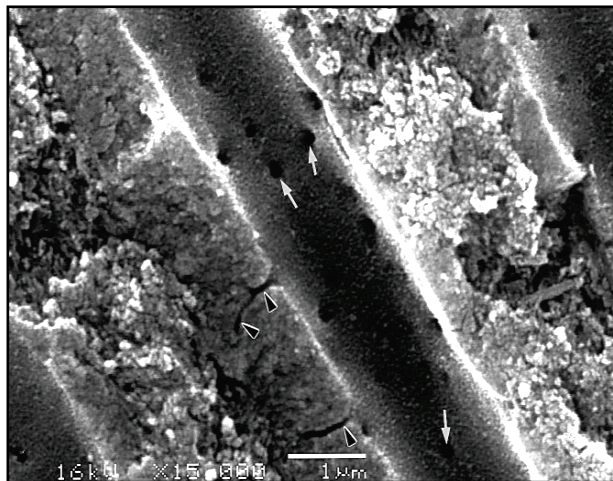
**Fig. 6.** General view of dentin on the occlusal surface of a molar with a high degree of enamel and dentin wear: 1 – crystalline substance; 2 – amorphous substance; scanning electron microscopy; accelerating voltage 25 kV, scale bar = 5  $\mu\text{m}$

In demineralized tooth specimens, the walls of longitudinally fractured dentinal tubules, even in the absence of dentinal tubules, had a pronounced framework fibrillar network of collagen fibers (Fig. 7). The main direction of these fibers was tangential to the longitudinal axis of the dentinal tubules.



**Fig. 7.** General view of dentinal tubules on a longitudinal fracture: 1 – dentinal tubule, 2 – framework fibrillar network, 3 – inter-tubular dentin; scanning electron microscopy; accelerating voltage 15 kV, scale bar = 5  $\mu\text{m}$

In native, non-demineralized teeth, collagen fibers surrounding the tubules and interweaving into the walls of the dentinal tubules were observed. These fibers were only seen in the central portions of those tubules adjacent to the pulp chamber of the tooth (Fig. 8). At the periphery of the dentinal tubule, there was an inner reticular lining on its walls, which was associated with the laminae limitantes, and its outer fibers penetrated into the peritubular dentin through small openings. These small branches perpendicularly penetrated the peritubular dentin and ended blindly within it.

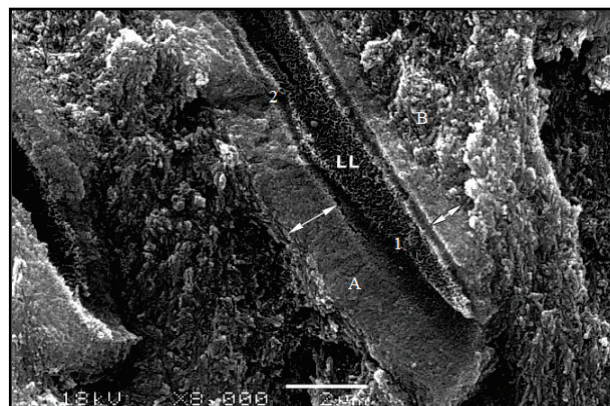


**Fig. 8.** Native preparation of a dentinal tubule in the outer third of dentin: the peritubular dentin is permeated by small openings (arrows) leading into short tubules (indicated by arrowheads), which end blindly in the peritubular dentin at uneven intervals; scanning electron microscopy; accelerating voltage 16 kV, scale bar = 1 μm

In individual preparations (Fig. 9), a cylindrical structure of the laminae limitantes was observed inside the dentinal tubule, and the dentinal tubule itself was surrounded by a layer of peritubular dentin with a perpendicularly oriented microtubule. The structure of the laminae limitantes is interpreted by some authors (Bertassoni et al., 2012; Bertassoni, 2017; Sui et al., 2018) as peritubular dentin, which regulates the permeability of

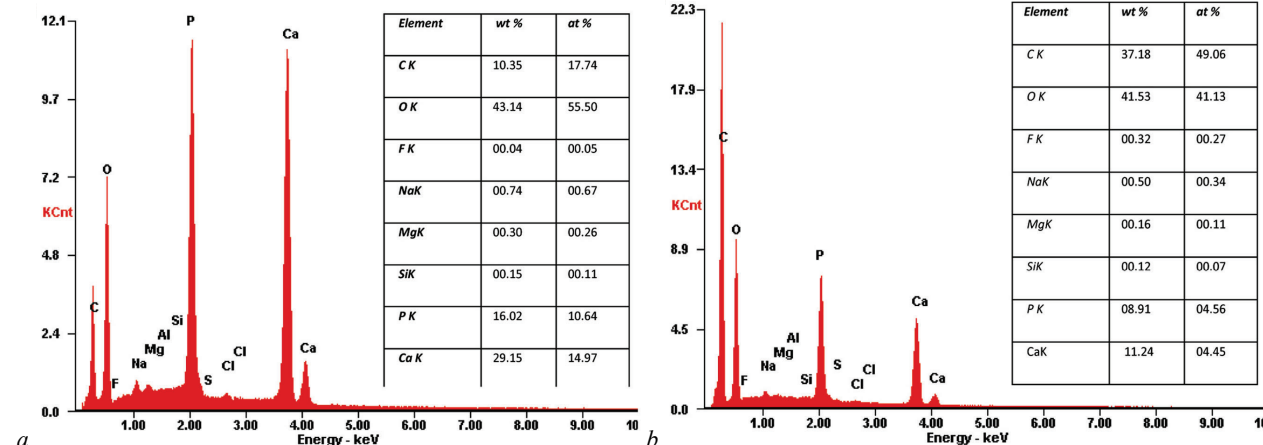
substances (Lin et al., 2019) at the predentin-dentin border and plays a crucial role in the mineralization processes of dental tissues.

In some cases, a cylindrical structure, possibly laminae limitantes, was observed inside longitudinally fractured dentinal tubulus in the inner third of the dentin (Fig. 9). The dentinal tubulus itself is surrounded by a layer of peritubular dentin with a perpendicularly oriented microcanaliculus. The laminae limitantes structure can be interpreted as peritubular dentin that regulates the permeability of substances at the predentin-dentin boundary and plays a crucial role in the mineralization processes of dental tissues.



**Fig. 9.** Native specimen of longitudinally fractured dentinal tubulus (I) in the inner third of the dentin: smooth compact (A) and finely granular (B) structure of peritubular dentin; cylindrical structure of laminae limitantes (LL); dentinal tubulus (double-headed arrows) with a perpendicularly oriented microcanaliculus (2); scanning electron microscopy; accelerating voltage 18 kV, scale bar = 2 μm

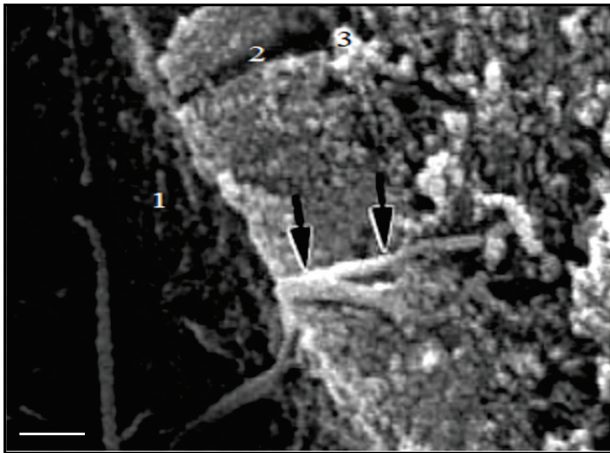
Our data indicate that the peritubular dentin, represented by a hyper-mineralized layer, can have a smooth or fine-granular structure with different contents of essential chemical elements, as confirmed by energy-dispersive X-ray microanalysis data (Fig. 10). Different surface areas of peritubular dentin with different structures have a significant difference in their micro- and macroelement composition.



**Fig. 10.** Quantitative distribution of the main essential chemical elements of peritubular dentin in the zone of smooth compact (a) and fine-granular (b) dentin according to energy-dispersive X-ray microanalysis data

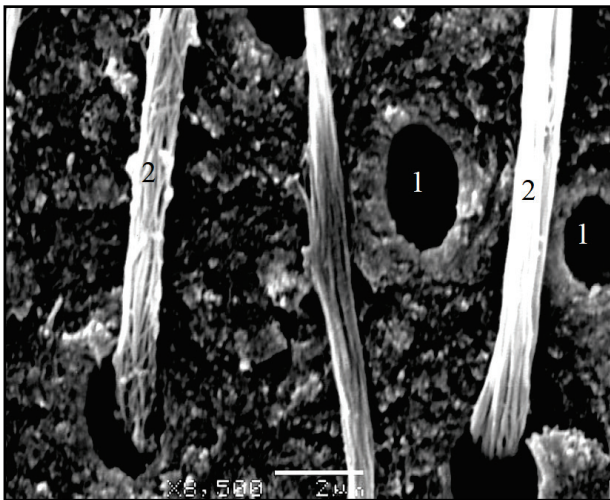
The results of our energy-dispersive X-ray microanalysis revealed differences in the inorganic components of peritubular dentin in its compact and fine-granular zones. Since in the hard tissues of teeth, inorganic substances, especially calcium and/or phosphorus, play an important role in caries resistance, disruption of the chemical composition profile of enamel and dentin creates conditions for their demineralization. It is now known that risk factors for dental caries, like most non-infectious diseases, are associated with disruptions in trace element homeostasis – insufficient intake of essential trace elements into the body and an excess of toxic elements, inevitably leading to increased environmental burden caused by them. Therefore, a comprehensive study of the effects of essential trace elements and inorganic and organic ecotoxins on human health, and the

development of preventive measures, remains an important issue. We noticed that small openings are located in the wall of the dentinal tubule, which continue into peritubular microtubules. The maximum diameter of these microtubules is 200 nm, and thin collagen fibers enter their lumen (Fig. 11), which, in our opinion, play an anchoring function for the entire fibrillar network of the dentinal tubule. The main anchoring collagen fibers in the outer third of the dentin are oriented parallel to the longitudinal axis of the dentinal tubules. However, the lumen of some peritubular microtubules is empty, or contains only the terminal fragments of anchoring collagen fibers, which is likely due to the imperfection of the specimen preparation method during SEM examination.



**Fig. 11.** Native dentinal tubule (1) in the middle third of dentin; the terminal anchoring collagen fibers (arrows) are oriented perpendicular to the wall of the dentinal tubules; the lumen of some peritubular microtubules (2) is empty, or contains only the terminal fragments of anchoring collagen fibers (3); scanning electron microscopy; accelerating voltage 20 kV, scale bar = 2 μm

The intratubular collagen fibers are oriented parallel to the wall of the dentinal tubules and, at high magnifications, showed periodic transverse striations. Some tubules contained individual fibers, while others contained fibers that were gathered into densely aggregated bundles (Fig. 12). In intertubular dentin, the amount of mineral substances is lower than in peritubular dentin. This has important clinical significance, as during the demineralization of dentin in the process of dental caries development, the destruction of intertubular dentin and an increase in its permeability occur.

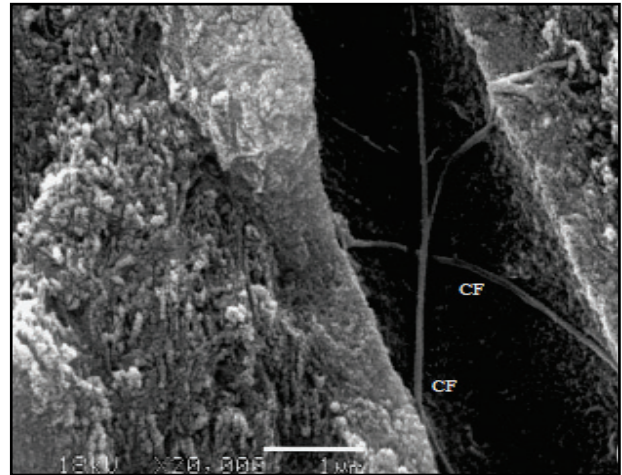


**Fig. 12.** Native preparation of a 36-year-old human premolar, transverse fracture of dentinal tubules (1); intratubular collagen fibers are gathered into thin bundles (2); scanning electron microscopy; accelerating voltage 20 kV, scale bar = 2 μm

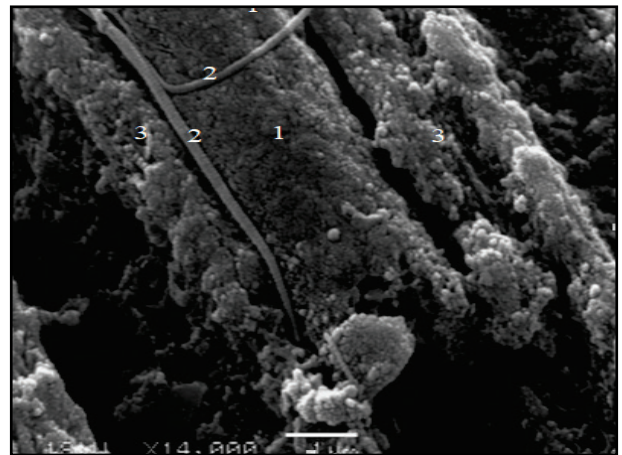
The largest number of tubules containing these fibers was found in the peripheral regions of the dentin. In these portions of the dentinal tubules, the fiber bundles were thicker than in the parapulpal portions of the dentinal tubules (Fig. 13, 14). In parapulpal dentin, there are fibers running tangentially to the enamel-dentin junction and perpendicular to the dentinal canalculi.

In the outer portions of the dentinal tubules, individual fibers passed from the lumen of the tubules through small openings into microtubules in the intertubular dentin. It was often observed that the fibers ended blindly in the peritubular dentin and represented special anchoring structures to which the entire tubular fibrillar network was attached. In addition to collagen fibers, the extracellular matrix of peritubular dentin is represented by a ground substance that primarily contains proteoglycans, which are associated with hydroxyapatite crystals. This has important clinical significance,

as the structure of both intertubular and peritubular dentin determines the structural and functional resistance of the hard dental tissues to caries.



**Fig. 13.** Native preparation of dentin in its outer third: small anchoring intratubular collagen fibers (CF) pass into the lumen of the peritubular microtubule; scanning electron microscopy; accelerating voltage 18 kV, scale bar = 1 μm



**Fig. 14.** Native dentinal tubule in the outer third of dentin: the peritubular laminae limitantes appears as a semicircle (1) inside the dentinal tubule; small collagen fibers (2) are located between the semicircle of the laminae limitantes (1) and the peritubular dentin (3); scanning electron microscopy; accelerating voltage 18 kV, scale bar = 1 μm

## Discussion

For many years, it was believed that the odontoblast process extended the entire length of the dentinal tubule and was present throughout the thickness of dentin. The origin of this assumption stems from a study conducted by Tomes (1857), who described connective tissue fibrils extending over the entire surface and along the full length of human dentinal tubules, which became known as Tomes' fibers. Although Tomes (1857) could not find a connection between these fibrils and pulp cells, other authors claimed to have seen this (Niño-Barraera et al., 2013; Martín-de-Llano et al., 2019; Nijakowski et al., 2023), and the term "Tomes' fiber" later became synonymous with odontoblast processes in dentin (Kawashima & Okiji, 2016; Weerakoon et al., 2022). In the modern literature, there are differing views regarding the true extent of the odontoblast process within dentin. The classical view holds that dentinal tubules are occupied by these processes from their pulpal end to the dentinoenamel junction. Garcés-Ortiz (2015) first questioned this assumption after examining human teeth using SEM. The authors noted that odontoblast processes were never observed beyond 0.7 mm from the pulp wall. Other SEM and TEM studies have also shown that odontoblast processes are confined to the inner third of dentin thickness (Orsini et al., 2007; Couve et al., 2013;

Wang et al., 2018). However, some SEM and immunofluorescence studies support the classical view that in human teeth, the odontoblast process extends the entire length of the dentinal tubule up to the dentinoenamel junction (Szabó et al., 1984; Rathfon, 2011; Wang et al., 2018). In several studies concerning the contents of dentinal tubules in animal teeth, there are also discrepancies regarding the level at which the odontoblast process is located (Kelley et al., 1981). Saberi et al. (2024) claim that in teeth of Merino sheep, odontoblast processes are present along the entire length of the dentinal tubule. In feline teeth, Ghodduzi (2003) found that the odontoblast process was limited to the inner third of dentin, whereas Dorvee et al. (2016) did not observe odontoblast processes within the lumen of bovine dentinal tubules. Łuszczynski et al. (2019) and Roßgardt et al. (2022), who studied equine molars, noted that odontoblast processes were observed and extended from the pulp to the dentinoenamel junction. The authors even claimed that there could be more than one odontoblast process within the lumen of a single tubule. We could not demonstrate similar results in our study of human teeth, since practically all dentinal tubules contained only one more or less preserved dentinal tubule, or its derivative, indicating the pronounced resistance of human dentinal tubules to mechanical impact, and therefore such dentin retains its sensitivity even with significant wear on the occlusal surface (West et al., 2013; Kabartai et al., 2015; Passos et al., 2015).

In all teeth, both young individuals and mature adults, the odontoblast processes were mostly limited to the parapulpal portions of the dentinal tubules. Unlike brachydont teeth, hypsodont teeth have an occlusal surface consisting mainly of dentin, which is five times softer than enamel. Therefore, such dentin can be damaged by excessive chewing, transforming into an unstructured calcified plate, or the so-called smear layer (Vadachkoria et al., 2019). In view of this, it seems unlikely that viable odontoblast processes could extend to the periphery of dentin, where dentinal tubules are subjected to a high degree of wear (Wetselaar & Lobbezoo, 2016; Tomes, 2019; El-Khoder et al., 2023). However, our observations indicate that in humans, odontoblast processes are likely mostly confined to the parapulpal portion of dentinal tubules, and this requires further justification and confirmation by subsequent transmission electron microscopy and immunofluorescence studies (Sigal et al., 1984; Orsini et al., 2007; Cavacas et al., 2013).

However, our SEM studies have shown that the odontoblast process is not the only structure present in the dentinal tubule, and there is an entire framework fibrillar system with anchoring microtubules to support it. The fibrillar system is arranged in the following order: in the peritubular dentin, there are microtubules in which the terminal fragments of anchoring microfibrils are located. In turn, they form the basis for creating a dense fibrillar network that is located on the wall of the dentinal tubule and interweaves with the laminae limitantes, which serves to hold the odontoblast process in place. Our studies have also demonstrated the presence of a ring-shaped cylindrical structure in the dentinal tubules, which extends from the pulpal end of the tubule to the dentinoenamel junction and, in its structure, resembles the laminae limitantes. According to Bertassoni (2017), this structure can mimic the appearance of an odontoblast process in SEM specimen preparations, which we are inclined to call the dentinal tubule. It is therefore not surprising that some authors, when describing odontoblast processes in peripheral dentin, have misinterpreted the laminae limitantes as an odontoblast process (Thomas, 1985). Pagavino et al. (1991) claim that the laminae limitantes is an organic inner lining of the peritubular dentin, and its appearance and visualization are enhanced by prior demineralization. According to the results obtained by these authors, the laminae limitantes, which usually adheres to the inner wall of the dentinal tubule, detaches from the walls and is freely located within the dentinal tubule due to the prior acid dissolution of the hypermineralized peritubular dentin.

In our study, we showed that between the wall of the dentinal tubule and the laminae limitantes, there are thin process-like anchoring structures that were shown in the dentinal microtubules of previously demineralized teeth. The appearance of these cylindrical structures closely resembles the laminae limitantes, as demonstrated by Kuntze et al. (2020) in human dentin. Although in native human tooth preparations, these structures were rarely observed in a cylindrical form, as shown in Figure 9, they are likely structures similar to the odontoblast process (or dentinal tubule). Since in

our study, the process-like structures had no connection to odontoblasts and were consistently detected only on etched specimens and in peripheral segments of dentinal tubules, they were probably not true odontoblast processes but rather the laminae limitantes. Indeed, it is unlikely that in mature human teeth, where unprotected dentin is exposed on the occlusal surface due to natural wear, odontoblast processes would be present in the outer dentin (Weerakoon et al., 2022). The striated pattern of fibrillar structures observed in the dentinal tubules of humans of different ages allows us to assert that these are collagen fibers, and their orientation parallel to the walls of the dentinal tubule only confirms their role as anchoring structures. According to Weerakoon et al. (2022), collagen is an important component of the contents of the dentinal tubule, present in more than 60% of human dentinal tubules. Since intratubular collagen is most pronounced in the parapulpal dentin and present in non-carious and minimally worn teeth, it is likely formed not only in response to irritation (Khatibi Shahidi et al., 2015; Weerakoon et al., 2022) but is also a physiological product of the odontoblast.

In this regard, Couve et al. (2013) claim that neither age nor tooth function are determining factors for the presence of intratubular collagen. According to these authors, intratubular collagen is a normal product of the odontoblast, so its distribution reflects the activity of this cell. It is therefore not surprising that the amount of collagen is greatest near the body of the odontoblasts. Odontoblasts also produce intertubular collagen fibers, which form a dense fibrillar network on the walls of the dentinal tubules, visible in demineralized tooth samples (Fig. 4). The mechanism that triggers the change in orientation of the intratubular collagen adjacent to the walls of the dentinal tubules can be applied to the extratubular collagen fibers, which are perpendicular to the odontoblast process (Martín-de-Llano et al., 2019). However, data on them still remain little known and fragmentary, and therefore the relationship between the non-collagenous peritubular dentin and the nature of the orientation of the intratubular collagen fibers also remains unclear (Hong et al., 2022; de Castro Oliveira et al., 2023), although we tend to attribute an anchoring function to them.

The topographic arrangement of the intratubular collagen fibers, documented in our study (Fig. 12), may be relevant in this regard. It should be noted that ultrastructural studies of human dentin remain fragmentary and insufficiently in-depth, so information about the anatomy and pathology of human dentin is still limited. Inorganic substances in the human body, especially calcium and/or phosphorus-based materials, play an important role in hard tissues (Xu & Wang, 2012; Hong et al., 2022). There is a minimum of information about the chemical composition of enamel and dentin. It is known that dentin, which is formed from the mesoderm, is a flexible mineralized tissue consisting of 70% inorganic material, 20% organic material, and 10% fluid. The crystals are mainly composed of calcium hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  and a small percentage of carbonate and fluoride (Nanci, 2016). In the study by Hong et al. (2022), the mineral content in the organic matrix, enamel-dentin junction, and dentin (matrix/mineral ratio) was studied using 2D confocal Raman microspectroscopic mapping/imaging technique. The Raman scattering results obtained by the authors demonstrated the presence of differences in the organic and inorganic components of the enamel-dentin junction and dentin between the occlusal and cervical regions of the tooth.

The distribution of major chemical elements in peritubular dentin (PTD) and intertubular dentin (ITD) was investigated by Sui et al. (2018) using 3D correlative focused ion beam scanning electron microscopy (FIB-SEM) and energy dispersive spectroscopy (EDS). It was found that peritubular dentin has a higher concentration of minerals compared to ITD (~1.52). The authors were able to establish a difference in chemical composition, clearly visualizing biopolymers and hydroxyapatite (HAp) crystallites with a larger mean crystallite size in ITD ( $32 \pm 8$  nm) than in PTD ( $22 \pm 3$  nm). Our energy dispersive X-ray microanalysis results showed differences in the content of inorganic components of peritubular dentin in its compact and fine-granular zones. The obtained results allow one to use changes in microstructure for modeling the process of chemical etching of enamel and dentin, which is used in the treatment of dental caries.

Considering that in humans, dentin constitutes the main part of the occlusal surface of teeth, it is directly exposed to the oral environment. For this reason, it is important to know its anatomical and functional features. Dentin in general, and especially its intertubular part, is considered a

rather static tissue. However, dentinal tubules and their contents remain viable and capable of responding to various stimuli even with high wear of the occlusal tooth surface (El-Khoder et al., 2023). Thus, the microstructure of human dentin is an important material for analyzing the reaction of tooth dentin to clinical intervention during dental procedures.

## Conclusions

The study of dentinal tubules of permanent teeth showed that three different types of structures are observed inside their lumen: odontoblast processes, cylindrical structures, and collagen fibers. Odontoblast processes are visualized along the entire length of the dentinal tubules; their peripheral parts contain cylindrical structures, which likely correspond to laminae limitantes. Collagen fibers often form a fibrillar network, which on one side intertwined into the dentinal tubule, and on the other side was connected to the wall of the dentinal tubule. Collagen fibers were most numerous in the parapulpal parts of the dentinal tubules. The results of energy dispersive X-ray microanalysis showed differences in the inorganic component of peritubular dentin in its compact and fine-granular zones. Since intratubular collagen is most pronounced in parapulpal dentin, it is likely that it may become a scaffold for the formation of reactionary/repairative types of dentin in response to dental caries and other external factors that may affect tooth tissues. This study only partially explained certain aspects of the dentin microstructure, but further in-depth studies of dentin ultrastructure are necessary to more fully understand the pathology of hard tooth tissues in humans and the possibilities for its prevention.

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