

Interpretation of the concepts of dentinal tubule and dentinal canaliculus

S. L. Popel*, N. O. Gevkaliuk**, N. I. Sydliaruk**, Y. M. Martyts**,
M. Y. Pynda**, V. Y. Pudyak**, V. Y. Krupey**

*Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine

**Ivan Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

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Vasyl Stefanyk Precarpathian
National University,
Shevchenko st., 57,
Ivano-Frankivsk, 76018, Ukraine.
Tel.: +38-097-874-14-46.
E-mail: serhii.popel@pmu.edu.ua

Ukraine Ivan Horbachevsky
Ternopil National Medical
University, Maydan Voli, 1,
Ternopil, 46001, Ukraine.
Tel.: +38-096-560-14-45.
E-mail: gevkaliuk@tdmu.edu.ua

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The biology of dentin occupies a central place in the theory of tooth integrity as an organ, and knowledge about the microstructure of dentin changes with the development of new research methods, such as scanning electron microscopy. The structures that form dentin, and the odontoblast processes, are an extremely complex biological structure due to cellular polarization. The structure of the dentin of crowns from 30 intact teeth extracted for orthodontic reasons has been studied. Dentin was examined on longitudinal chips using scanning electron microscopy, determining the average number of dentinal tubules per unit area of dentin, the length and diameter of the dentinal canaliculi along the dentinal tubule. Scanning electron microscopy of demineralized teeth showed that numerous fine fibrillar structures were found in the predentin, connecting the odontoblast processes (dentinal canaliculi) and the walls of the dentinal tubules. The odontoblast processes were always in close contact with the microfibrillar network and were located within the dentinal tubule. It was found that the dentinal canaliculi have their own anchoring microfibrillar system, which allows each dentinal canaliculus to be held in a central position. The microfibrils are attached to the wall of the dentinal tubule and form a continuous mesh structure among the dentin tissue and are directly fixed on one side to the wall of the dentinal tubule, and on the other side to the wall of the dentinal canaliculus, which is a derivative of the odontoblast process. In different areas of the dentin, the length, number, and diameter of the microfibrils have a wide variative range. Microstructures were numerous in different regions of the dentinal tubule. Their number decreased with distance from the level of the outer dentin. In the inner third of the dentinal tubule, microfibrillar structures are observed that form a dense network of different types, thicknesses and diameters. The most common direction of the microfibrils was from the surface of the odontoblast process to the wall of the dentinal tubule, and in other cases these microfibrils were attached to both opposite inner surfaces of the dentinal tubule. The base of the microfibril is attached to the wall of the dentinal tubule, as if forming a continuous structure among the dentin tissue and the surface of the odontoblast process. It is shown that the microfibrillar structures may be a previously unknown framework system that ensures the stabilization of odontoblast processes inside the dentinal tubule. In the scientific classification of dentin ultrastructure, it is advisable to use the term "dentinal tubule" and "dentinal canaliculus", since this structure is a derivative of the odontoblast process.

Keywords: teeth; dentin; dentinal tubule; dentinal canaliculus; odontoblast processes; microfibrils.

Introduction

The biology of dentin occupies a central place in the theory of tooth integrity as an organ, and knowledge about the microstructure of dentin changes with the development of new research methods, such as scanning electron microscopy (SEM). Dentin is a mineralized connective tissue with the ability for self-renewal. Dentin is structurally and functionally related to the dental pulp, since it is formed by cells located in the peripheral layer of the pulp – odontoblasts and their processes, which are located in the dentinal tubules (DTs) (Berkovitz et al., 1992; Fagundes et al., 2015).

The composition of dentin includes non-collagenous proteins and mineralized collagen, which forms the basis of dentin. According to the periods of activity of dentin formation, it is divided into 3 types: primary, secondary, and tertiary. Primary dentin is formed during the period of tooth formation and eruption, secondary (regular, physiological) dentin is formed after tooth eruption, with its deposition rate decreasing with age. Only primary and secondary dentin form the tissue of normal mineralized, structurally formed dentin of intact (caries-free) teeth. Tertiary dentin (reparative, substitute) is formed locally in response to the action of irritating factors. A characteristic feature of the structure of dentin is that throughout its existence, it is an acellular tissue. Odontoblasts play an important role in

the process of dentin calcification. Through their processes, they provide delivery of mineral salts from the blood into the developing dentin matrix. Mineralization of dentin occurs in such a way that discrete spherical calcification areas (dentinal globules) are formed in it, which do not completely merge. Areas of slightly or completely uncalcified dentin, called interglobular dentin, may remain between these globules. In contrast, around the odontoblast processes, a collar of highly mineralized dentin is formed, which is called peritubular dentin (Berkovitz et al., 1992).

Odontoblasts take an active part in the process of dentin formation. These cells form thin precollagen fibers, which later turn into collagen and form the organic basis of predentin. Odontoblasts synthesize and secrete type I collagen (the main organic component of dentin), glycoproteins, phosphoproteins, proteoglycans, glycosaminoglycans. Specific products of odontoblasts are the so-called phosphorins – phosphorylated proteins that are found only in dentin. They are thought to play an important role in controlling the rate of dentin mineralization. Odontoblasts also produce calcium-binding proteins – osteocalcin and osteonectin, which are found in both dentin and bone tissue. Odontoblasts possess not only secretory but also lytic activity. About 15% of the collagen they synthesize is destroyed by the odontoblasts themselves with the help of the lysosomal apparatus (Carda & Peydró, 2006). The deposition of the first collagen fibers

occurs directly in the amorphous intercellular substance of the dental papilla. When the predentin layer reaches a thickness of 40-80 μm , it is displaced to the periphery by newly formed layers of predentin, in which the fibers have a different direction – they are arranged parallel to the surface of the dental papilla. Subsequently, these inner layers of dentin, rich in tangential fibers, form the pulp dentin in the formed tooth. The radial fibers, which lie in the outer layers of dentin, form the mantle dentin. As the dentin layer thickens, the odontoblasts are gradually displaced inward into the papilla, leaving thin processes – dentinal processes of odontoblasts, surrounded by a thin cytoplasmic membrane (Fox et al., 1984).

Dentinal tubules protect the odontoblast processes from the harmful effects of environmental factors, and the odontoblast processes themselves secrete a protein matrix for the formation of collagen and non-collagen proteins, from which a mineralized protein complex is mainly developed (Carda & Peydró, 2006). Odontoblasts are cells that form dentin – tall columnar (cylindrical) cells located at the border between the pulp and primary dentin. The functions of odontoblasts include the secretion of dentin matrix proteins and ensuring the processes of dentin mineralization (Carda & Peydró, 2006; Lee et al., 2023). Moreover, odontoblasts participate in the transmission of stimuli from the environment to the pulp, helping to reduce pain sensitivity by transforming and modulating external impulses (Naoum et al., 2015; Osmari et al., 2018; Liu et al., 2020). The receptor hypothesis suggests that odontoblasts are cells that perceive stimuli with their own processes and transmit it to nerve fibers in the dentinal tubules (DTs) or in the peripheral areas of the dental pulp.

Since Tomes' (1857) first description of odontoblast processes as a simple cytoplasmic extension located inside the dentinal tubule and secreting all the protein and non-protein components of dentin involved in dentin mineralization, several structures forming dentin have been described, and the odontoblast processes have been revealed to be an extraordinarily complex biological structure due to cell polarization, which is located inside the dentinal tubule and extends over varying distances at different levels of dentin. Peritubular dentin surrounds this important structure, and finally, intertubular dentin encompasses both structures (Maniopoulos & Smith, 1983; Fagundes et al., 2015; Lin et al., 2021). Some researchers have claimed to have found dentinal tubules located only in the predentin zone (Niño-Barrera & Garzón-Alvarado, 2012). Others have reported the presence of dentinal tubules in the first third of the length of the dentinal tubule (Schilke et al., 2000; Garcés-Ortiz et al., 2015; Gevkaliuk et al., 2023), while in other studies dentinal tubules were observed in the outer third of its length, reaching the dentinoenamel junction (Brenna et al., 2003; Kuntze et al., 2020). The study of dentinal tubules in the roots of extracted human molars (Fox et al., 1984) revealed that they contact the cementodentinal junction. In another study of dentinal tubules in the teeth of *Macaca mulatta* monkeys (Kelley et al., 1981), it was noticed that dentinal tubules were present in the inner and outer thirds of dentin, while the middle third of dentin was devoid of them.

In a large retrospective literature review, we found that as early as (Johansen & Parks, 1962) the presence of thin sheet-like membranes throughout the thickness of dentin, except for predentin, was described. After that, individual scientists studied dentin and border structures using the SEM method (Szabó et al., 1984; Goracci et al., 1999; Al-Saud & Al-Nahedh, 2012). The microstructures are described as bifurcating lateral branches and ramifications of the odontoblast processes, and have been found to be associated with openings and windows in the wall of the dentinal tubules. In other studies, these structures were described near the dentinoenamel junction and are described as bifurcations of dentinal tubules. Interestingly, the results of other studies report a more or less complex microfibrillar network attached to the odontoblast processes within the dentinal tubules (Garcés-Ortiz et al., 2015; Ryou et al., 2023). The aim of the work is to study the microfibrillar network that connects the wall of the dentinal tubules and the odontoblast processes, observed at different distances of the dentinal tubules of the human tooth.

Materials and methods

The research was carried out in accordance with the ethical principles of the latest revision of the Helsinki Declaration on Biomedical Research

(2000), the Council of Europe Convention on Human Rights and Biomedicine, and the relevant laws of Ukraine, in compliance with all necessary legal and administrative requirements, as confirmed by the conclusion of the Bioethics Commission Expertise Committee on Bioethics of I. Horbachevsky Ternopil National Medical University (protocol No. 75 dated 01.11.2023). Patients were informed about the purpose of the study, for which their written informed consent was obtained.

We studied 30 caries-free teeth of patients aged 15–21 years undergoing orthodontic treatment. Immediately after extraction, the crowns of the upper or lower premolars were separated from the roots by making a groove at the cemento-enamel junction with a water-cooled tungsten carbide bur and a high-speed handpiece. Splitting was carried out with a chisel and a prosthetic hammer. The crowns were divided into two halves in the mesiodistal direction and immediately immersed for 24 hours in Kamovsky's fixative solution at 4 °C. Then the samples were rinsed in cacodylate buffer (pH = 7.4) and demineralized in a 5% aqueous solution of nitric acid. After critical point drying, the samples were glued with conductive glue onto aluminum stubs and coated with a 20 nm thick layer of chemically pure aluminum [assay 999] and examined using a scanning electron microscope (JEOL-25M-T220A, Japan, 2006).

Results

The features of dentin of the studied teeth adjacent to the dental pulp did not differ from those previously reported. In the inner non-mineralized dentin, each dentinal tubule (DT) contained only one odontoblast process, which had the appearance and structure of a dentinal canalculus connected to the walls of the dentinal tubule by numerous fine microfibrillar structures forming a dense mesh (Fig. 1a). In the inner third of the dentin, one end of the microfibrils was always attached to the odontoblast process, and the other end contacted the wall of the dentinal tubule. The spaces among these microfibrils were only 0.32–0.51 μm , and the microfibrils themselves were so numerous that they occupied most of the dentinal tubule, forming a dense network (Fig. 1b). In other areas, these fibrils merged, became wider, and formed flattened wide structures, often appearing as a homogeneous sheet-like material (Fig. 1c). In the aforementioned areas, the odontoblast processes (dentinal canalculi) were always in close contact with this microfibrillar network and located within the dentinal tubule. The most common direction of these microfibrils was from the surface of the odontoblast process to the wall of the dentinal tubule at an acute angle, while in other cases, these microfibrils were attached to both opposing inner surfaces of the dentinal tubule. We did not visualize boundaries between the fibrillar or sheet-like structures and the peritubular dentin. We found that the base of the microfibril was attached to the wall of the dentinal tubule, as if forming a continuous structure among the dentin tissue and the surface of the odontoblast process.

In different areas of dentin, the length, number, diameter, and area of microfibrils varied widely. Microfibrils located in the inner third of the dentin thickness had an average length ranging from 0.04 to 3.60 μm , with a mean value of $2.35 \pm 0.99 \mu\text{m}$. The number of microfibrils varied from 30 to 52 microfibrils/10 μm^2 , with an average value of 42.01 ± 6.80 microfibrils/10 μm^2 . The diameter of the microfibrils ranged from 0.03 to 0.46 μm , with an average value of $0.32 \pm 0.14 \mu\text{m}$, and their number ranged from 34 to 40 microfibrils per 10 mm^2 , with an average value of 36.61 ± 2.31 microfibrils per 10 mm^2 . In predentin, the area of microfibrils ranged from 0.01 to 2.51 μm^2 , with an average value of $1.09 \pm 0.63 \mu\text{m}^2$. The diameter of the microfibrils ranged from 0.03 to 0.51 μm , with an average value of $0.33 \pm 0.01 \mu\text{m}$.

In contrast, the length of the microfibrils in the middle third of the dentin thickness was between 0.01 and $0.21 \pm 0.05 \mu\text{m}$; the diameter of the microfibrils ranged from 0.03 to 0.52 μm (mean value $0.05 \pm 0.13 \mu\text{m}$), and their number varied from 8.01 to 18.02 ± 11.91 microfibrils/10 μm^2 ; 2.91 microfibrils/10 μm^2 . In the outer third of the dentin, almost all the broken defective microfibrils were located, and we assumed that this measurement would give false data: their diameter ranged from 0.01 to 0.02 μm with a mean value of $0.01 \pm 0.007 \mu\text{m}$. Moreover, their number ranged from 0 to 10 ± 3.61 microfibrils/10 μm^2 , 3.02 microfibrils/10 μm^2 .

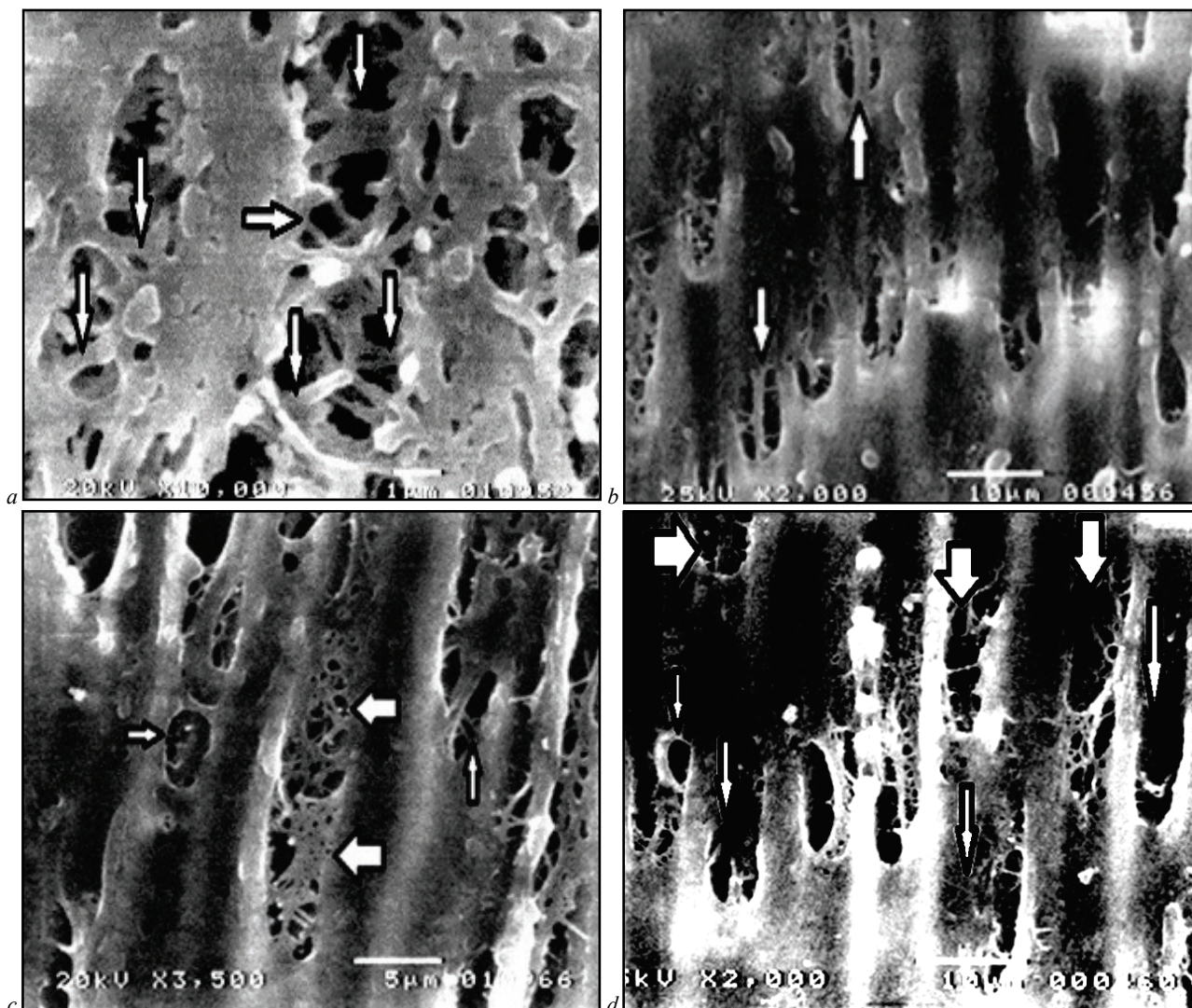


Fig. 1. Inner and middle third of dentin: *a* – unmineralized dentin with fine microfibrils (arrows); *b* – inner third of dentin with microfibrillar structures (arrows); *c* – reticular structures formed by lamellar structures (wide arrows) and microfibrils (thin arrows); *d* – middle third of dentin with microfibrils (wide arrows), empty areas (thin arrows)

As can be seen from the presented SEM images, in unmineralized dentin, thin microfibrils of various diameters fill the dentinal tubules. In the predentin zone, a very dense network of thin and numerous microfibrils is formed. When analyzing the surface areas of dentin, this structure was relatively less dense in the inner third of dentin, where the empty spaces of the fibrillar framework network were wider and larger. In the inner third of dentin, microfibrillar structures were present in the dentinal tubules, connecting and "suspending" the odontoblast process on the walls of the dentinal tubule. In the dentinal tubules, there were reticular structures formed by lamellar structures and some microfibrils, and pores could be seen in the wall of the dentinal tubule. In the middle third of dentin, fewer microfibrils were observed, and some areas of the dentinal tubules were empty. In addition, compared to the inner third of dentin, in its middle third we observed a small number of microfibrils and lamellar structures, as well as many areas devoid of the microstructures described above (Fig. 1d). These microfibrillar and lamellar structures were almost absent in the dentinal tubules located in the outer third of dentin (Fig. 2a), and they almost disappeared when we observed dentinal tubules near the dentino-enamel junction.

In the predentin zone, a very dense network of thin and numerous microfibrils is formed. This structure was relatively less dense in the inner third of the dentin, as the empty spaces of the fibrillar framework network were wider and larger, as more superficial areas of the dentin were analyzed. Furthermore, compared to the inner third of the dentin, in its middle third, we observed a small number of microfibrils and sheet-like structures,

as well as many areas devoid of the microstructures described above (Fig. 1d). These microfibrillar and sheet-like structures were barely present in the dentinal canaliculi located in the outer third of the dentin (Fig. 2a), and they almost disappeared as we observed dentinal canaliculi near the dentino-enamel junction.

A feature of the DTs in the more outer areas of the dentin was that almost all DTs did not have microfibrils attached to their surface (Fig. 2b), or their number was very small. Furthermore, the DTs in these areas rarely contained odontoblast processes. Interestingly, we managed to observe that in some areas in the middle third of the dentin, the tip of some odontoblast processes was smooth with a flat or round end. The body of the terminal parts of these odontoblast processes contained a few microfibrils attached to their surfaces (Fig. 3a), and in a few cases, microfibrils were attached to the tip of the odontoblast process (Fig. 3b).

In addition, we observed the presence of some round or nodular, smoothly surfaced formations of varying size. In the intertubular dentin, these structures were numerous and appeared as isolated or conglomerate nodules. More often, they appeared as large structures and rarely as small nodules protruding into the lumen of the dentinal tubule. The inner surface of the dentinal tubules frequently protrudes, which may be smooth or contain very fine microgranules. This structure was not detected in the outer third of the dentin, as the granules in these areas were coarse, which is explained by the so-called "butterfly effect". Since 1983, "butterfly" images (Fig. 4) have been observed on root fractures and are considered an "optical phenomenon".

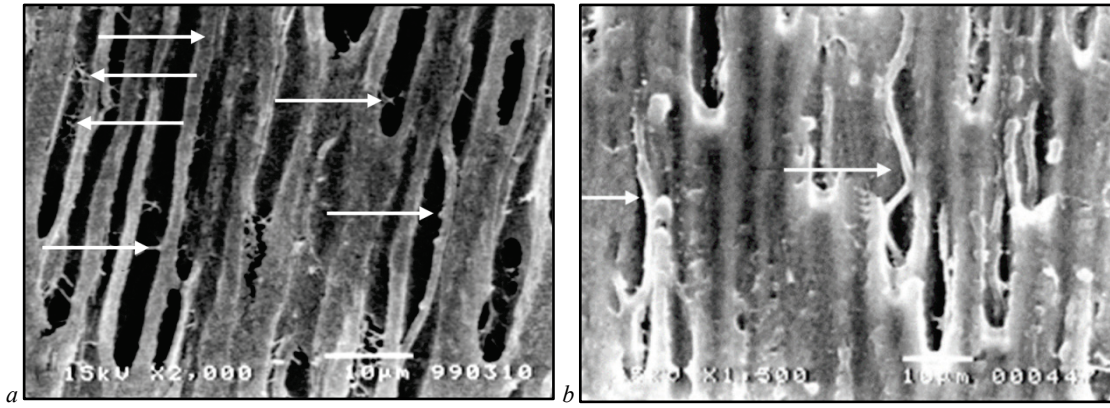


Fig. 2. Outer third of dentin: *a* – dentinal tubules contain a few microfibrils with the odontoblast process (arrows); *b* – odontoblast processes without microfibrils (arrows), scale bar = 10 µm

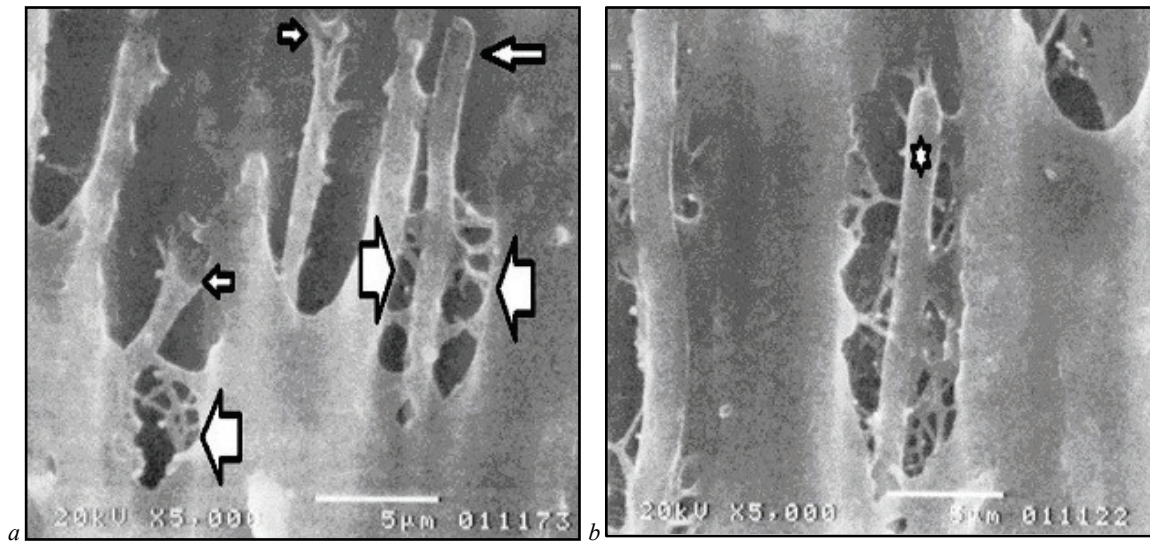


Fig. 3. Terminal regions of the odontoblast process: *a* – some microfibrils (wide arrows) that are preserved in the terminal part of this odontoblast process, the end of the odontoblast process is round (thin arrow) compared to the other two (small arrow); *b* – some microfibrils are attached to the terminal part of the odontoblast process (asterisk), scale bar = 5 µm

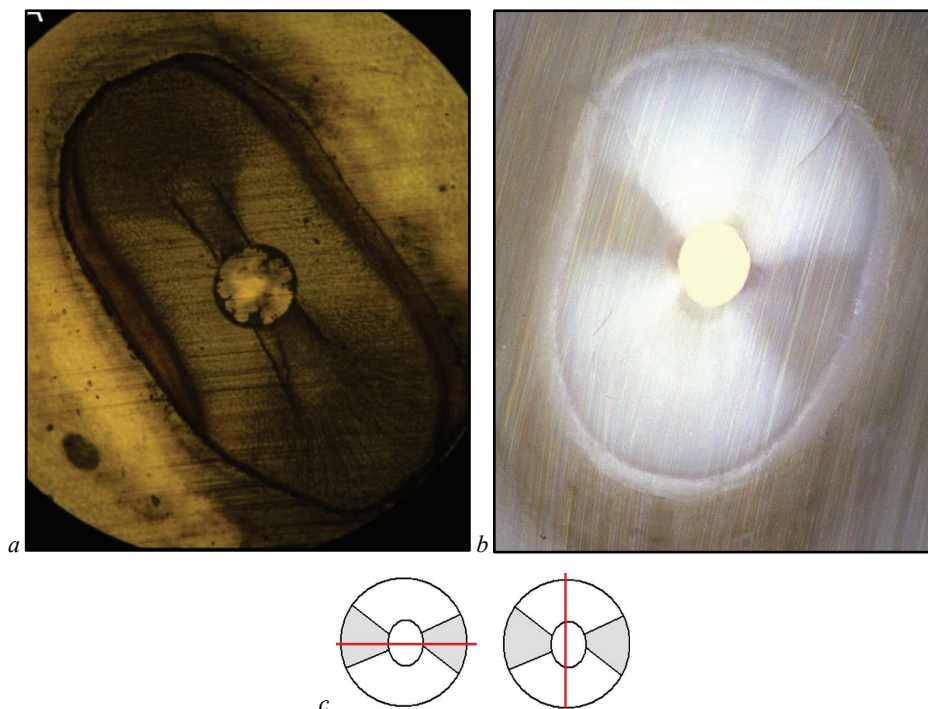


Fig. 4. Actual image of the "butterfly phenomenon" (*a*, *b*) and their schematic modeling (*c*) as an SEM feature of root dentin sclerosis

We have shown that the spatial distribution of dentinal tubules (Fig. 5) determines the differentiation of odontoblasts in the process of dentinal tubule formation with a 1:1 distribution of the latter and their processes (Fig. 6). It is also necessary to take into account the difference in the density of dentinal tubules, as we pointed out in previous publications.

As can be seen in Figure 6b, dentinal canaliculi are membrane formations located in dentinal tubules and are processes of odontoblasts, while the dentinal tubule itself is a calcified structure of the dentin. The diameter of the dentinal canaliculus is always smaller than the diameter of the tubule, so the dentinal canaliculus requires a supporting subsystem for its stabilization, which is a fibrillar, protein structure. We have established that dentinal canaliculi are quite fragile and often break down during the preparation of dentin fractures after freeze-drying during the transition of the critical point (Fig. 7). Therefore, to preserve dentinal canaliculi, it is necessary to avoid angular displacements of adjacent parts of the fracture, which naturally occur in these cases, leading to their destruction, which explains the difficulty and sporadic nature of their visualization by the SEM method.

On individual preparations, a rather motley SEM picture of dentinal canaliculi and dentinal tubules is revealed (Fig. 8). The diameter of most dentinal tubules is 4–5 μm , and dentinal canaliculi - only 1 μm . In some dentinal tubules, there is a fibril-like substance between their wall and the dentinal canaliculus, in others – a crystalline substance. The lumen of some dentinal canaliculi is sealed with an amorphous or crystalline substance, while in others, the dentinal tubule has a clear cavity without con-

tent. Furthermore, in some dentinal tubules, the canaliculus contacts the wall of the tubule on one side, tightly adhering to the wall.

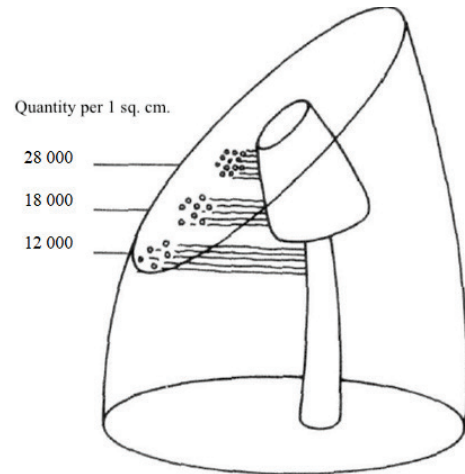


Fig. 5. Schematic representation of the density of dentinal tubules in three locations of the root surface fracture: near the root canal (upper part); in the middle between the canal and the cementum (middle part); near the dentinocemental junction (lower part)

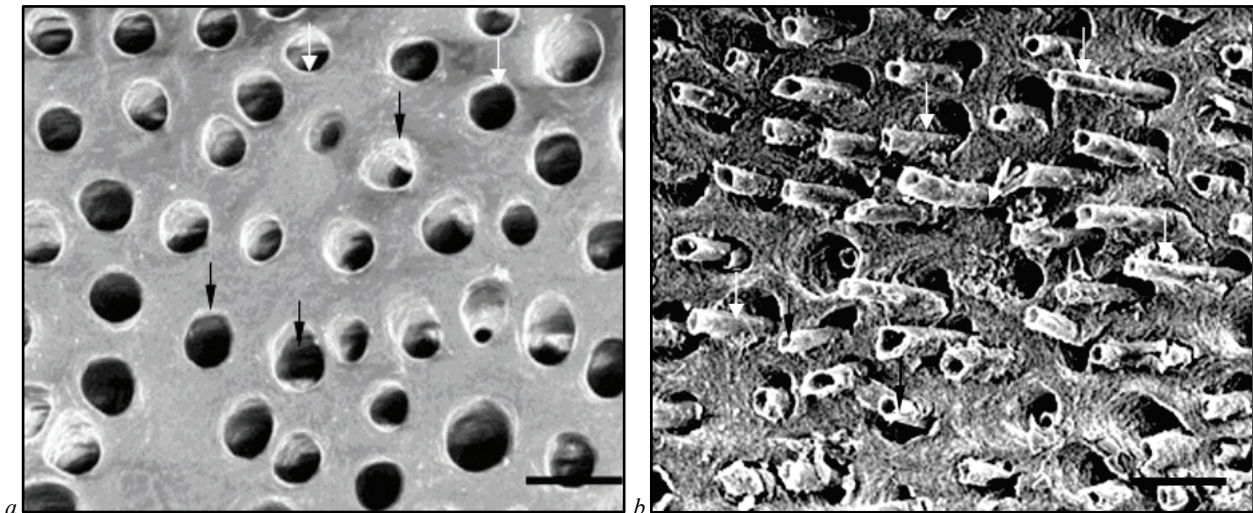


Fig. 6. Structure of the root dentin in the middle part of the tubule length on the transversal ridge of tooth: *a* – dentinal tubules foramina (arrows); *b* – dentinal canaliculi in tubules (arrows)

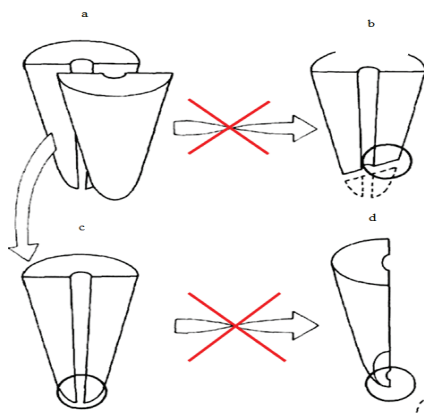


Fig. 7. Scheme of the stages of dentin preparation for SEM-study (*a* → *c*) and unacceptable angular displacements (*b* → *d*) that should be avoided for visualization of dentinal canaliculi, which are analogues of odontoblast processes

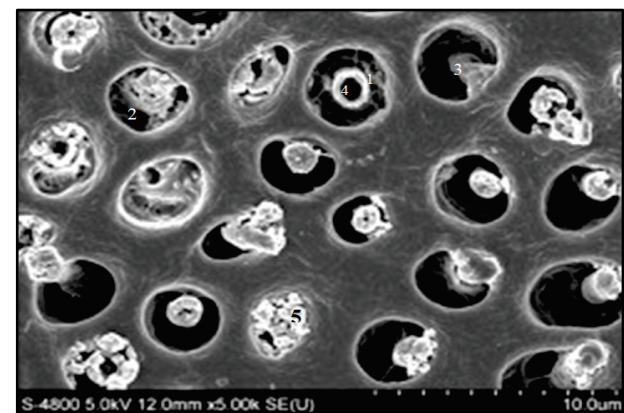


Fig. 8. SEM image of dentinal canaliculi and dentinal tubules: fibrillar (1) and crystalline (2) substance in dentinal tubules; lumen of dentinal canaliculus sealed with amorphous or crystalline substance (3), cavity of dentinal canaliculi without content (4); dense contact of the dentinal canaliculus with the wall of the dentinal tubulus (5)

In a previously published work (Gevkaliuk et al., 2023), we reported the existence of an independent fibrillar microsystem of the dentinal tubule, as indicated by micrographs with a dense microfibrillar network present on the walls of the prepared dentinal tubule in the absence of an odontoblast process (Fig. 9).

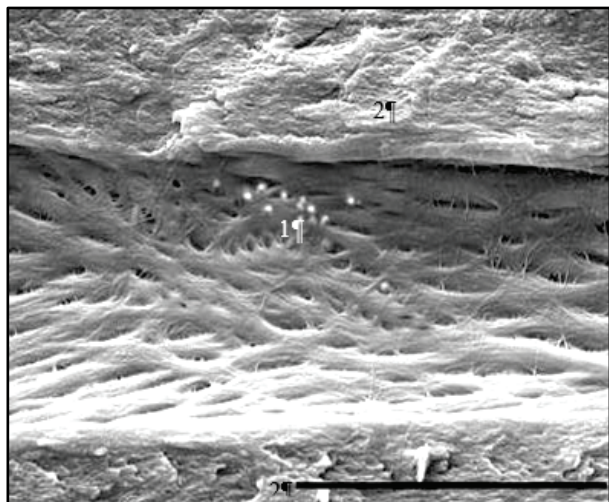


Fig. 9. Densely interwoven microfibrillar network on the wall of a dentinal tubule with local coccal microflora (1); 2 – peritubular dentine; accelerating voltage 25 kV; scale bar – 100 μm

Discussion

For some time, the structure of dentin has been thoroughly studied by SEM and was the subject of many published articles, in which details of newly discovered dentin structures were described (Brenna et al., 2003; Garcés-Ortiz et al., 2015; Ryou et al., 2023). Previously (Scott, 1955) odontoblast processes were described in the inner third of dentin, which were associated with fine fibrils and were considered to be ramifications of the odontoblast process. Analysis of previously published data on SEM studies of dentin architecture revealed that some of them (Kelley et al., 1981; Fox et al., 1984; Goracci et al., 1999) included micrographs showing microfibrillar structures of similar morphology to the structures described in detail in our study. In most of the considered articles, there were no comments on the presence or possible function of these structures, and the authors did not consider them an important discovery. In some articles, their existence was commented on very briefly (Thomas, 1983; Grötz et al., 2000; Carda & Peydró, 2006).

Only a few authors offered their views on these microstructures, and they considered them as ramifications or lateral branches of the odontoblast process (Goracci et al., 1999), or as a dense fibrous lamina limitans network (Tabata et al., 1994), ramifications of lamina limitans (Thomas & Carella, 1983), a system of anastomosing ramifications, processes of lamina limitans (Bertassoni et al., 2012) and collagen fibrils (Garcés-Ortiz et al., 2015). Comparing the features of the microfibrillar structures found in our study with the micrographic material in previously published articles (Grötz et al., 2000; Carda & Peydró, 2006; Kuntze et al., 2020), we concluded that they are identical. Our results showed that these microstructures are microfibrils that form a more or less dense network, in which isolated thin fibrils were often observed, and sometimes these fibrils were wider, appearing as a thick sheet-like membrane covering part of the inner surface of the dentinal tubule and intertwining with the odontoblast process. Sometimes these structures were absent or appeared as isolated microfibrils in areas of dentin that did not have odontoblast processes.

Our results do not support the hypothesis that microfibrils are ramifications or branches of odontoblast processes (Szabo et al., 1984) and that these ramifications are always associated with openings in the wall of the dentinal tubule, connecting one odontoblast process through these tubules with a neighboring odontoblast process (Vasiliadis et al., 1983; Carda & Peydró, 2006; Bertassoni et al., 2012). In our material, this microfibrillar network had no relation to openings on the surface of dentinal tubules. For

many years, it was believed that the odontoblast process in the dentinal tubule is immersed in a liquid medium (dentinal fluid, or lymph), and accordingly, Figure 2a, shows that the dentinal tubules in the outer third of dentin have a few micrometers, and according to this hypothesis, the odontoblast process "freely floats" in this fluid. Our results show that the described microfibrillar network is part of a complex subsystem, and we can assume that its function is to support-suspend the odontoblast process, and this microfibrillar subsystem is created on the principle of periodontal "ligamenta suppositoria", and to fix the odontoblast process in a stable position within the dentinal tubule. In our study, these microfibrillar structures were never fixed and never observed in lateral openings, which are often described in the wall of dentinal tubules by other authors (Marshall et al., 1997; Schilke et al., 2000). The results of our study convincingly show that they are not ramifications of the odontoblast process. Moreover, their fibrillar structure also indicates that they are not related to the previously described lamina limitans (Garcés-Ortiz et al., 2015).

In our study, we could not rule out the probable collagen nature of the microfibrillar subsystem of dentinal tubules described in the previous article (Gevkaliuk et al., 2023). Furthermore, these microfibrils were found to be attached to the surface of the odontoblast process; this function suggests that the microfibrillar subsystem is a part of the odontoblast process. Moreover, the results of our study convincingly show that the reticulated and sheet-like structures are also part of this subsystem, helping the microfibrils to perform the function of reliably supporting the odontoblast process on the walls of the dentinal tubule. The location of the aforementioned microfibrillar reticulated and sheet-like structures exclusively within the dentinal tubule suggests that these microstructures may arise as a cellular product that is a specific product of the odontoblast process. It can be assumed that the results we present may be related to methodological flaws, and they may just be artifacts.

There are several reports from other authors about changes caused by immersing dentin samples in various acids for demineralization (Pashley et al., 2000; Poggio et al., 2013; Klitynska et al., 2023; Lavrin, 2023; Ryou et al., 2023; Noenko et al., 2024). Acid demineralization removes the mineral component of dentin, and its first effect is to increase the diameter of the dentinal tubules due to changes in peritubular dentin (Ren et al., 2023) without changing the center-to-center distance of adjacent tubules (Louie et al., 2010; Dorvee et al., 2017). Previously, Marshall et al. (1997) calculated that during dentin demineralization, the change in the width of peritubular dentin was linear and occurred at a rate of approximately 0.005 mm/s. Also, the study (Paciornik et al., 2007) showed that the final diameter of the dentinal tubule is in the range of 3.5 to 5.5 μm . It is also known that shrinkage-drying is a constant phenomenon that occurs during this process (Scott, 1955; Poggio et al., 2013), and some studies have shown that the volume loss of dentin varies when using different demineralizing solutions (Pashley et al., 2000; Abou Neel et al., 2016), and some substances, such as HEMA (hydroxyethyl methacrylate) and ethylene glycol, can prevent such "shrinkage-drying" (Pashley et al., 2000). It is important to comment that, according to the study (Habelitz et al., 2002; Poggio et al., 2013), this shrinkage and volume loss are associated with the removal of minerals and dehydration with the formation of more or less wide gaps between collagen fibers.

In our study, we used Karnovsky's fixation methods and sublimation drying, which involve partial volume loss of dentin, and on our micrographs, as expected, there are obvious widenings of the dentinal tubules and narrowing of the peritubular dentin structure. In our opinion, the changes in dentin in this case are expected, and we do not consider these changes to be serious since no overall distortion of the material is observed. There are some issues that are not entirely clear from the point of view of histological examination of dentin sections (Gevkaliuk et al., 2023), so SEM studies can explain them. One of them is the so-called "butterfly effect" (Russel et al., 2014; Lin et al., 2021; Sodvadiya et al., 2021). Before, "butterfly" images (Fig. 4) have been observed on root fractures and are considered an "optical phenomenon" associated with dentin sclerosis (Carvalho & Lussi, 2017), as a result of which dentinal tubules undergo certain morphological changes. Russell et al. (2013) clarified this issue by concluding that in SEM examination, the buccolingual root area contains a larger number of dentinal tubules (Fig. 5), and clinically, restoration on the buccal or lingual surface may have better conditions for retention and

durability of the filling material than on proximal surfaces. Furthermore, the study by Niño-Barrera & Garzón-Alvarado (2012) using SEM and a mathematical model showed that the spatial distribution of dentinal tubules accordingly determines the differentiation of odontoblasts and the formation of dentinal tubules with a 1:1 distribution of tubules and processes (one tubule-one canaliculus) (Fig. 6).

The results of our studies explained the development of a greater or lesser number of dentinal tubules in different areas of the tooth root; this situation may be related to lateral canal shaping, which during endodontic treatment can affect the appearance of recurrent infections (Ricucci et al., 2014; Zan et al., 2018). This structure of dentinal tubules may be the cause of ineffective treatment of hard tissue diseases of the tooth (Vieira et al., 2012). We assume that new studies are needed to confirm our results. Therefore, we define the prospects for further research in analyzing new material to search for patterns in the architecture of human tooth dentin and its morphometric justification.

Conclusions

Dentinal tubules contain many microstructures in the form of thin and thick microfibrils, which connect the wall of the dentinal tubules and the odontoblast process, while in other cases we observed framework, mesh-like structures or sheet-like membranes in the non-mineralized predentin and in the inner and middle third of the dentin thickness. They were found in greater numbers in the inner third of the dentin, somewhat less as they approached the cementum-dentin junction, because the analysis was carried out closer to the root surface of the tooth. These microstructures may represent a previously undescribed subsystem that holds and protects the odontoblast process within the dentinal tubule and is an integral part of the odontoblast process itself. The concept of a "dentinal canaliculus", which is revealed after preparing dentin fractures for SEM study, should be included in the scientific histological classification of the ultrastructural organization of dentin and not considered an artifact resulting from the specific effects on dentin structures during their drying by freeze-drying through the critical point transition, and the walls of this canaliculus are nothing more than the remains of the "sublimation-dried" membrane of the odontoblast process.

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